

ASSESSMENT OF SURFACE BACTERIA CONTAMINATIONS AND SENSORY QUALITY OF SELECTED VEGETABLE FRUITS EXPOSED TO PULSED X-RAY IRRADIATION

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ABSTRACT

This study assessed the impact of pulsed X-ray irradiation on the bacteria contaminations and sensory quality of carrot and tomato. Fifty (50) kg each of carrot and tomatoes were collected into sterile sample polythene bags aseptically and were transported to the Food microbiology laboratory of the Ibrahim Badamasi Babangida University, Lapai for analysis. Bacteriological quality assessment of the vegetable fruits were investigated using standard techniques. Carrot and tomato were exposed to different doses of 42, 50, 60, 67, 71, 75, 80, 85, and 90kv irradiation and non irradiated samples were analyzed microbiologically using pour plate technique. Odour, texture, color and general acceptability of irradiated and non irradiated samples were analyzed by 20 trained panelies for sensory evaluation using 7 point hedonic scale. Different bacteria isolates from the surface of the carrot and tomatoes samples were observed culturally and microscopically. The result showed that carrot samples exposed to doses of 42,50, 67, 71, 75, 80 and 85kv and 90kv completely free of bacteria while tomato samples exposed to doses of 42, 50, 60, 67, 71, 75, 80, 85 and 90kv led to complete elimination of microorganisms present in the samples. Bacterial reduction rate of the exposed samples were directly propotional to the voltage and time exposed. There was gradual reduction in sensory profile of carrot within the voltage range of 5.0-3.5v and exposure rate of 42kv and 90kv. There was also a gradual reduction in sensory profile of tomato at the voltage range of 3.7-3.5v within the exposure rate of 42kv and 90kv respectively. The pulse x-ray is an effective non thermal treatment for vegetable fruits which could also preserve the sensory quality, however further studies are recommended to perfect this novel approach of food preservations.

Keywords: Pulse X-ray, vegetables, hedonic scale, microorganisms, sensory quality

INTRODUCTION

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well-being. Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006). Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Carmo *et al.*, 2004).

The carrot (*Daucus carota subsp. sativus*) is a root vegetable,

usually orange in colour, though purple, black, red, white, and yellow cultivars exist (Sifferlin *et al.*, 2018). Carrots are a domesticated form of the wild carrot, *Daucus carota*, native to Europe and southwestern Asia.

All over the world, tomatoes occupy a significant position in agriculture and as well human diet. Second to potatoes, tomatoes are the most produced and consumed vegetable (Moreno *et al.*, 2006). There are thousands of varieties of tomatoes, the mostly widely available are classified in to three group; cherry, plum and slicing tomatoes. A raw sweet variety like the cherry tomato is the grape tomato really wonderful to eat in raw or in salad.

Fruits and vegetables are important components of a healthy and balanced diet. Their sufficient daily consumption could help prevent major diseases such as cardiovascular diseases and certain cancers (WHO/FAO Report, 2004). However, major outbreaks involving fresh fruits and vegetables have been associated with common food borne pathogens such as *Salmonella* sp., *Shigella* sp., *Campylobacter*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium* sp., *Bacillus cereus* as reported by Beuchat *et al.* (1998), Yaun *et al.* (2004) and Berger *et al.* (2010). Common traditional disinfectants (chlorine, chlorine dioxide, bromine, iodine, trisodium phosphate, sodium chlorite, sodium hypochlorite, quaternary ammonium compounds, acids, hydrogen peroxide, ozone, permanganate salts etc.) are partially effective in removing pathogens, each type of disinfectant varying in efficiency and in allowable maximum concentration (Beuchat, 1998; Yaun *et al.*, 2004; Allende *et al.*, 2009 and Oms-Oliu *et al.*, 2010). These Shortcomings of those traditional methods demands an alternative non-thermal approach to effectively disinfect the fruits without causing serious sensory changes. Other attempts in reducing the number of microorganisms on the surface of fresh fruits and vegetables and extending the shelf life were modified atmosphere packaging Soliva-Fortuny *et al.* (2004), the use of edible films Viña *et al.* (2007), and the low temperature storage Abadias *et al.* (2012). These treatments are selective in reducing the number of pathogens on the surface of fresh fruits and vegetables. Therefore, the use of nonselective treatments for the destruction of pathogens on the surface of fresh fruits and vegetables would be a better option. Such alternative processes are the irradiation of food and the use of germicidal ultraviolet light and pulse x-ray as reported by Gardner *et al.* (2000). At low dose levels (1 keV or less), most fresh-cut vegetables and fruits show little change in sensory properties such as appearance, flavor, color, and texture, although some

