Novel Use of UV-C: Sanitation of Fresh and Fresh-Cut Produce

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Abstract
A UV-C system with a column of water passing through a circle of UV-C lights, called the UV Turbulator, has provided a practical means of using UV for sanitation in the fresh and fresh-cut produce industry. Studies on several different fruits and vegetables demonstrated the efficacy of UV-C in reducing microbial counts on the produce item itself. In commercial processing conditions, UV-C reduced the aerobic plate counts (APC) by 1.0 log cfu/g and coliforms by 1.4 log cfu/g on apple slices. Yeast counts were reduced by UV-C and remained lower throughout the 15-day shelf life. Increasing the UV-C fluence did not further reduce the log count of inoculated Enterobacteriaceae on apple slices and lettuce pieces. Using UV-C as a step in a multi-hurdle sanitation approach resulted in a greater log reduction in Enterobacteriaceae on lettuce pieces than a chemical sanitizer alone. Higher temperatures as the final step in the hurdle system were more effective in reducing APC and yeast growth during storage of pomegranate arils.

Keywords: fresh produce, fresh-cut, wash water, apple slices, pomegranate arils, lettuce, APC, yeast and molds, *Citrobacter freundii*.

Introduction
Sanitation of wash water is an important step in fresh and fresh-cut produce processing; however, despite marketing claims to the contrary, this does not result in a log 5 reduction of pathogens on the fresh produce itself. The aim of wash water sanitation is to prevent cross-contamination of produce during processing by sanitizing the wash water. The Center for Produce Safety key learnings states that “even properly managed wash systems do not sanitize the surface of fruits and vegetables.” (Anon., 2014) This is clearly demonstrated by the data in Gonzalez et al. (2004).

Ultraviolet light has been shown to inactivate a wide range of microorganisms, including bacteria, fungi, viruses and parasites, and has been evaluated on fresh produce. In addition to reducing foodborne pathogens, UV-C also can reduce spoilage microorganisms and other plant pathogens and thereby extend shelf life of fresh and fresh-cut produce (Gómez-López, 2012). UV technology has been approved by the US Food and Drug Administration (21CFR179.39; Anon., 2016) for surface microorganism control in food and is not prohibited by the National Organic Program (7CFR205.105; Anon., 2017). In the European Union, there is no unified legislation about the use of UV-C light in foods (Gómez-López, 2012).

Despite the benefits of UV, the adoption of this sanitation method by the produce industry has been slow because of practical limitations. Typically dry systems with overhead lamps have been used, and exposure times have been several minutes to hours. Produce was damaged by high temperatures and water loss, and it required manual or mechanical turning of the produce item to improve surface exposure. The efficacy of UV-C depends on the fluence or UV dose which is difficult to determine accurately (Bolton and Linden, 2003), and published fluences have lacked a clear explanation of their measurement.

Fresh Appeal introduced a patented wash water system, the UV Turbulator, to wash whole or cut fruit and vegetables in a column with a spinning vortex of water, which has overcome these limitations. A ring of UV-C (254 nm) lamps is positioned around the column to optimize surface exposure (Figure 1). The water recirculates through the system, and UV-C transmittance is managed by replenishing water when the transmittance reaches a predetermined lower limit. The Turbulator usually is used in conjunction with other sanitizers, usually as a prewash, which helps remove much of the dirt and cell contents in the water so the transmittance of the water in the Turbulator remains higher and the need for water replenishment is reduced.

The prewash sanitizers, which include hypochlorite, chlorine dioxide or peroxyacetic acid (PAA), are used at rates allowed by the FDA for washing of fresh produce (21CFR173.315) (Anon., 2016). Of these, PAA is the preferred sanitizer because of the synergy between PAA and UV-C (Waites et al., 1988). This combination of sanitation methods, or hurdles, is an improvement on current commercial processing methods.

