

Original Article

Microbial Safety, Visual Quality and Consumers' Perception of Minimally-Processed Ready-to-eat Salad Vegetables Prepared and Stored at Room and Refrigeration Temperature

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Raw salad vegetables are evaluated for the consumer's perceptions on taking ready to eat fresh cut-vegetables and the effectiveness of some non-chlorine disinfectants [peracetic acid (PAA), shell powder (SP) and hydrogen peroxide (H₂O₂)] in improving the microbial safety, quality and shelf life of ready to eat fresh-cut vegetables (lettuce, carrot and cucumber) at ambient and refrigeration temperature. Consumer's perception study results identified three clusters of consumers, whose preferences are related to purchasing styles and socio-demographic variables. The overall positive attitude of consumers was evident towards convenience, taste and appearance, but safety and health benefit attributes get importance while buying the ready to eat fresh-cut vegetables. The microbiological and visual observation result demonstrated that, all the non-chlorine sanitizers used were able to decrease the bacterial population in fresh-cut vegetables initially; however, microbial population increases or remain constant or decrease depending on the types of vegetables, storage temperature and duration. In addition, among the wash-sanitizers, PAA and H₂O₂ showed better microbial reduction for fresh-cut lettuce, and cucumber, and SP showed better microbial reduction for fresh-cut carrot. Irrespective of sanitizer treatment refrigerated storage showed better visual quality, microbial safety and shelf life of fresh-cut produce. Therefore, this study results suggested that washing fresh-cut vegetables with produce specific sanitizer and stored at refrigerated temperature keep the quality of fresh-cut produce better compared to ambient storage.

Keywords: fresh-cut vegetables; consumer's perceptions; Microbial safety; and storage temperature.

Introduction

Fresh-cut products are fruits or vegetables that have been trimmed, peeled and/or cut into an entirely usable product, subsequently packaged to offer consumers high nutrition, convenience and flavour while maintaining freshness^{1,2}. According to Food and Drug Administration³, fresh-cut fruit and vegetable products are defined by being minimally processed (already washed, cut, mixed and packaged) and ready for consumption⁴.

The fresh-cut produce market in Asia has witnessed dramatic growth in recent years, primarily stimulated by consumer demand for fresh, healthy, convenient and additive-free foods which are safe and nutritious^{5,6,7}. The market has shown a steady growth trend since the late 1980s and 1990s⁸. Fresh-cut vegetables command a larger market share in Thailand⁹, Japan¹⁰, and Korea¹¹. With growing consumer demand for ready-to-eat products, the market for fresh-cut products in these countries is likely to show a continued growth trend. However, in South Asian countries, fresh-cut produce is sold in open-air markets and food stalls and is increasingly sold in supermarkets. Fresh-cut fruits, in particular, have gained popularity in urban centres and among young consumers. Often these products are displayed without

the benefits of refrigeration, so their shelf-life is frequently not extended beyond the day of display. Fresh-cut vegetables for cooking constitute the most significant part of the fresh-cut produce industry. Fresh-cut salads are another major category as consumers perceive them to be healthy. However, with increasing demand for fresh cuts at the retail level, the fresh-cut industry is facing challenges to extend shelf-life and enhance food safety^{12,13,14}.

Since the preparation of traditional dishes also necessitates a variety of fresh ingredients in South Asia. The drudgery of peeling vegetables, shelling peas, trimming herbs and vegetables, and combining these ingredients often deters the busy housewife from preparing these traditional foods. Similarly, the difficulty of peeling fruits such as pineapple, jackfruits and watermelon and sometimes their large size deters the consumer from purchasing them. Fresh-cut processing addresses all of these issues by making products available in a convenient, easy-to-use format with minimal waste¹⁵. Cottage industry suppliers are increasingly preparing packs of fresh-cut fruits and vegetables. They are being sold in wet markets in response to consumer demand for produce in a ready-to-use format.

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Consumers generally purchase fresh-cut produce for convenience, freshness, nutrition, safety and the eating experience. Indeed, consumer demand for these attributes has led to considerable innovation and diversification in the fresh-cut industry¹⁶. Apart from presenting the consumer with a range of options in a single package, freshcuts reduce wastage at the household level. They allow the consumer to procure only the quantities of fresh produce required. While fresh-cut produce requires relatively little product transformation, it requires investment in technology, equipment, management systems and strict observance of food safety principles and practices to ensure product quality^{17,18,19}.

In Bangladesh, due to rapid urbanization, population growth, urban centres expansion occurred in the past two decades. At the same time, traditional food supply chains and food habits have been changing to keep up with these changing trends. These social changes include increases in single-person households, increases in middle-income populations; less time for meal preparation; increased demand for convenience food items; increased sales of ready-to-eat meals; and increases in the restaurant and fast-food operations^{20,21,22}. Growth in the market opportunities for fresh-cut produce will continue only if consumers believe that fresh-cut produce is safe and of high quality with sufficient shelf-life²³. Other challenges to the marketing of fresh-cut produce include: preserving product quality through the marketing chain; maintaining the cold chain and proper logistics; adequacy of processing equipment, refrigerated storage and processing facilities; availability of technology to set up processing plants and conduct research to preserve the quality of fresh-cut produce. Therefore, in this study, we evaluated the consumer's perceptions on taking ready to eat fresh-cut vegetables and the effectiveness of some non-chlorine disinfectants in improving the microbial safety quality and shelf life of prepared to eat fresh-cut vegetables at ambient and refrigeration temperature.

Materials and methods

Consumer's perception study

To understand the status of minimally processed ready-to-eat salad vegetables listing of the products have been done by visiting the five popular places for the street item, five salad bars and five chain shops (10 respondents of each and total 150 respondents). A short interview was taken to the stakeholders about processing environments, customer's behaviour, awareness about the safety and quality of minimally processed ready-to-eat salad vegetables etc. Three types of the questionnaire were prepared; 1) consumers who take minimally processed fruit and/vegetables from the street (Group A), 2) consumers of modern salad bars (Group B), and 3) consumers who purchased minimally processed ready-to-eat salad vegetables from the chain shop (Group C). The framework of the survey prepared (Fig 1) is extracted from a classic attitude-behaviour model based on Engel, Blackwell and Minland⁸. Attitudes are classified and called "search attitudes" (like freshness, color, and appearance), "experiences attitudes" (like taste and flavour) and "credence

attitude" (like shelf life, health and microbiological safety). To gain insight into consumer decision making towards purchasing minimally processed vegetables and fruits cross-sectional data were collected through a consumer survey. Respondents were selected through non-probability judgmental sampling (population was selected based on personal judgment), and attribute data were as follows:

Perceived attribute importance in the purchasing (ImpPur) and consumption (ImpCon) stage of minimally processed fruits and vegetables; the average was produced on a scale from 1 (not at all-important) to 5 (very important). All form of data was collected and processed and analyzed.