products can lose firmness. Some vegetables such as fresh-cut cilantro can tolerate 3.85 keV of radiation Foley *et al.* (2004). In fact, the destruction of spoilage organisms increases the shelf life of most fresh and fresh-cut vegetables (Prakash *et al.* (2004). Irradiation (x-ray) may induce the loss of firmness (softening) in some fruits as reported by Gunes *et al.* (2000) and Palekar *et al.* (2004). Irradiation-induced loss of firmness is related to partial depolymerization of cell-wall polysaccharides, cellulose, and pectin and to changes in activity of the cell-wall enzymes pectinmethylesterase and polygalacturonase that act on pectic substrates. However, the loss of firmness can be mitigated by dipping diced tomatoes and fresh-cut apples in calcium Solution prior to x-ray irradiation as indicated by Gunes *et al.* (2000) and Prakash *et al.* (2007) and by storing the products in modified-atmosphere packaging (Boynton *et al.*, 2006). Irradiated (1 kGy) cilantro and lettuce showed some softening, but after a few days of storage, there was no significant difference between irradiated and non irradiated samples (Fan *et al.*, 2003b). At low dose levels (≤ 1 kGy), few if any effects on flavor and aroma are observed in fresh and fresh-cut vegetables. A decrease in characteristic aroma of cilantro (Fan *et al.*, 2003a) and off-flavor of Bell peppers (Masson, 2002) has been observed at doses of ≥ 3 kGy. Changes in flavor and aroma of fresh vegetables are highly correlated with microbial spoilage. Thus, x-ray irradiation generally inhibits or delays development of off-flavors related to growth of spoilage organisms. In general, the effect of x-ray irradiation on quality of fresh and fresh-cut vegetables is minimal. In those cases where significant changes are seen at effective dose levels, effects on texture, color, or browning can be minimized by combining irradiation with other technologies such as calcium dips, modified-atmosphere packaging, or anti browning agents.

Considering the adverse impact of traditional methods of disinfection process of vegetable fruits such as Carrot, and Tomatoes. Traditional methods of vegetable fruits disinfection mechanism of using water and other chemical agents, have series of shortcomings such as limited efficiency to remove microorganism (Yaun *et al.*, 2010). Thus the methods are partially effective in removing pathogens because types of disinfectant varies in efficiency and maximal concentration. These shortcomings of traditional methods demand an alternative by Non-thermal approach of food preservation process by X-ray Irradiation to effectively kill or reduced microorganism causes serious contamination and sensory changes in fruits. Owing to the fact that the consumption of fruits and vegetables are important components of health and balance diet. However, major outbreaks involving fresh and vegetables fruits have been associated with common food borne pathogens such *Escherichia coli*, *Shigella* sp, *Salmonella* sp. Each type of ionizing radiation has its own advantages and disadvantages. For example, gamma rays and X-rays have higher penetration ability than electron beams. However, gamma rays are emitted by radioactive materials, such as cobalt-60 and cesium-137, while generation of X-rays is a relatively inefficient and energy intensive process. Most energy (about 90%) is lost to heat during the conversion of electron beams into X-rays. Electron beams have a low penetration ability, even though the electron beam generators can be switched on and off and do not involve radioactive materials. At low dose levels (1 kGy or less), most fresh-cut vegetables show little change in appearance, flavour, colour, and texture, although some products can lose firmness. As an example, the

