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 freshness¹,². 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 freshcuts 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 waste.¹⁵ 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.
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 industry. Apart from presenting the consumer with a range of options in a single package, fresh cuts reduce waste 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.

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. 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-life.

Other challenges to the marketing of fresh-cut produce include: preserving product quality through the marketing chain; maintaining the cold chain and proper logistics; 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 Minland. Attributes 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 sativus) 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., 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% HzO2 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.

**Fig 2.** Summary of the washing procedures and methods.
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 emphasize washing (Fig 4 B). The chain shop consumers gave equal importance on all attributes during purchase but less on packaging (Fig 4 C). Inevitably, 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

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**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 ImPur=important during purchase , black line ImCon=Important during consumption (scale 1-5, 10 respondents from each group).
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.
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 refrigerated (4°C) and room temperature were evaluated after washing with each of the

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**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.
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. 8A). 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. H₂O₂ 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 H₂O₂ 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. 7C. Visual quality of various sanitizer-washed carrot stored at ambient and refrigerated temperature up to 13-days.

Fig. 8A. Microbial quality of various sanitizer-washed cucumbers stored at ambient 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-sanitizer 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 psychrotrophic 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. 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 storingat refrigerated temperature keep the quality of fresh-cut produce better compared to ambient storage.

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