Microbial Safety and visual quality study

Sample collection and processing: Fresh Carrots (*Daucus carota* L.), lettuce (*Lactuca sativa* var. *capitata* L.) and Cucumber (*Cucumis sativa*) were purchased from a local kitchen market (Gazipur, Bangladesh) and were transported to the laboratory within 30 min and upon arrival, they were manually processed. These three vegetables were included because of their initial microbial load, different topology of the vegetable tissue and their economic relevance in the fresh-cut produce industry. The carrots and cucumber were briefly peeled and pieces using a sharp Knife (DORCO, Korea). The outer leaves of lettuce were manually removed and cut into 1 cm pieces by a sharp knife. After cutting, each batch of vegetables was washed with each sanitizer (Distilled water, 150 ppm PAA, 200 ppm Cl water, and 0.5% H₂O₂) and air-dried for 2 hours. After drying, one batch undergoes microbial analysis and two batches of each were kept for storage study. A detailed illustration of washing procedures is given in Figure 2.

Preparation of solution and washing

The PAA solution was prepared following the methods of Vandekinderen *et al.*²⁴, with little modification. Initially the sample was diluted in a solution (50/50, v/v) of potassium iodide (10 g/L, Merck, Germany) and methanol (Merck, Germany) at -10°C. The liberated iodine was titrated with a standardized 0.01 N sodium thiosulfate solution (Merck, Germany), and appropriate dilutions based on the active PAA concentration were made. The PAA concentrations were determined after optimizing the efficiency of varying PAA concentrations (0, 50, 100, and 150 ppm) with fixed contact time (5 minutes) to remove the native microflora in three fresh-cut vegetables. The contact times were chosen after a screening of the relevant literature and taking into account practical considerations. H₂O₂ (0.5%) solution was prepared by adding deionized water (DW) to commercial 30% H₂O₂ and to prepare 0.1% SP solution, 0.1 g SP powder in 100 ml of DW was added. The utensils used (cutting board, knife, stainless steel tray, perforated trays etc.) in this Study, were dipped in 200 ppm NaOCl solution for 2 minutes. Afterwards, the materials were turned upside down for another 2 min, and utensils were cleaned with 200 ppm NaOCl manually in the last minute. The working surface was sterilized by being sprayed with 70%

ethanol (EtOH). The detailed activities from samples collection, processing of the vegetables up to microbial enumerations were given in Figure 3.

Statistical Analysis

All trials were replicated three times. Reported plate counts observed in all agar media were converted into colony forming

unit (CFU)/g. The numbers represent the mean values obtained from three individual trials, with each of these values obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington, DC, USA). Significant differences in plate count data were established by the least significant difference at the 5% significance level.

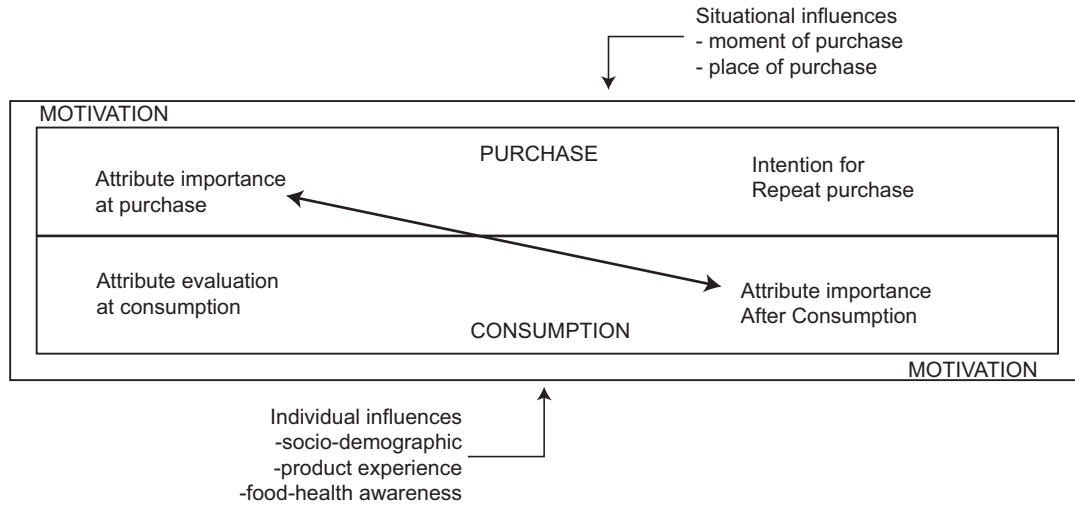


Fig 1: Framework and specific objectives (arrows) of the Study.