appearance of irradiated spinach was similar to that of the non-irradiated samples after 14 days storage at 4°C. Some vegetables such as fresh-cut cilantro can tolerate 3.85 kGy of radiation (Foley *et al.*, 2004). In fact, the destruction of spoilage organisms increases the shelf life of most fresh and fresh-cut vegetables (Prakash and Foley, 2004; Niemira and Fan, 2005). The response to irradiation is specific to product, and even similar varieties, as shown in studies on various lettuce types (Niemira *et al.*, 2002), exhibit differences in texture and respiration rates. At low dose levels (≤ 1 kGy), the effects on nutritional quality are minimal. Irradiation can reduce ascorbic acid thus increasing their antioxidant capacity (Fan, 2005). However, since phenolic compounds are also responsible for the browning reactions in vegetables, their increase is not a desired outcome. In general, the effect of irradiation on quality of fresh and fresh-cut vegetables is minimal. In those cases where significant changes are seen at effective dose levels, effects on texture, colour, or browning can be minimized by combining irradiation with other technologies such as calcium dips, modified-atmosphere packaging, or antibrowning agents. Adoption of irradiation for food applications has been a slow process. The limited number of foods approved by regulatory agencies, cost, consumer reluctance to accept irradiated foods, and the public's uncertainty of this technology may contribute to its minimal commercialization. Studies on marketing of irradiated foods have demonstrated that consumers are more willing to buy irradiated foods after they are provided information about the process (Bhumiratana *et al.*, 2007).

Spoilage microorganisms exploit the host using extracellular lytic enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage (Miedes and Lorences, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas, 2005b). Spoilage microorganisms also can enter plant tissues during fruit development, either through the calyx (flower end) or along the stem, or through various specialized water and gas exchange structures of leafy matter. Successful establishment, however, requires the spoilage microbe to overcome multiple natural protective barriers. Fruits and vegetables possess an outer protective epidermis, typically covered by a natural waxy cuticle layer containing the polymer cutin (Lequeu *et al.*, 2003). This study aimed at assessing the surface bacteria and sensory quality of selected vegetable fruits (Carrot, and Tomato) exposed to pulsed X-ray irradiation.

MATERIALS AND METHODS

Study Area

Research was conducted in Lapai and Minna, Niger State, Nigeria.

Collection of Samples

A total of one hundred (100) kg consisting of fifty (50) kg each of carrot and tomatoes vegetable fruit samples were purchased from Lapai market, Niger State and were deposited into sterile polythene bags for each sample respectively.

Preparation of Vegetables for Microbial Analysis

Before samples were taken out of their polythene (packaging), the possible contact surfaces were carefully sterilized using polyurethane sponges to prevent cross contamination. Damaged samples were discarded before analysis. Each sample (whole) were aseptically transfer into a stomacher bag fill with equal weight of buffer pepton water. Each whole sample was then be agitated and rubbed by hand in the stomacher bag for 2 minutes to suspend surface microbes as described by FDA (1998) and Abadias *et al.* (2012).

Preparation of Media

Nutrient agar, MacConkey Agar, Sabrouad-Dextrose agar, *Salmonella-Shigella* agar, peptone water (all from Fluka, Germany), Urea agar base, tryptone broth (both from Lab M, UK), mannitol salt agar, Methyl red voges-proskeur broth and Simmons-citrate agar and *Salmonella-Shigella* agar were prepared according to manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes.

Isolation of Bacteria from Selected Vegetables

Twenty-five (25) g of carrot and tomatoes samples each were weighed and washed in 225 ml of sterile distilled water. MacConkey, *Salmonella-Shigella* agar and nutrient agar were inoculated with 1 ml of the rinse water using the pour plate technique. The mixtures in the plates were allowed to solidify, inverted and incubate at 37°C for 24 hours for colony formation. Each colony were sub cultured to get pure culture for further studies and identification. Distinctive morphological properties of each pure culture such as colony formed, elevation of colony and colony margin were observed and Gram stained for microscopy. The same procedures were adopted on the analysis of the vegetable fruits after pulse X-ray exposure.