The initial validation of the UV Turbulator on microorganisms was conducted on ‘Braeburn’ apple slices, which were inoculated with a known load of *Lactobacillus* bacteria. The

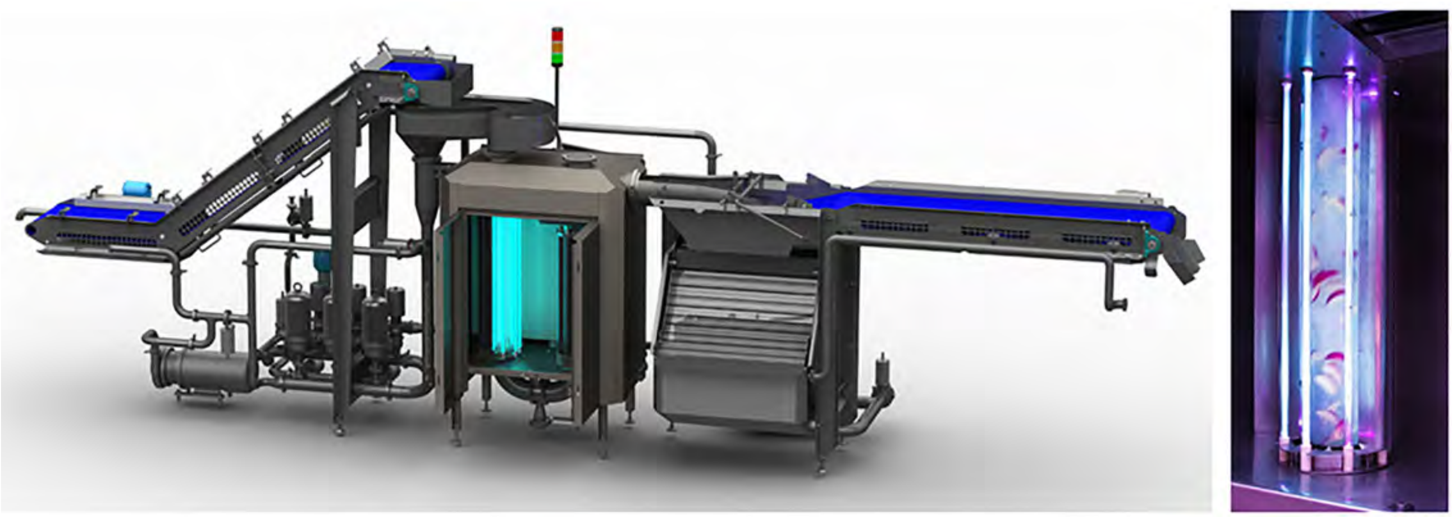

Figure 1. From left, a diagram of Fresh Appeal's UV Turbulator within a processing line and the Turbulator with apple slices.

application of the UV Turbulator achieved about a 2 log reduction (Woolf, 2007). Since its commercial introduction in 2008, there has been positive feedback on the effect of the UV Turbulator against microorganisms, but this data has been proprietary and confidential. This paper presents research conducted during the last three years to quantify the effect of the UV Turbulator, alone or in conjunction with the other hurdles, on naturally occurring and/or inoculated bacteria under research and commercial settings. The proprietary information on the technical specifications of the UV Turbulator and the fluences used are not part of the scope of this paper.

Materials and methods

Exp. 1: Commercial fresh-cut apples

Apple slices were processed in a commercial fresh-cut apple plant in the US, where incoming whole apples were washed in PAA sanitizer (not exceeding 80 ppm as specified in CFR 173.315) (Anon., 2016), mechanically sliced into a flume containing a sanitizer, and exposed to UV-C in the UV Turbulator for approximately 10 seconds with the transmittance of the water managed automatically. The apple slices were then subjected to a mild heat treatment followed by rapid cooling in a commercial antioxidant solution (Natureseal, Mantrose-Haeuser, Westport, CT).

Slices were collected at three different stages: washed in PAA before and again after the Turbulator, and slices after the heat treatment and subsequent cold flume. Each set of samples was collected four times during a commercial production run. Naturally occurring aerobic plate counts, coliforms and yeast were measured by DonLevy Laboratories, Crown Point, Ind., and reported as colony-forming units per gram of tissue (cfu/g). A separate set of commercial samples treated with or without UV-C were stored at 4°C, and yeast counts were measured after 7 and 15 days. Data were analyzed statistically with JMP (SAS Institute Inc., Cary, NC).