Materials and Methods of Vegetables Washing

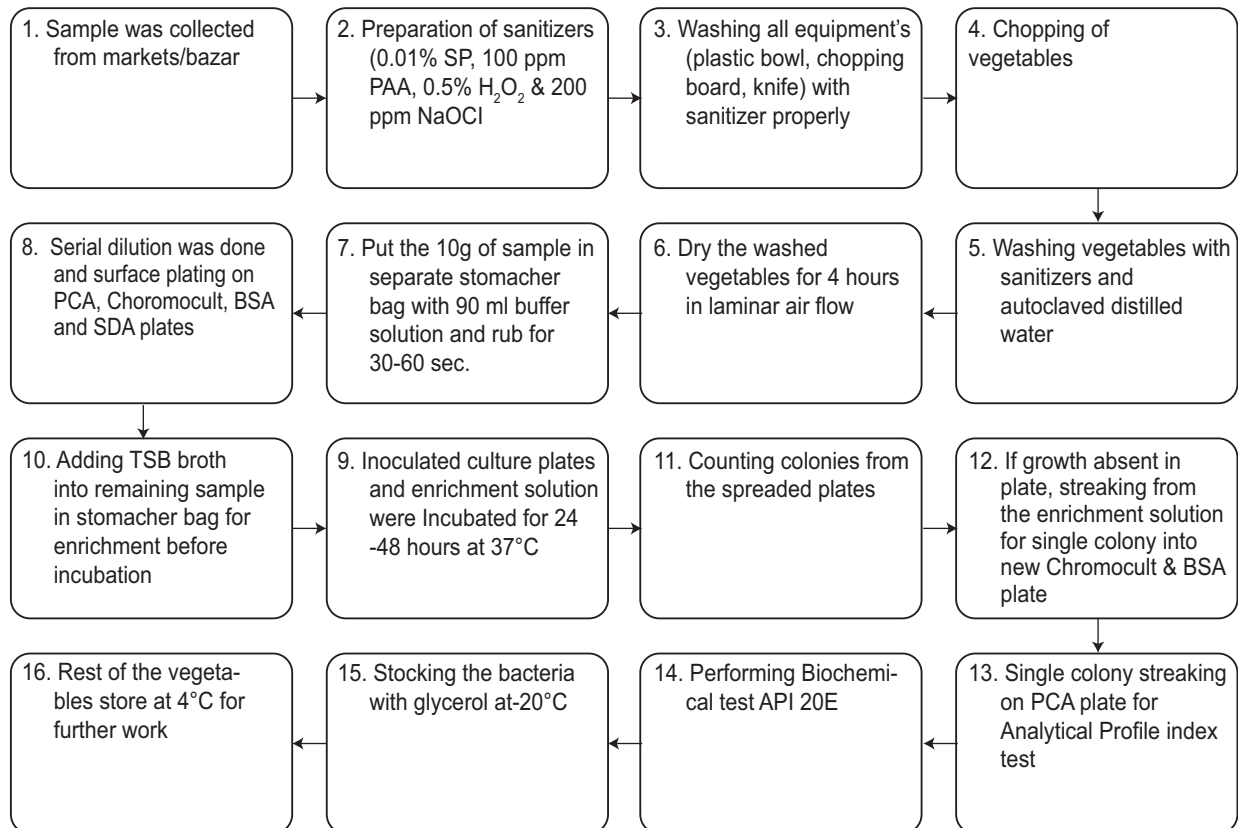


Fig. 2. Summary of the washing procedures and methods.



Fig. 3. Detailed activities from samples collection, processing of the vegetables including microbial enumerations 1. Lettuce 2. Carrots, & 3. Cucumber; 4-5. Sanitizer preparation, 6-7, chopping of vegetables, 8-10. Lettuce, cucumber and carrot washing 11-13. Drying after treatments, 14-16. Stomaching of samples 17. Serial dilution, 18. Plating 19. Incubation at 37R°C 20-21. enumerations.

Results

Consumer's perception study

In Bangladesh various types of consumers who take minimally processed ready to eat fruit and vegetables. However, three categories of consumers dominate or cover the maximum of the population. These consumers take processed fruits and vegetables

at the street, at the modern salad bar, and buy from chain shops. This Study collected data from total 150 respondents, and the information was compiled and illustrated a fig 4 (A-D). The results demonstrated that the consumer who consumes street vendor's minimally processed fruits and vegetables gave importance in taste, odor, and appearance equally during purchase and consumption while giving safety the maximum importance during

consumption (Fig 4 A). The salad bar consumers preferred safety, health benefit, nutritional value, odor, shelf life, and taste in deciding on purchase and consumption. But during the purchase, they emphasized washing (Fig 4 B). The chain shop consumers gave equal importance on all attributes during purchase but less on packaging (Fig 4 C). Invariably, safety and health benefit got the highest priority (Fig 4 D).

Optimization of PAA concentration

The influence of varying PAA concentrations (0, 50, 100, and 150 ppm) with fixed contact time (5 minutes) to remove the native microflora was tested in three types of fresh-cut vegetables: fresh-cut cucumber, carrots, and iceberg lettuce. It was observed that the efficiency of PAA to remove the native flora was highly dependent on the type of fresh-cut produce: the highest microbial reductions were obtained for cucumber (0.9–2.7 log cfu/g) and carrot (0.6–1.8 log cfu/g) followed by iceberg lettuce (0.3–0.8 log cfu/g) (Table 2). All the treated samples, regardless of the type of vegetable and concentration of the PAA treatment,

were within the recommended concentrations of common disinfectants and acceptable for consumption (Table 1). Furthermore, it was also observed that raising the concentration of PAA, increase the reduction efficiency of the bacterial count, but the effect was not persisted during both the room temperature and 4°C refrigeration storage condition, the microbial count increased significantly and reached to maximum permissible level for fresh-cut carrot (Fig 5). Based on these results, 150 ppm of PAA was used in all the subsequent studies.

Microbial Safety and visual quality study

Lettuce: As lettuce is a highly perishable vegetable thus 6 days of storage in refrigerated (4°C) and room temperature were evaluated after washing with each of the sanitizer or its combinations. The fresh cut lettuce samples were found relatively better in microbiological quality & safety. The total aerobic bacterial count (TABC) count was recorded as 4.8 log CFU/g, TCC count was found present but non-detectable, *E. coli* was absent but the presence of fecal coliform was evident and recorded