Irradiation and Storage of Selected Vegetable Exposed to Pulse X-Ray

Before treatment, samples of vegetable fruits were selected to form different uniform lots. During PL treatment, the samples lots of each vegetable fruits were placed in the centre of the tray and aligned with their main axis parallel to the lamp tube at the maximum vertical distance allowed. At this distance, the average energy dose per pulse delivered on the upper surface of the samples were ranged from 0.35 J/cm² to 0.45 J/cm². During the treatment, the samples were rotated in order to expose each side of the fruit to the same dose.

Experimental protocol

DOSE (mAs/ms)	GRP								
	A	B	C	D	E	F	G	H	I
	42kv	50kv	60kv	67kv	71kv	75kv	80kv	85kv	90kv
12/0.03	2	2	2	2	2	2	2	2	2
20/0.05	2	2	2	2	2	2	2	2	2
32/0.07	2	2	2	2	2	2	2	2	2
50/0.10	2	2	2	2	2	2	2	2	2
80/0.13	2	2	2	2	2	2	2	2	2

GRP: Group, Kv: kilo volts

Physicochemical Analysis of Selected Vegetables

Periodically, at each of the specified period (0, 7, 14, and 21 days), fruits from each lot were selected and analyzed for color CIE L*a*b* (color scale for the measurement), pH and temperature.

Sensory Evaluation of Selected Vegetable Exposed to Pulse X-Ray

The sample of vegetable fruit (carrot and tomatoes) exposed to irradiation were accessed for colour, odour, consistency and acceptability using 7 point hedonic scale by 10 panelists.

Statistical Analysis of Data

Statistical analyses were performed using one way analysis of variance (ANOVA), to test the effect of the applied x-ray radiation dose on the studies parameters, and the differences between the mean value were identified by Duncan's multiple range test using SAS *et al.* (2002). mean values and standard errors of the mean were reported.

RESULTS

Cultural and microscopic identification of bacteria isolated from the selected vegetable are presented in table 1. The bacteria isolates present varying cultural and microscopic characteristics ranging from colour, appearance, consistency and shape of the colonies. The steady increase in the dosage level of carrot exposure shows that the microbial load had reduced from log¹⁰ - log¹⁰ cfu/ml with high dose from 45,000-5,000 with a linear trend of y=4697+45,00 (Figure 1). While the constant increased in time of carrot exposure to radiation shows a steady decrease in microbial load (0.055) with a linear trend of y=0.83.32+7 (Figure 2). It was observed that at constant increase in the dosage level of tomato exposure, there were steady reduction in the microbial load from log¹⁰ - log¹⁰ CFU/mL with increasing dosage level at the linear trend of y=4697+40.000 even with time (Figure 3 and 4). There was also gradual reduction in sensory profile of carrot within the voltage range of 5.0-3.5v and exposure rate of 42kv and 90kv. However, good sensory profile was observed with 4.0v from non-exposed sample and lowest voltage of 1.0v from the exposed + treated samples. There was a uniform pattern of sensory profile of carrot at 3.3A within the current range of 400-615 Amp. In addition to the exposed + treated samples in relation to time of exposure (Figure 5 and 6). Gradual reduction in sensory profile of tomato was recorded at the voltage range of (3.7-3.5)v within the exposure rate of 42kv and 90kv respectively, However, with the highest voltage of 4.0v from non-exposed sample. There was also reduction in sensory profile of tomato at the voltage range of (5.0-3.5)v within the exposure rate of 42kv and 90kv, with voltage of 4.0v from non-exposed samples and lowest voltage 1.0v from the exposed + tested samples with time. (Figure 7 and 8).

