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Research Article

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Artificial Organic Load for Produce Safety Research: The Need for Standardized Method

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Abstract

The Produce Rule has generated interest in produce safety. Ensuring proper control of the water quality used throughout a production chain is a critical step in ensuring food safety. Properly validated food safety processing controls are needed for effective food saftey management for produce processing water, such as the use of sanitizer in washing systems to avoid possible pathogenic cross-contamination. While previous work has been conducted to assess the influence of sanitizer in the presence of organic matter, there are a wide spectrum of approaches to mimic vegetative organic matter, as well as various methods to analytically characterize the presence of organic load and its effects on sanitizer efficacy. In order to provide better technical support to the produce industry about effective antimicrobial solutions available that work in the presence of organic matter, there is a need for a standard artificial organic load replication method. This review examines the physiochemical properties used to analyze organic load on bench top settings such as turbidity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), oxidation-reduction potential (ORP) and UV254 in efforts to make a comparison between quantitative tools used for measuring organic load and how these compare to measurements seen in on-farm processing conditions. It also looks at procedures used to replicate the organic load and how these affect the physiochemical properties of the water in sanitizer efficacy trials, which has become an important issue in light of the Food Safety Modernization Act (FSMA) regulation where farmers are required to test their agricultural water for the presence of *Escherichia coli*. Current research approaches that include vegetative material to mimic the organic load found in production water are done artificially using a variety of methods, which makes it challenging to make efficacy comparisons among existing work, and there is minimal published work that utilized production process water conditions. The most common methods of generating artificial organic load utilize fresh produce processed via two different routes: processing with blenders or paddle mixers. While there are various methods currently being used in bench top settings to quantify artificial wash water quality, not all are representative of measures seen on–farm, therefore presenting a challenge to assessing sanitizers most effective in real-world application. Based on published work, COD and TDS provide a more effective indicator to quantify water quality because their respective values provide a linear relationship with the amount of produce that is washed. Understanding the physicochemical properties of agricultural wash water and how they affect the efficacy of sanitizers, would enable the industry to develop a standardized method for organic load replication for the screening of commercially available sanitizers.

Keywords: Foodborne illness; Artificial organic load; *Escherichia coli;* Sanitizers

Introduction

Over 45 percent of the foodborne illness in the United States every year are related to produce [1]. Fresh produce, being a ready-to-eat product, is processed without a kill step, which means that potential pathogenic contamination must be minimized through good agricultural and postharvest handling practices. As of 2013 the Food and Drug Administration (FDA) established the "Produce Rule" as part of the Food Safety Modernization Act (FSMA), requiring that science-based minimum standards be established for any handling of produce intended for human consumption, including growing, harvesting, holding, and packaging, which went into effect January of 2017 [2]. With the implementation of this rule, the FDA established that no *E. coli* can be present in processing water, and that treating the wash water is an acceptable means of reducing microbial loads [2]. Postharvest wash water is used as a method to remove sand, soil, and debris but does not provide sufficient removal of microorganisms; therefore, water maintenance is needed to reduce the microbial load [3]. If the processing water is not properly maintained it can become a vector to introduce or spread pathogens to produce rather than reduce them [4]. The use of sanitizer is one method of controlling microbial load in wash water and it has proven to reduce the risk of cross contamination of the bacteria present [5].

While changing produce wash water on a daily basis has been shown to be common, it is not a universal practice [6]. Many growers reuse water in an effort to minimize costs and water usage, and not all producers add sanitizer to their postharvest processing water [6,7]. In a recent survey of the Mid-Atlantic region, 47% of the growers that responded wash their produce first by washing with plain water, and only 22.4 % wash their produce with a disinfectant of some sort [8]. Having a step that is lacking disinfectant allows for cross contamination to occur and reuse of water will allow for bacteria to accumulate in the water. Implementation of the Produce Rule has put into place that there be no detectable generic *E. coli* in wash water, requiring there to be controls in place to ensure that no *E. coli* is present [2]. One issue with the implementation of this requirement is that there is no guidance on how

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or which sanitizers to use. It was seen in a survey of several southeast states that many small to medium growers are not testing their water supply and are using these sources to wash produce [9]. In order to properly implement this method of control, more research is needed for the design of a validated method to control sanitizer concentrations used for on farm processing lines. Therefore, it is critical to understand which sanitizers, and at what levels, are effective at preventing cross contamination within the produce wash water [10].