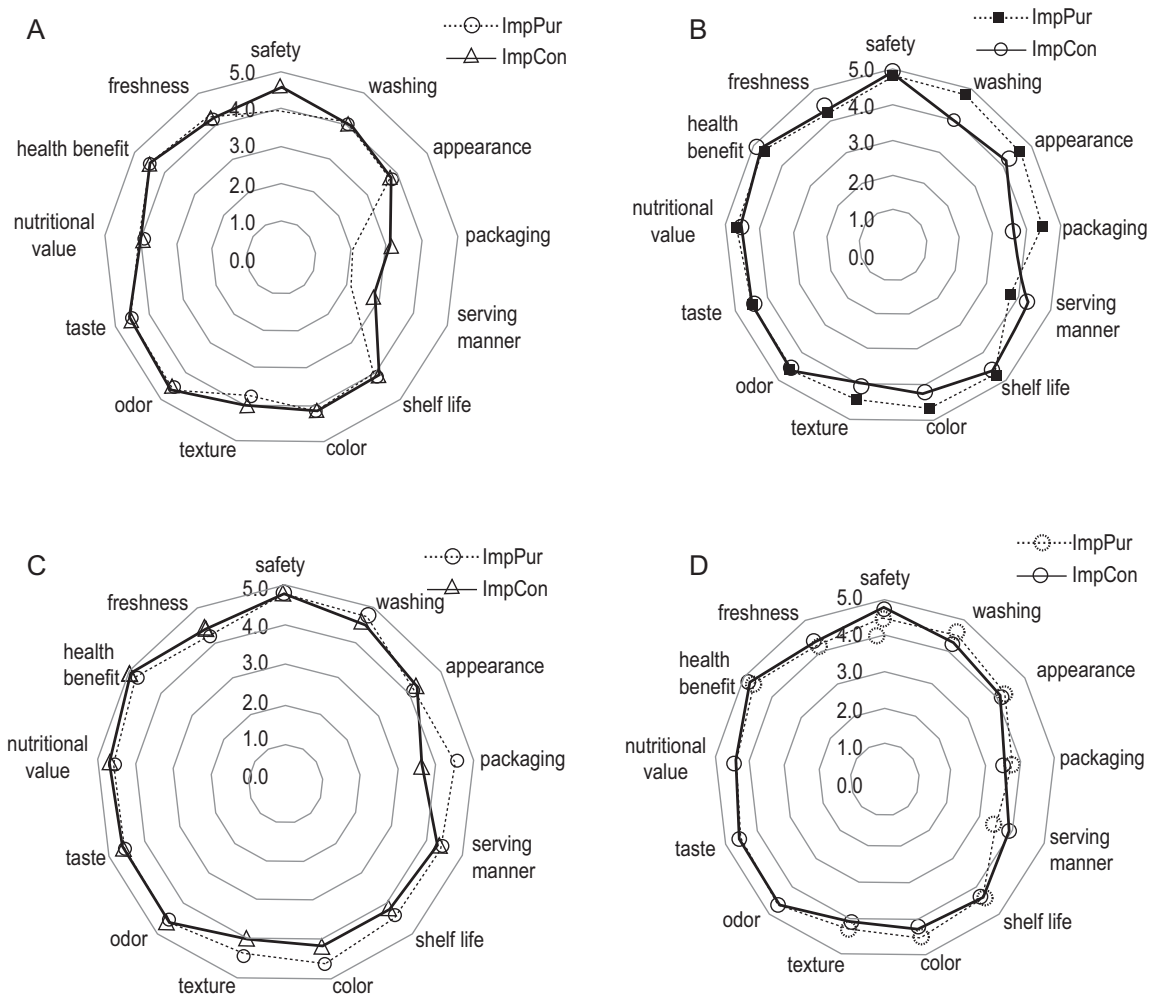


Fig. 4. Perception on minimally processed fruits and vegetables; street (A), modern salad bar (B), chain shop (C), average of all (D) of modern chain shop consumers (dot line possess ImpPur=important during purchase , black line ImpCon=Important during consumption (scale 1-5, 10 respondents from each group).

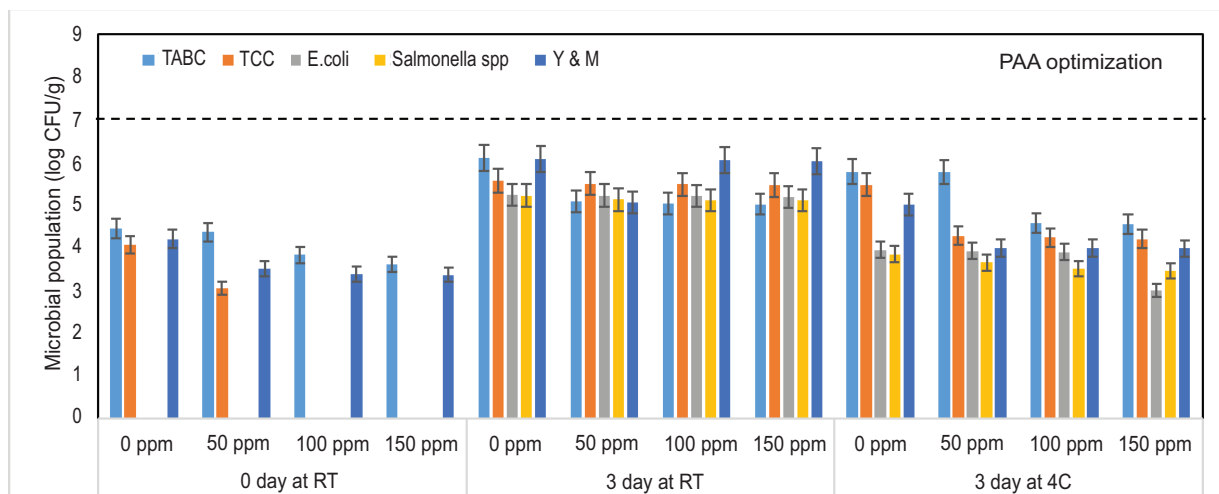
Table 1: The recommended concentrations of common disinfectants (Huss 2003)

Sanitizers	Food contact surfaces	Non-food contact surfaces
Chlorine (ppm)	100-200*	400
Quats (ppm)	200*	400-800
Peroxyacetic acid (ppm)	200-315*	200-315

The higher end of the listed range indicates the maximum concentration permitted without a required rinse (surfaces must drain)

Table 2: Optimization of PAA concentration in washing ready to eat salad vegetables for 5 minutes contact time

	Lettuce				Cucumber				Carrot			
	PAA concentration in ppm				PAA concentration in ppm				PAA concentration in ppm			
	Control	50	100	150	Control	50	100	150	Control	50	100	150
TABC	4.4± 0.5	4.1± 0.7	3.8± 0.3	3.6± 0.1	4.8± 0.0	3.9± 0.1	3.8± 0.0	2.1± 0.6	3.9± 0.0	3.3± 0.0	2.9± 0.4	2.1± 0.2
TCC	4.0± 0.8	3.0± 0.4	0	0	3.1± 0.1	2.8± 0.1	2.5± 0.2	2.3± 0.7	4.0± 0.8	2.9± 0.0	1.8± 0.0	0
<i>E. coli</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Salmonella</i> spp	0	0	0	0	0	0	0	0	0	0	0	0
TFC	4.2± 0.0	3.5± 0.2	3.3± 0.8	3.3± 0.6	4.4± 0.8	2.5± 0.2	0	0	4.2± 0.8	3.1± 0.0	2.3± 0.1	0

**Fig. 5.** Efficiency of PAA concentration during 3 days of storage fresh cut carrots at ambient and refrigeration temperature (4°C).

as 3.3 log CFU/g, Y and M count was recorded as 3.75 CFU/g (Fig 6 A). Washing lettuce with distilled water was found unable to reduce the bacteria and Y & M. On the other hand, SP water wash reduces the TABC by approximately 1.0 log CFU/g, but failed to reduce yeast and mould count. H₂O₂ wash was able to lower TABC about 2.0 log CFU/g and Y&M count to an undetectable level. PAA wash was found most effective in reducing TABC count and Y&M count to an undetectable level at Day 0.

When all this sanitiser-washed lettuce was stored at ambient temperature for three days and 6 days, the TABC, TCC, *Salmonella* spp., and Y&M count increased to an unacceptable level at day-3. He spoiled at day-6 in control and distilled water washed lettuce. Although SP waterwashed, H₂O₂ and PAA washed lettuce could retain acceptable microbiological criteria at day-3.

Still, all these values were either crossed or about to cross the permissible microbiological limit except H₂O₂ washed lettuce at day-6 (Fig 6 A).

In the case of refrigerated storage, little increase or decrease of TABC, total coliform count (TCC), *Salmonella* spp., and Y&M count was observed in control and distilled water washed lettuce at Day-3 and Day-6. However, the microbiological counts of lettuce treated with various sanitizers were far below the acceptable microbiological limit up to 6 days of storage at refrigeration temperature. This finding suggested that washing the vegetables with sanitizers and stored at refrigerated temperature up to 6 days keeps the quality of lettuce better than ambient storage (Fig 6 B).