Exp. 2: Increasing UV-C dose on apples and lettuce

Cut apple pieces and organic Romaine lettuce (10 cm x 10 cm) (Figure 2 on page 6) were dip-inoculated with *Citrobacter freundii* (a surrogate for *Listeria monocytogenes*), spun in a domestic salad spinner, air dried for 2 hours in refrigerated conditions and washed for 30 seconds in sterile water to remove unattached bacteria. Pieces were attached to wire hooks and held in the UV Turbulator for 10, 60, 120, 240 and 480 seconds. The Turbulator contained fresh water, and the UV transmittance did not drop below 80 percent.

Enterobacteriaceae counts, which include *Citrobacter freundii*, were measured on the samples after treatment by AsureQuality, Auckland, New Zealand. Results were analyzed by JMP (SAS Institute Inc., Cary, N.C.).

Exp. 3: Inoculated Romaine lettuce

Romaine lettuce was purchased from an organic grower. The unwashed lettuce was cut into pieces (approximately 10 cm x 10 cm) with sharp knives and pieces were dipped in a *Citrobacter freundii* suspension at 11,400,000 cfu/g or 7.1 log cfu/g. The pieces were drained and dried in a salad spinner before being packed in macroperforated plastic bags and stored overnight at 4°C. The lettuce pieces were treated as specified in Table 1, dried in a sanitized salad spinner, packed in bags (75 g/bag) and stored at 4°C for 6 days. The

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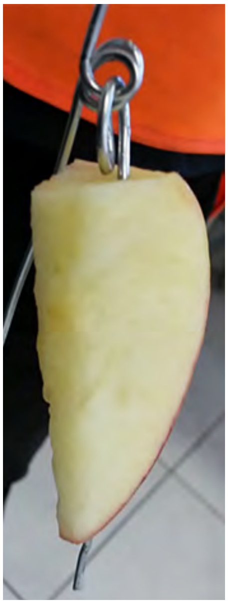

Figure 2. Apple slices and Romaine lettuce pieces on wire hooks used to extend the time in the UV Turbulator.

UV Turbulator had fresh water at the start of every treatment, and the UV transmittance did not drop below 80 percent. Because of logistical reasons, the treatment with heat was conducted a day later than the remainder of the treatments. A separate baseline measurement of inoculated but unwashed product was measured for each set of samples.

Table 1. Details of different treatments used on Romaine lettuce pieces.

Abbreviation	Treatment	Treatment details
PAA	Peroxyacetic acid (Tsunami 100)	60 ppm for 90 s
UV	Ultraviolet-C	10 s in UV Turbulator
PAA+UV	PAA followed by UV	Sequential steps are described above
PAA+UV+Heat	PAA followed by UV followed by hot water (with PAA) followed by rapid cold water cooling	PAA and UV (as above) followed by 40.5°C for 30 s, then 0-2°C for 30 s

Enterobacteriaceae (which includes inoculated *Citrobacter freundii*) and aerobic plate (APC) counts were measured by AsureQuality (Auckland, New Zealand) the day after treatment and again after 6 days of storage. At each date, 3 reps (bags of lettuce) were analyzed per treatment. The data was reported as log counts and log reductions from the inoculated but unwashed lettuce samples. Log reductions were analyzed by JMP (SAS Institute Inc., Cary, N.C.).

Exp. 4: Pomegranate arils

'Wonderful' pomegranates were grown in California and stored for 6 months before being shipped to New Zealand, where the UV Turbulator was located. The arils (seed and surrounding fleshy tissue) were extracted from the fruit and subjected to five different treatments (Table 2) before being dried in UV sanitized air. Hydrogen peroxide is only allowed in wash water when combined with acetic acid to form peroxyacetic acid and should not exceed 59 ppm in wash water (21CFR173.315) (Anon., 2016).