Table 1: Cultural and Microscopic Characteristics of bacteria isolated from Vegetables

S/NO	Cultural Morphology	Microscopic characteristics
1	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
2	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
3	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
4	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
5	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
6	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli
7	opaque-blackish spreading colony on Salmonella Shigella Agar	Gram -ve bacilli

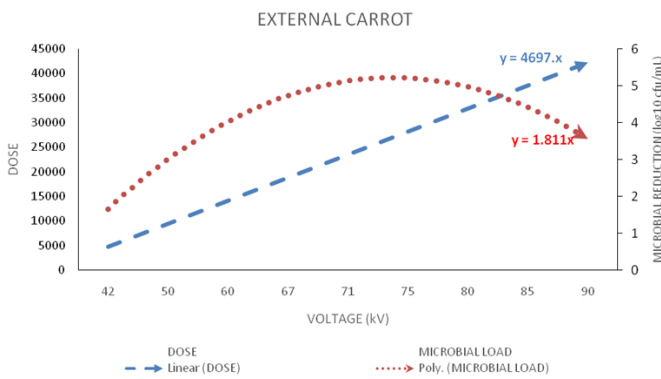


Figure 1: microbial load of carrot after pulsed x-ray exposure in relation to dose

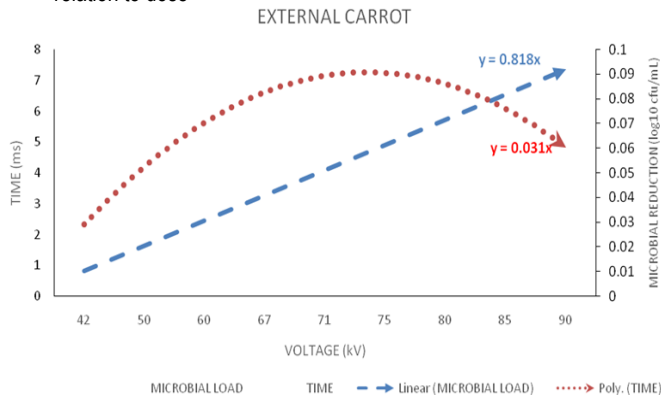


Figure 2: Microbial load of carrot after pulsed x-ray exposure in relation to time

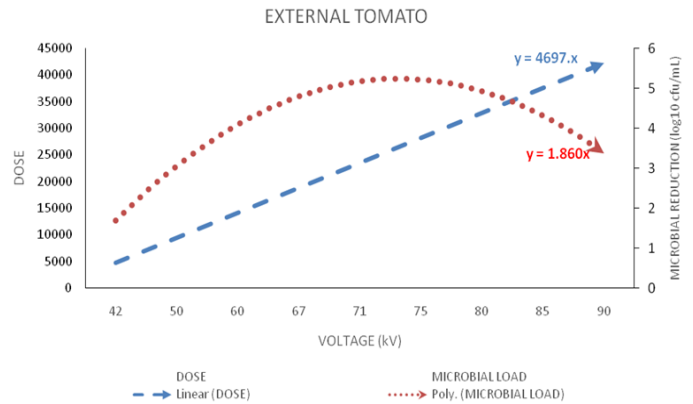


Figure 3: Microbial load on tomato after pulsed x-ray exposure in relation to dose

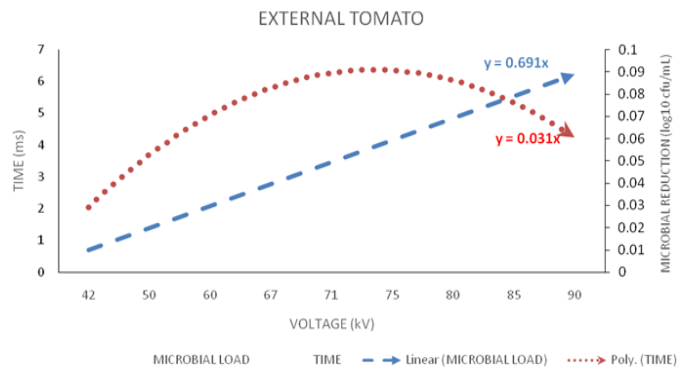


Figure 4: Microbial load on tomato after pulse x-ray exposure in relation to time