Visual Quality of Lettuce: The visual observation of treated and non-treated lettuce stored at ambient and refrigerated

temperature, results demonstrated that irrespective of washing treatment, ambient temperature stored lettuce changes its color from green to pale green, hence visually becomes non-attractive to the consumers. On the contrary, visual quality of refrigerated stored lettuce at day 3 and day 6, was relatively better, but non-

attractive, despite various sanitizer treatment was done (Fig 6 C). This finding and microbial quality data demonstrated that washing with sanitizer and stored in refrigerator retain its visual quality little better than that of ambient temperature stored lettuce.

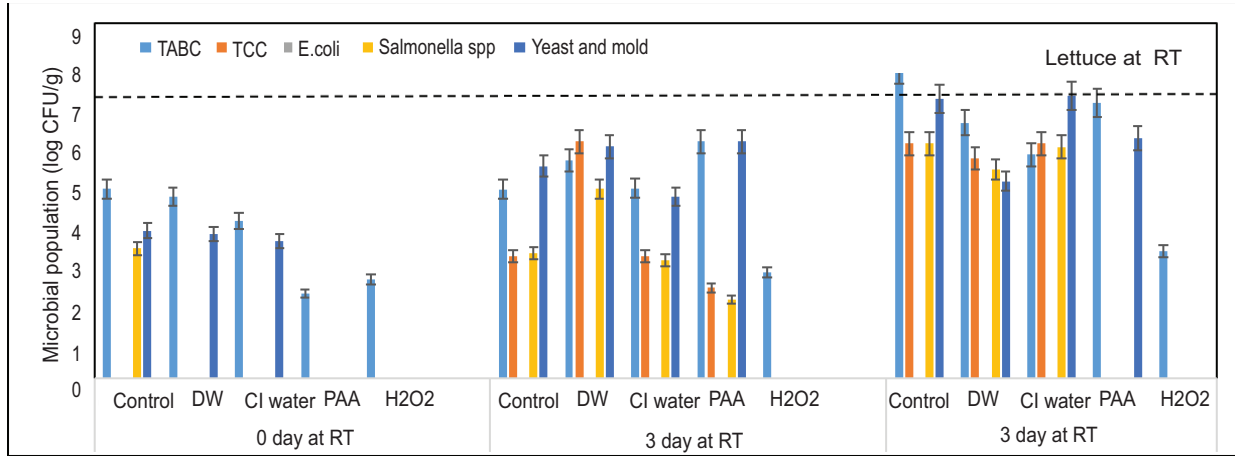


Fig. 6A. Microbial quality of various sanitizer-washed lettuces stored at ambient temperature up to 6-days.

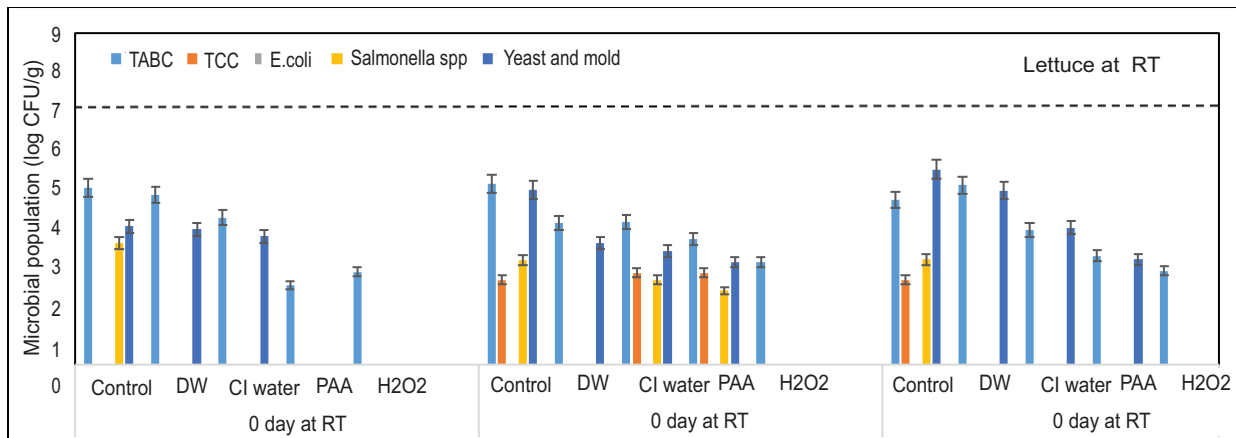


Fig. 6B. Microbial quality of various sanitizer-washed lettuces stored at refrigerated temperature up to 6-days.



Fig. 6C. Visual quality of various sanitizer-washed lettuces stored at ambient and refrigerated temperature up to 6-days.

Carrot: Carrot is a perishable vegetable but not as highly perishable like lettuce and cucumber thus extended storage of 13 days both at refrigerated (4° C) and room temperature were evaluated after washing with each of the sanitizer. The total aerobic bacterial count (TABC) was recorded as 3.9 log CFU/g, total coliform bacteria was recorded as 4.08 log CFU/g, and yeast and mould count were recorded as 4.28 log CFU/g (Fig 7 A). Although no *E. coli* was found in the fresh-cut carrot samples, a higher presence (3.0 log CFU/g) of *Salmonella* spp was recorded in fresh-cut carrot samples. When fresh cut carrot was washed with sanitizers and stored at ambient temperature for 13-days, a gradual increase of TABC from 3.9 to 6.1 log CFU/g at day 10 was observed and crossed the maximum permissible limit (7.0 log CFU/g) at day 13. Among the wash sanitizers, H₂O₂ showed better reduction of TABC followed by PAA washed fresh-cut carrot. The lowest efficacy was observed for 0.1% SP water washed fresh-cut carrot stored at ambient temperature throughout the storage period (Fig 7 A).

On the other hand, in the case of 4°C storage, irrespective of wash-sanitizer used, H₂O₂ washed fresh-cut carrot showed a better reduction in TABC and other microbiological counts to below detection level, compared to PAA and SP water and was able to retain the TABC count lower throughout 13-days storage at 4°C. The reduction of microbiological count was found in the

following order H₂O₂> PAA> SP water> DW wash (Fig 7 B).

Nonetheless, the microbiological counts of carrot treated with various sanitizers were far below the acceptable microbiological limit up to 13 days of storage at refrigeration temperature. This finding suggested that washing the vegetables with sanitizers and stored at refrigerated temperature up to 13 days keep the quality of fresh cut carrot better compared to ambient storage.