Table 2. Details of the five treatments used to wash pomegranate arils (where PAA = peroxyacetic acid, trade name Tsunami 100, Ecolab, St. Paul, Minn.; and HP = hydrogen peroxide).

Treatments	Wash (60 s)	UV (10 s)	Hot water	Cooling (2°C)
Control	Water	No	21°C	Water
Tsu48	80 ppm PAA	Yes	48°C	80 ppm PAA
Tsu55	80 ppm PAA	Yes	55°C	80 ppm PAA
HP48	1.5% H_2O_2	Yes	48°C	1.5% H_2O_2
HP55	1.5% H_2O_2	Yes	55°C	1.5% H_2O_2

The arils (122 g/cup) were packed in sealed plastic containers. Three containers (reps) were analyzed per treatment per day after 0, 7, 10, 14, 18 and 21 days at 4°C. Aerobic plate counts, yeast and molds counts, and aril quality data were measured. The microbial data were analyzed statistically (ANOVA test) using R statistical package. This experiment was designed by Fresh Appeal and conducted by Plant and Food Research, New Zealand, as a contract research project.

Results

Exp. 1: Commercial fresh-cut apples

The APC on apple slices were reduced by 1.0 log after treatment with the UV Turbulator, and coliform counts were reduced by 1.4 logs ($p < 0.05$), despite the prewash with PAA (Figure 3). The hot flume did not significantly reduce the microbial counts.

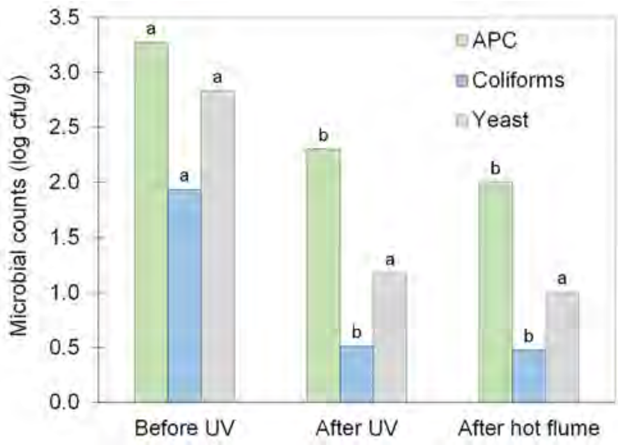

Figure 3. Aerobic plate counts, coliforms and yeast counts (log cfu/g) on apple slices sampled at three stages in a commercial plant, after PAA wash but before the Turbulator, after the Turbulator and after the heat treatment. Different letters denote significant difference ($p < 0.05$) using Tukey-Kramer HSD test.

The yeast counts were reduced by the UV-C step (Figure 3), but the results were not significant because of the high variability of the samples taken before the Turbulator. Two of these samples had undetectable yeast counts and two had higher counts. However, all the yeast counts were low after treatment (<20 cfu/g or <1.3 log cfu/g). Despite low yeast counts after treatment, these counts usually increase during storage. The UV-C sanitation step was effective at reducing this rate of increase, and after 15 days of shelf life, the yeast counts were 1.9 log lower than comparable slices without the UV-C step (Table 3).

Table 3. Yeast counts (log cfu/g) on apple slices processed in a commercial plant with and without the UV-C sanitation step after 7 and 15 days of storage at 4°C.

	Yeast count (cfu/g)	
Turbulator	**Day 7**	**Day 15**
No UV	3.2 a*	5.1 a
UV	1.8 c	3.2 b

*Different letters denote significant difference ($p < 0.05$) using Tukey-Kramer HSD test.

Exp. 2: Increasing UV-C dose on apple and lettuce

Increasing the UV-C exposure time from 10 seconds to as much as 480 seconds did not further reduce the log counts of the Enterobacteriaceae (which includes the inoculated *Citrobacter freundii*) on either apple (Figure 4) or lettuce (data not shown). On both commodities, there was at least a 2 log difference in Enterobacteriaceae counts between the washed sample and UV-C treated sample.