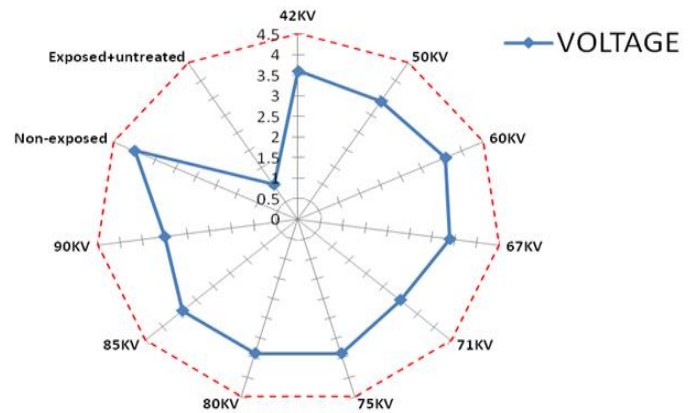


Figure 5: Sensory profile of tomato after pulsed x-ray exposure (voltage)

1=EDL, 2=VDL, 3=DL, 4=N, 5=L, 6=VL, 7=EL.

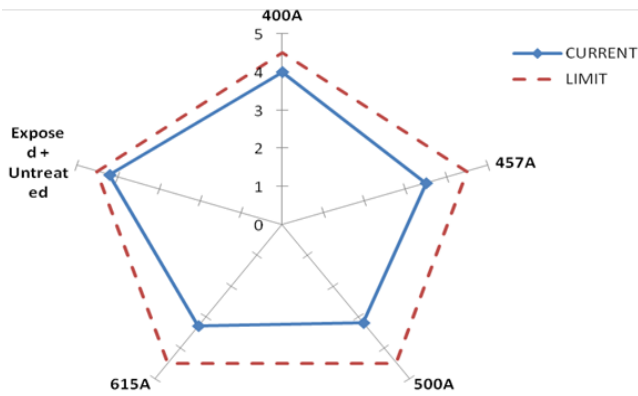


Figure 6: Sensory evaluation of tomato after pulsed x-ray exposure (current)

1=EDL, 2=VDL, 3=DL, 4=N, 5=L, 6=VL, 7=EL.

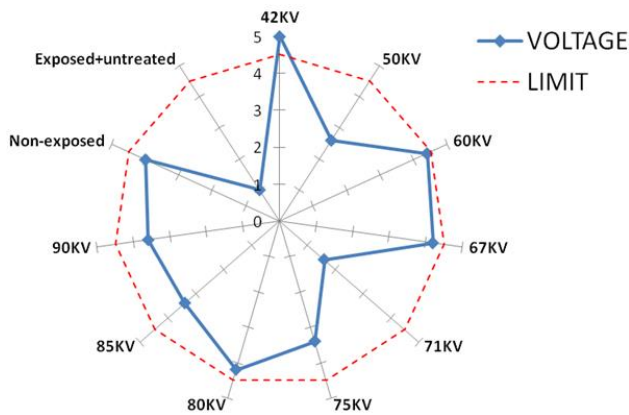


Figure 7: Sensory evaluation of carrot after pulsed x-ray exposure (voltage)

1=EDL, 2=VDL, 3=DL, 4=N, 5=L, 6=VL, 7=EL.

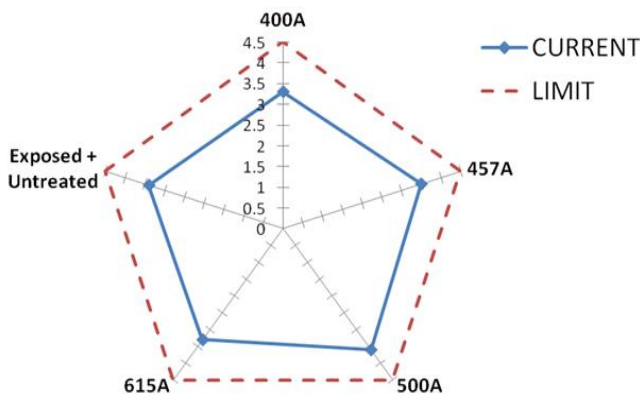


Figure 8: Sensory evaluation of carrot after pulsed x-ray exposure (current)

1=EDL, 2=VDL, 3=DL, 4=N, 5=L, 6=VL, 7=EL.