A major hurdle in determining sanitizer efficacy is the presence of pollutants, and organic and inorganic loads in the wash water, as these may limit the effectiveness of the sanitizer [11]. A high organic load in produce wash water can result in an increased transfer of pathogens to uncontaminated plants [12,13]. Pathogens can be trapped in organic material that accumulates in the water leading to an increase in the potential for cross contamination [14]. Over time the addition of more produce to the washing tanks decreases the quality of the water and increases the concentration of contaminants [15] thereby increasing a risk in produce safety. Of the approved sanitizers, chlorine has shown to be the most commonly used, where the residual chlorine in wash water systems is the main factor in preventing cross-contamination [16]. However, due its nature, being a strong oxidant, chlorine based sanitizers dramatically lose efficacy in the presence of organic material [17-19]. Peracetic acid, coupled with hydrogen peroxide, is an emerging interest as a sanitizer, as it has not been seen to produce harmful disinfection by disinfection by-products (DBP's) or lose efficacy in the presence of suspended solids, up to 80 mg/L total dissolved solids in the water [20]. As sanitizers are added to process water there is an initial rapid decay in sanitizer concentration [21]. This makes the identification and evaluation of new sanitizers, as well as the need to validate effective and safe sanitizers a critical issue in produce safety [10].

While there has been work conducted in food safety to assess the influence of sanitizer in the presence of organic matter, there are a wide spectrum of approaches to mimic vegetative material and various methods of analytically characterizing the presence of organic load. Different commodities used to create organic load can render varying results, based on the nature of the produce used, making it difficult to create and implement a standard replication method that helps to make assessments on validity among sanitizers for industrial application. In order to provide the best technical support for on-farm applications, the processing water conditions used in validation of sanitizer efficacy should mimic the processing conditions of the produce industry. It is critical for sanitizers to be tested in the presence of organic matter, even with small-scale research, to better understand their efficacy in order to render more comparable results to on-farm process settings and account for the respective sanitizers quenching capacity of processing water [22]. Before transferring research to the field, it is best to evaluate the bench top results under various conditions, that replicate on farm conditions to ensure that when transferred to a farm all interferences have been evaluated [23]; simulating organic load is essential in bench top work in order to be prepared for interferences that may be present on farm. There are a variety of conditions that make translation of laboratory bench top research into effective industry application difficult, which could be eased with a standardized testing method [10,12,14]. This review aims to investigate the different research approaches used to replicate produce processing water and the physiological methods to quantify these conditions in order to better understand what properties may be used for future validation research.

Review

Organic load and the physiochemical properties of wash water

Sanitizer efficacy can be affected by organic matter present in the wash water, increased temperature, exposure to light and contact with metals, such as iron found in spinach. Organic load can be increased by different elements during washing of the produce, including dirt or soil, or organic matter that is released from the edges or damaged areas of the produce being washed, which can cause different reactions from different sanitizers [12]. Attempts in quantifying the effect of organic load have relied on measurements of water quality; including turbidity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), conductivity, oxidation reduction potential (ORP) and UV254 [16,24-27]. While these methods have shown to be useful tools in measuring water quality, previous work has shown that different researchers are conducting experiments under different conditions. This variability can render different results making it more challenging to compare research in the development of a standard organic load replication method.

Turbidity is a measure of the particulate matter present in water that can be composed of plant material, inorganic, and organic particles reported in NTU (Nephelometric Turbidity Unit) [28]. Some studies have shown that in certain applications, increased turbidity corresponds in a linear manner with the amount of produce processed in the wash water system [15]. During processing turbidity can vary throughout production, and depending on production days, which needs to be taken into account when using this as a measure of water quality [12], which in turn also needs to be considered when using this as a measure for generating artificial wash water. It was seen by Luo et al. [15] that when washing spinach and lettuce respectively, there was a linear increase in both the turbidity of the water as well as the chemical oxygen demand (COD) in relationship to the amount of produce that was washed [15]. COD is a standard test that uses water to consume oxygen in the form of potassium dichromate, during organic matter degradation [29]. It is quantified by introducing an excess of a strong oxidizer into the test sample to determine the remaining oxidant [30,31], which in turn will measure the organic pollutants in water that are capable of being oxidized [32]. COD measures the oxygen consumption of both biological substances and inert organic matter, whereas biological oxygen demand (BOD) only measures the oxygen consumption of biological substances, such as microbial oxidation. Luo [33] observed that COD and BOD increase rapidly in wash water with repeated use over time, as well as with large amounts of produce washed, indicating poor water quality. In produce samples washed in water of poor quality there was significantly more microbial growth when compared to those washed with clean water. Reporting COD values helps to provide a more comprehensive characteristic of the water quality [34]. In addition, BOD, has limited applications in water quality as the analysis requires extensive time to execute the assay and the reported values can have a significant variation depending on the bacterial seed be used in the process. These may limit the value of BOD for produce wash water analysis. While COD is related to BOD, they are not directly comparable methods of determining organic pollutant levels [32].