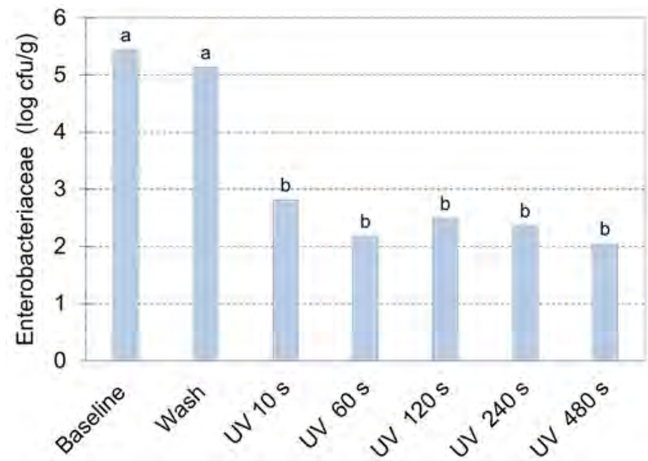

Figure 4. Enterobacteriaceae (which includes the inoculated *Citrobacter freundii*) (log cfu/g) on apple slices washed in the Turbulator with increasing UV-C fluence. Different letters denote significant difference ($p < 0.05$) using Tukey-Kramer HSD test.

Exp. 3: Inoculated Romaine lettuce

When inoculated Romaine lettuce was subjected to commercial wash treatments and compared to an inoculated but unwashed baseline sample, the treatments with UV-C resulted in higher reductions in Enterobacteriaceae (log cfu/g) than PAA alone (Table 4). There was no additional effect on Enterobacteriaceae with PAA followed by UV or PAA+UV+heat.

Table 4. Enterobacteriaceae counts (log cfu/g) (including inoculated *Citrobacter freundii*) and the log reduction from the relevant baseline on Romaine lettuce pieces after treatment and again after 6 days of storage at 4°C.

Treatments	Initial		After 4 days at 4°C	
	Log cfu/g	**Log reduction**	**Log cfu/g**	**Log reduction**
Baseline 1	5.67		5.34	
PAA	4.23	1.44 b*	4.58	0.76 b
UV	3.53	2.14 a	3.60	1.74 a
PAA+UV	3.36	2.31 a	3.60	1.74 a
Baseline 2	5.50		5.34	
PAA+UV+Heat	3.22	2.28 a	3.36	1.98 a

*Different letters denote significant difference ($p < 0.05$) using Tukey-Kramer HSD test.

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Exp. 4: Pomegranate arils

All four treatments were superior to the control in reducing APC (Figure 5). Similar trends were observed in yeast and mold counts (data not shown) on pomegranate arils for at least 14 days of storage. The increase in yeast and mold counts are particularly important on pomegranate arils as they are responsible for decreasing the shelf life by causing off odors prior to obvious contamination. Microbial counts on arils treated with Tsu48 were similar to the control after 18 days while the treatments at the higher temperature (55°C) maintained lower counts.

Of these treatments, HP55 was most effective in controlling APC and yeasts and molds; however, treatments containing hydrogen peroxide, regardless of the temperature, resulted in more severe aril browning (Figure 6). Consequently, the treatments with PAA (Tsunami) were superior in terms of microbial control and aril quality and could extend shelf life to 14 days (Tsu48) or 18 days (Tsu55).

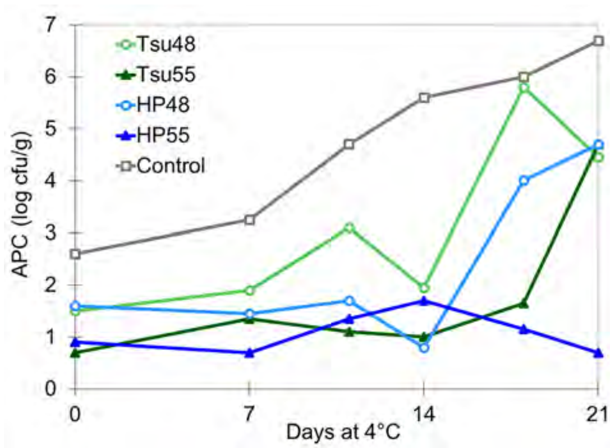

Figure 5. Aerobic plate counts (log cfu/g) on pomegranate arils treated according to Table 2 measured after 0, 7, 10, 14, 18 and 21 days at 4°C.