DISCUSSION

The bacteria species isolated from the vegetable fruits are in correspondance with those previously reported from fruits and vegetables by Uzeh *et al.* (2009). The low bacteria count obtained disagrees with those obtained in vegetable fruits as reported by Bukar *et al.* (2010). The bacteria isolates may be part of natural flora of the vegetable fruits or contaminant from soil, irrigation, water, the environment during transportation (Ofn *et al.*, 2009). The progressive reduction in microbial loads with increase in exposure time may be due to high rate of irradiated dose in some samples. thus low dose irradiation is a reliable technology capable of killing human pathogens such as *E.coli* O157:H7 and *Salmonella* sp. by 2-8 logs with out causing significant deterioration in product quality as reported by Ofn *et al.* (2009). The softening observed in some samples at doses greater than 42kv is totally contradictory to the findings that dose of 1kv cilantro and lettuce showed some softening, but after a few days of storage, there was no significant difference between irradiated and non- irradiated samples (Fan *et al.*, 2003). Other Product, such as celery (Prakash *et al.*, 2000), mushroom slices (Koorapati *et al.*, 2004), and Shredded carrots (Baker, 1998), also showed no change in firmness (Alekar *et al.*, 2004). There were no significant difference ($P < 0.05$) between irradiated and non irradiated carrot and tomato. This is similar to the findings of Song *et al.* (2007). The sensory quality (texture) of the pulse X-ray irradiated vegetable fruits could be attributed to the time and dose of exposure which agrees with the findings of Gunes *et al.* (2000) and Alekar *et al.* (2004) who reported that irradiation induces the loss of firmness (softening) in some fruits and vegetables. The effect of low dose on flavour and aroma of the vegetable fruits could be due to the reaction on the fruits compositions which conformed with decrease in characteristics aroma of some vegetable fruits like cilantro as reported by Fan *et al.* (2003) and off-flavor of bell peppers (Massom, 2002). At high doses, changes in flavor and aroma of fresh vegetables are highly correlated with microbial spoilage. Thus, irradiation generally inhibit or delay development of off-flavor related to growth of spoilage organisms. The effect of irradiation on permeability of cell membranes can resist in electrolyte linkage and loss of tissue integrity. These effects are limited at low dose, but higher dose levels, electrolyte leakage may cause a soggy and wilted appearance. However, the sensory quality of non-irradiated carrot and tomato decreased with storage time. This is in agreement with Bibi *et al.* (2006) who reported that the appearance and flavour scores for tomatoes showed similar trends as that of carrot store at 5°C. the appearance score was lower for non-irradiated samples. The flavor of tomatoes was enhanced with irradiation in this study. The effectiveness of pulse -x-ray observed against the micro-flora on the samples agrees with the finding of Song *et al.* (2006) who studied the effectiveness of gamma irradiation for inactivating *Salmonella typhimurium* and *E.coli* in carrot and tomatoes store at 10°C.

Conclusion

Pulsed x ray radiation doses of 42, 50, 60, 67, 72, 75, 80 and 85kv resulted in complete inactivation of microorganisms in carrot, while at all exposure doses of x-ray radiation with tomato resulted in complete inactivation of bacteria infected organism. Thus, this current research shows that at high and extreme dose of pulsed x-ray radiation, wave length would be too large to inactivated or eliminated the microorganism, also the pulsed x-ray radiation at

small, minimum, and optimal doses would be able to inactivated the microorganism, thus render fruits/vegetables free of microorganisms.

Recommendations

It is recommended that further highlights be provided which is needed to safeguard the health of the consumers' nutritional consequences.

Possible formation of toxic by-products and the applicability of photosensitization on food products, For example, vegetables should be further studied to give more safety preferences.

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