Visual Quality of Carrot: The visual observation of treated and non-treated fresh-cut carrot stored at ambient and refrigerated temperature results showed that irrespective of washing treatment, ambient temperature stored carrot did not change its colour and visual quality significantly, thus the visual quality was still attractive to the consumers throughout the storage period (Figure 7C). However, visual quality of freshness of fresh cut carrot was found relatively better in refrigerated stored samples, despite various sanitizer treatment was done. This finding along with microbial quality parameters data demonstrated that refrigerated stored fresh cut carrot was better than ambient temperature stored fresh cut carrot (Figure 7 C).

Cucumber: In this Study, cucumber is an intermediary perishable vegetable compared to carrot and lettuce thus, intermediary extension storage time (9 days) both at refrigerated (4°C) and room temperature were evaluated after washing with each of the

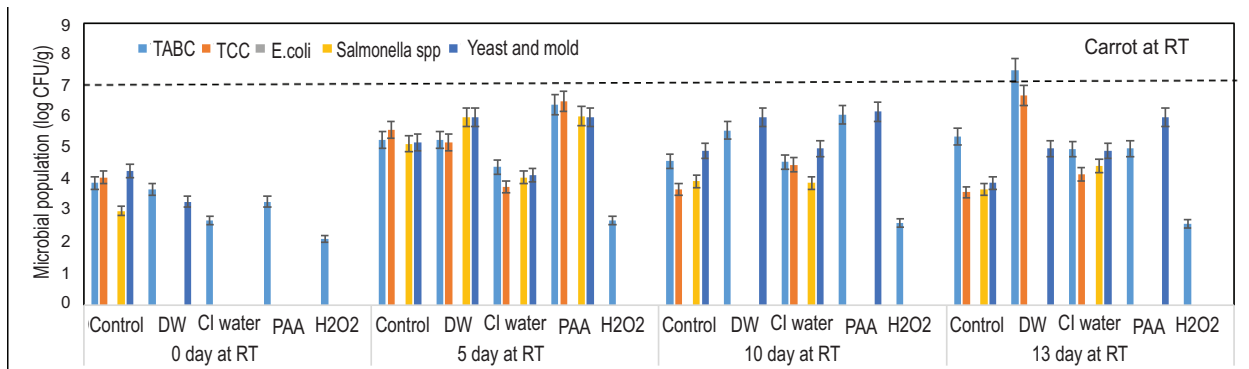


Fig. 7A. Microbial quality of various sanitizer-washed Carrot stored at ambient temperature up to 13-days.

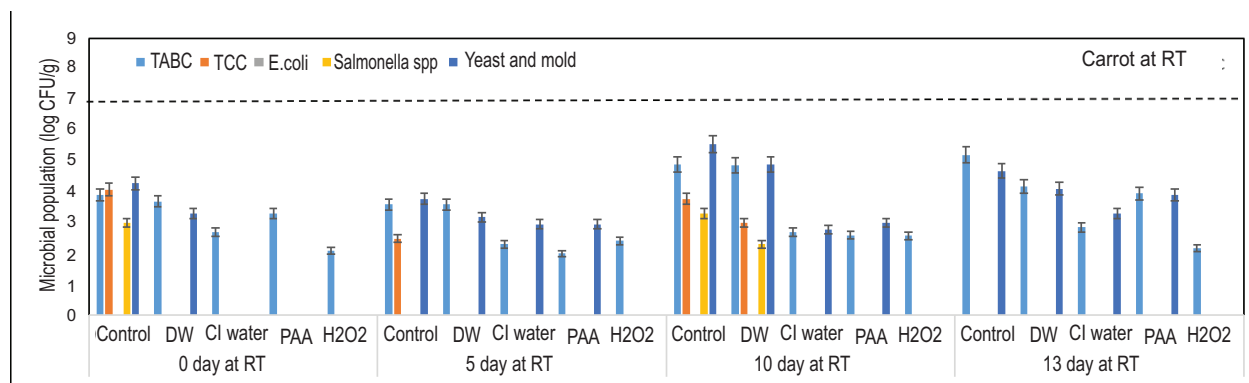


Fig. 7B. Microbial quality of various sanitizer-washed carrot stored at refrigerated temperature up to 13-days.



Fig. 7C. Visual quality of various sanitizer-washed carrot stored at ambient and refrigerated temperature up to 13-days.

sanitizer. The initial microbial load in fresh-cut cucumber was recorded as, 4.88 log CFU/g, total coliform count was recorded as 3.0 log CFU/g, yeast and mould count was recorded as, 4.48 log CFU/g. Although the presence of *E. coli* was not evident throughout the Study, the presence of *Salmonella* spp was recorded as 2.4 log CFU/g (Fig 8 A). In the case of ambient storage fresh cut cucumber, washing with sanitizer was able to reduce the microbial load at 0-day. However, a gradual increase was recorded at day-3 and crossed the maximum permissible limit from day-6 up to storage. H_2O_2 showed better reduction

and retained the microbial load lower compared to other sanitizer used in this study throughout the storage period.

In the case of 4°C storage, the situation was slightly better than the ambient temperature storage. When fresh cut cucumber was washed with H_2O_2 and stored at 40°C for 3, 5 and 9 days, consistent retention of the microbial count was observed on days 3, 6, and 9. On the other hand, a slightly increase of the microbial count was observed with distilled water wash, PAA, and SP wash fresh cut cucumber, indicating that the fresh-cut vegetables should

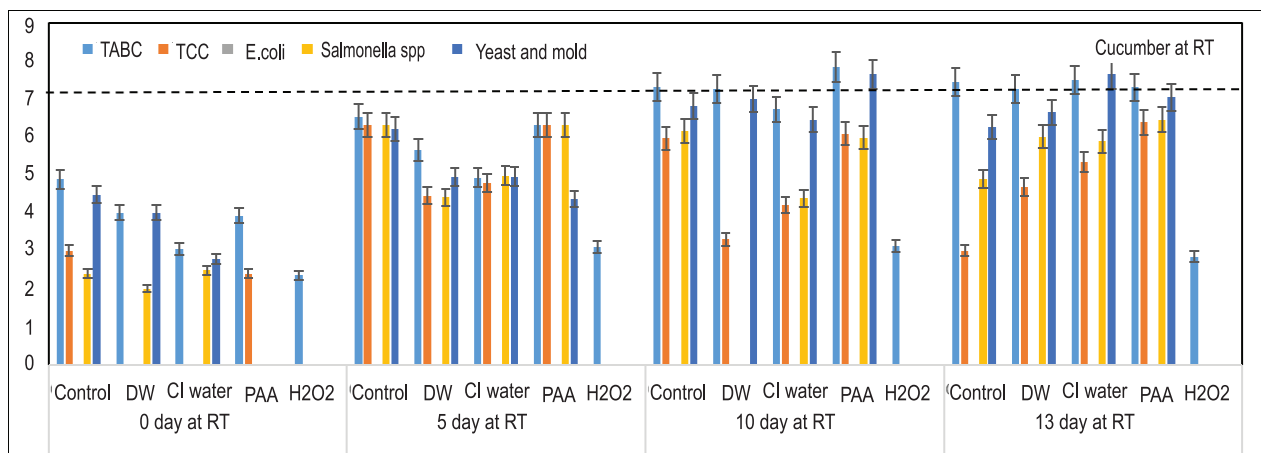


Fig. 8A. Microbial quality of various sanitizer-washed cucumbers stored at ambient temperature up to 9-days.