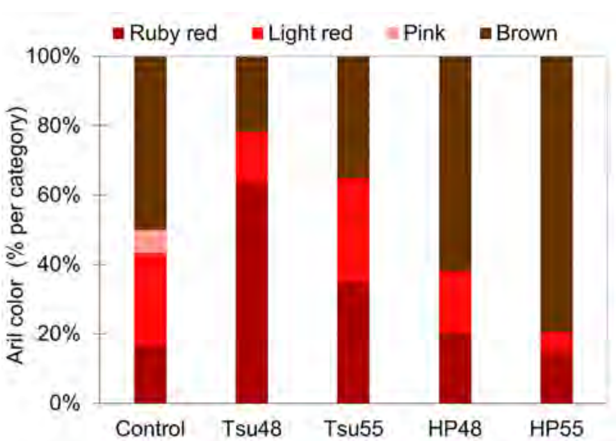

Figure 6. Pomegranate aril color (% red, light red, pink or brown) after different sanitation treatments (Table 2) and 18 days of storage at 4°C.

Discussion and conclusions

The UV-C wash step is an effective sanitation step for reducing initial microbial load on the produce itself and maintaining a lower load during storage (Table 3; Figure 4). UV-C is usually used as an additional hurdle to chemical sanitation. The advantage of an initial wash with a chemical sanitizer is that the UV-C transmittance of the water in the Turbulator remains higher for longer and fewer water changes are required. Since the efficacy of UV-C sanitation is determined by UV-C transmittance of the water this is an important step. The UV-C Turbulator automatically measures UV-C transmittance and replenishes or replaces the system with fresh water when it reaches a predetermined set point. The constant recirculation of the water in the UV-C Turbulator ensures that the water itself is sanitized.

Provided that the UV-C transmittance is well managed, the current fluence is appropriate, since increasing the time did not significantly affect the microbial loads. Very high fluences can damage plant tissue; however, no obvious damage was observed directly after processing. Future research will consider the effects of increasing UV-C fluence on the microbial counts and produce quality during storage. The hot flume did not further reduce the bacterial counts on apple slices (Figure 3) or on Romaine pieces (Table 4). However, increasing the temperature from 48°C to 55°C did further decrease APC and yeast and mold counts on pomegranate arils initially, as well as maintain lower microbial counts during storage. Mild heat treatment has benefits aside from the effect on microbial loads and been shown to reduce the respiration rate and ethylene production of the apple slices and maintain the firmness (data not shown).

This multi-hurdle system can be used to wash whole produce (e.g., apples), as well as fresh-cut fruits and vegetables. Produce items that can be singulated in the Turbulator, e.g. apples, are better candidates, as opposed to heads of lettuce, where there is a high risk of shading. However the data on inoculated lettuce pieces was very promising with reductions of about 2 logs initially and about 1 log after 6 days at 4°C. The system would not be appropriate for whole heads of lettuce, and the current Turbulator is not designed for very large produce items.

UV-C has proved to be a useful sanitation step for fresh and fresh-cut produce. This is usually part of a multi-hurdle processing method but also could be used alone. UV-C could replace chemical sanitizers with an acceptable physical means and satisfy the consumer demand for "chemical-free" produce. UV-C can be used in organic production systems. The UV Turbulator is a practical means of using this physical sanitation system in fresh produce and ensuring good coverage, a consistent exposure time and management of UV-C transmittance. The data presented here confirms the observations of commercial users of the Fresh Appeal system who have reported longer shelf life and superior quality of their fresh-cut produce. ■

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