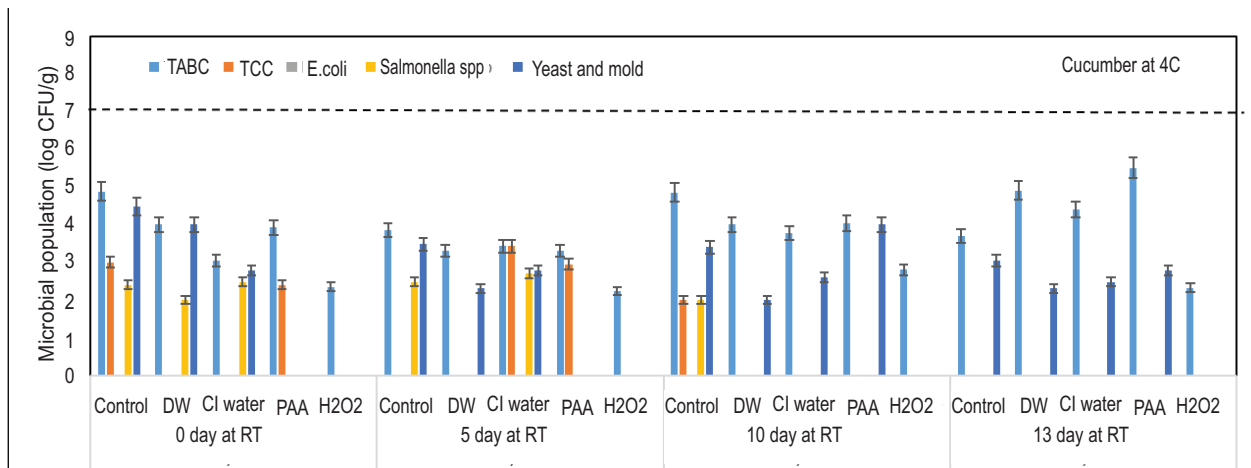


Fig. 8B. Microbial quality of various sanitizer-washed cucumbers stored at refrigerated temperature up to 9-days.



Fig. 8C. Visual quality of various sanitizer-washed cucumbers stored at ambient and refrigerated temperature up to 9-days.

keep at refrigerated temperature for better microbial quality and safety (Fig 8 B). However, compared to ambient temperature storage, the microbiological quality parameters of fresh cut cucumber treated with various sanitizers were still below the acceptable microbiological limit up to 9 days of storage at refrigeration temperature.

Visual Quality of Cucumber: The visual observation of treated and non-treated cucumber stored at ambient and ambient temperature results showed that irrespective of treatment condition, ambient temperature stored cucumber changes its

texture from hard to soft with storage duration and on day-9 fresh-cut cucumber is softest and thus the visual quality becomes non-attractive to the consumers. On the contrary, visual quality on the texture of fresh-cut cucumber was observed relatively better in refrigerated stored samples, despite various sanitizer treatments being done. PAA washed fresh cut cucumber among the wash-sanitiser showed good texture and visual quality acceptable at day-9 of 4°C storage (Fig 8 C). This finding and microbial parameters, demonstrated that refrigerated stored fresh-cut cucumber was better to close to adequate level than ambient temperature storage cucumber.

The overall result indicates that all the sanitizers could decrease the bacterial population in fresh-cut vegetables initially. However, with storage temperature and time, microbial population increases or remain constant or lower depending on the types of vegetables, storage temperature and duration. Since ambient temperature is the optimum temperature for most aerobic bacteria to proliferate due to the favorable conditions and presence of nutrition for bacteria to grow. On the contrary, at 4°C is not a favorable condition for most aerobic bacteria, and at 4°C, microorganisms can survive but were unable to increase. Only psychotropic bacteria can grow, which render the vegetables spoil or rotten with time. Therefore, washing fresh-cut vegetables with non-chlorine sanitizers and stored at refrigerated temperatures for a short time could be the marketing strategy for ready-to-eat fresh-cut salad vegetables.

Discussions

The basic form of primary processing, often known as minimal processing, involves sorting, grading, cleaning, drying, shelling/threshing, and packaging fresh agricultural products. Fresh-cut vegetables are examples of primary processed products, which don't require heavy machinery or investment, instead they need basic facilities for cleaning and freezing. However, good processing practices and handlers' good hygiene practices are imperative for the safe production of ready to eat fresh-cut vegetables^{25,26}. The use of secondary ingredients is almost non-existent in primary processing. In this study, after receiving the primary ingredients (lettuce, carrot, and cucumber), the ingredients were first sorted/trimming. For lettuce, the outer leaves were removed; for carrot and cucumber, dirt and debris were removed, then washed with tap water followed by distilled water, and peeled and then cut into small pieces. At this stage, microbial count was done. The results showed a higher number of *Salmonella* spp. in all the primary vegetables purchased, indicating that the receiving ingredients did not meet the quality standards and should be rejected. However, for the sake of the experiment we have accepted this primary ingredient to evaluate the effectiveness of non-chlorine sanitizers in eliminating pathogens and improving the shelf life at ambient and refrigeration temperature. SP, PAA and H₂O₂ washing successfully eliminated *Salmonella* spp. initially at ambient temperature, however, during storage, presence of *Salmonella* was evident in SP washed fresh cut lettuce at day 3 and day 6; and PAA washed fresh cut lettuce at day 3 but not at day-6; however, H₂O₂ washed fresh cut lettuce was seen eliminated *Salmonella* from day 0 up to day 6 at ambient temperature storage. Similar findings was observed in fresh cut lettuce stored at refrigerated temperature up to day 3, however, at day 6 no *Salmonella* was observed, this might be due to the cumulative stress of cold temperature and the commensal microbial growth inhibited or inactivated *Salmonella* spp. or resident microbial growth may alter or spoil the food, or release secondary metabolites which inactivated or inhibited *Salmonella* spp. In addition, no *Salmonella* was detected even in DW washed

fresh cut lettuce at day 6 under refrigerated storage support the causes of *Salmonella* inactivation. Furthermore, except in control lettuce, inhibition of *Salmonella* spp. in control carrot and cucumber samples at 13 days and nine days, respectively, was evident; this might be due to the disruption of the integrity of vegetables by slicing and peeling, thus facilitating enzymatic degradation on fresh-cut carrot and cucumber resulted in releasing some organic substances inhibitory to *Salmonella* spp., and lettuce may not have such enzyme to produce substances inhibitory to *Salmonella* spp.

The shelf life of fresh produce depends on the initial microbial load, holding temperature and duration. In this study, total aerobic bacterial count (TABC) and total fungal count were considered as indicators of the shelf life of the fresh produce. Comparatively higher TABC load was recorded in lettuce and cucumber. Thus, holding this lettuce and cucumber at ambient temperature crossed the maximum permissible limit (7.0 log CFU/g) within six days of storage (Fig 6A and 8A). On the other hand, carrots contained a low initial microbial load and thus delayed in crossing the maximum permissible limit (Fig 7A). Nevertheless, irrespective of vegetables, none crossed the maximum permitted limit up to 13 days of storage at 4°C.

Total coliform count indicates of processing places environment in which the processing of the vegetables was done. Lower to moderate levels of total coliform bacteria were recorded in the vegetable samples. The presence of *E.coli* and *Salmonella* indicates faecal contamination at any stages along the value chain. Although no *E. coli* was observed, the presence of *Salmonella* was recorded showing the post-processing faecal contamination. Thus, pre-washing the vegetable with sanitizer is imperative for the safety of the fresh-cut produce.

Conclusion

The quality and safety of minimally-processed fruits and vegetables are essential parameters to get consumers' confidence in the fresh, minimally processed agriculture products. The overall result indicates that all the sanitizers could decrease the bacterial population in fresh-cut vegetables initially; however, with storage temperature and time, microbial population increases or remains constant or lower depending on the types of vegetables, storage temperature, and duration. Irrespective of sanitizer treatment, refrigerated storage showed better visual quality, microbial safety and shelf life of fresh-cut produce. Therefore, this study results suggested that washing fresh-cut vegetables with produce specific sanitizer and storing at refrigerated temperature keep the quality of fresh-cut produce better compared to ambient storage.

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References

1. Johnston CS and Gaas CA. Vinegar: medicinal uses and anti-glycemic effect. *Med Gen Med*, 2006;8:61.

2. International Fresh-cut Produce Association. IFPA. 2000. "Fresh-cut Produce: Get the Facts!" www.fresh-cuts.org
3. FDA/CFSAN. 2007. Guide to minimize food safety hazards of fresh-cut fruits and vegetables. US Department of Health and Human Services.
4. Artes F and Allende A. Emerging Technologies for Food Processing. Elsevier; London, UK. Minimal fresh processing of vegetables, fruits and juices; 2005; pp. 677–716.
5. Blancou J. History of disinfection from early times until the end of the 18th century. *Rev Sci Tech*. 1995;**14**(21):39.
6. Slavin JL and Lloyd B. Health Benefits of Fruits and Vegetables. *American Society for Nutrition Adv Nutr*. 2012;**3**:506–516.
7. Steinmetz KA and Potter JD. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc*. 1996;**96**:1027-39.
8. Kim JG. Fresh-cut market potential and challenges in Far-East Asia. *Acta Hort*. 2007;**746**:53-60.
9. Sanguanpuag K, Kanlayanarat S and Tanprasert K. Trends of fresh-cut produce in Thai retail markets for identification of packaging for shredded green papaya. *Acta Horticulturae*, 2007;**746**:481-483.
10. Yamamoto K. 2017. Food processing by high hydrostatic pressure, *Biosci. Biotechnol. Biochem.*, <http://dx.doi.org/10.1080/09168451.2017.1281723>
11. Liu C, Hsu C and Hsu M. Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pretreatment and modified atmosphere packaging. *Packag. Technol. Sci*. 2007;**20**:337–343.
12. Garret EH. Fresh-cut produce: tracks and trends. In: Lamikanra O, editor. Fresh-cut fruits and vegetables: science, technology, and market. *Boca Raton Fla. CRC*. 2002;p 21–9.
13. Russell AD. 1990. Bacterial Spores and Chemical Sporicidal Agents. *Clinic Microb Rev*. Apr. 1990, p. 99-119.
14. Stranieri S and Baldi L. Shelf Life Date Extension of Fresh-Cut Salad: A Consumer Perspective. *J. Food Prod. Mark*. 2017;**23**: 939-954. doi: 10.1080/10454446.2017.1266545.
15. Plazzotta S, Manzocco L and Nicoli MC. Fruit and vegetable waste management and the challenge of fresh-cut salad. *Trends Food Sci. Technol*. 2017;**63**:51–59. doi: 10.1016/j.tifs.2017.02.013.
16. Ragaert P, Verbeke, W, Devlieghere F and Debevere J. Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Qual and Pref*. 2004;**15**(3):259-270.
17. Abadias M, Usall J, Oliveira M, Alegre I and Vinas I. Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. *Int J of Food Microb*. 2008;**123**:151–158.
18. Baldry MGC. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *J Appl Bacteriol*. 1983;**54**:417-423.
19. Entani E, Asai M, Tsujihata S, Tsukamoto Y and Ohta M. Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. *J Food Prot*. 1998;**61**:953–959.
20. Mahfuza I, Arzina H, Md. Kamruzzaman M, Afifa K, Md. Afzal H, Rashed N and Roksana H. Microbial status of street vended fresh-cut fruits, salad vegetables and juices in Dhaka city of Bangladesh. *Int Food Res J*. 2016;**23**(5): 2258-2264.
21. Strawn LK and Danyluk MD. Fate of *Escherichia coli* O157:H7 and *Salmonella* on Fresh and Frozen Cut Pineapples. *J Food Prot*. 2010;418-424.
22. Vijaya kumar C and Wolf-Hall CE. Evaluation of household sanitizers for reducing levels of *Escherichia coli* on iceberg lettuce. *J Food Prot*. 2002;**65**:1646–1650.
23. James JB, Ngarnsak T and Rolle RS. Processing of fresh-cut tropical fruits and vegetables. In: A technical guide, RAP PUBLICATION 2010/16; Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific Bangkok. 2011; 15-102.
24. Vandekinderen I, John VC, Frank D, Bruno De M, et al. Effect of Decontamination Agents on the Microbial Population, Sensorial Quality, and Nutrient Content of Grated Carrots (*Daucus carota* L.). *J Agri and Food Chem*. 2008;**56**(14):5723-31.
25. Calonico C, Delfino V, Pesavento G, Mundo M and Nostro AL. Microbiological Quality of Ready-to-eat Salads from Processing Plant to the Consumers. *J Food Nutr Res*. 2019;**7**:427–434.
26. de Oliveira MA, Maciel de Souza VM, Bergamini AMM and de Martinis ECP. Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Cont*. 2011;**22**(8):1400–1403.