Total dissolved solids (TDS) measures the organic and inorganic compounds dissolved in the water by measuring the mass of the minerals dissolved, which allows it to have a strong relationship to conductivity, reported in Siemens, and salinity, reported in PSU (practical salinity units), of the solution [35]. As more organic material is shed in the wash water the number of dissolved ions in the water increases, thus increasing the conductivity of the water, allowing for conductivity to give an estimate of the TDS in the water [36]. Conductivity is often an alternative to measuring TDS for water quality [35]. A disadvantage to TDS measurements is that it is possible to overestimate TDS levels due to the possibility of the sample crusting, and water trapping in the sample [31]. Estimates of TDS, as well as information about the ionic and mineral content of a solution can be generated using conductivity [31], which measures the ability of an electrical current to pass through water [37]. Conductivity is a sensitive test in that temperature, as well as inorganic solids dissolved in the water can affect conductivity measurements [31]. Conductivity is a valuable measurement for water quality, as it can also be used to detect damaged plant material [38].

Oxidation-reduction potential (ORP) is a measure of the relative intensity of electron activity in the sample [31]. ORP, when used as a water quality measure, allows for easy monitoring of disinfectant levels in a postharvest water system [26]. ORP is not a practical method for PAA systems because the ORP meter measures the oxidation potential of hydrogen peroxide, which has a dissociation constant (pKa) higher than the pH of water, meaning it gives low results [39]. ORP measurements, while being a rapid and single-value assessment tool for disinfection potential [26], can be affected by the material of the electrode being used in solutions due to pH, temperature, probe contamination, the presence of organic matter, as well as the formation of salt bridges, and artifacts on the electrode surface [31]. ORP has shown to be a valid measuring tool when chlorine and ozone based sanitizers are present due to their strong oxidizing properties.

Another measurement that is beneficial when using chlorine based sanitizers is UV254, due to its ability in predicting the amount of chorine that is needed in a water solution [27]. Compounds with higher phenolic compounds have been observed to have a higher UV254 absorbance, compared to those with fewer phenolic compounds [27]. However, one of the downfalls with using UV254 is that the presence of turbidity in the water sample can affect the results [40]. While UV254 can be affected by turbidity, when trying to predict chlorine levels, it is a good indicator of total organic compounds (TOC) and levels of disinfection by products (DBP) [40].

All of the methods mentioned above, briefly summarized in Table 1: "*Analytical Measurements to Quantify Organic Load*", have shown a relationship to organic matter present in a wash water system. Current methods used to test for the presence of organic matter in wash water have been adopted from those used to establish water quality and pollution [31]. Organic matter degrades over time through photochemical and biochemical reactions, so bodies of water that have a larger surface area are not as likely to have high levels of organic matter [41]. In produce washing systems there is less surface area for the organic matter to degrade over time, and more organic matter is constantly being introduced to the water. There is a strong need for a uniform physiochemical property analysis of produce wash water since there are many variables that can affect the results. Not all physiochemical parameters are the same for each commodity, and different produce have shown to require different free chlorine demands [27]. Table 2: "*Survey of Industrial Wash Water*" shows how physiochemical properties vary between commodities. If the overall presence of organic matter can be better characterized, validation methods for different sanitizers may be identified and established. However, further research is needed to determine the relationship between these physiochemical properties and sanitizer depletion in agricultural wash water, otherwise, it is difficult to compare the work being done on the efficacy of different sanitizers.

Comparing current methods in generating artificial wash water

The need for a standardized artificial wash water model becomes more apparent when researching previous and current work being done, due to the varying methods and commodities used for artificial wash water replication. Different produce contains different cellular content that can affect certain analytical methods for example, Table 2 shows that in the same experiment [25] spinach had higher NTU values than chicory. Methods for breaking down the organic material can also affect the properties of the water. Blenders have the ability to completely breakdown and homogenize a sample, allowing for all the cellular contents of the produce to be in solutions; while a paddle mixer partially breakdown the sample which may produce differences in organic load characterization. When replicating a wash water solution, the removal of the outer layer of produce, which removes dirt and other impurities that may be present during processing, can be an essential step for most commodities for decreasing any possible environmental contamination or interference with processing agents. Chemical coatings and waxes used to preserve produce may affect the organic load and potentially give incorrect COD readings [42,43]. However,

Method	Purpose	Approach	Limitations	Reference
Turbidity	Measure particulate matter in water	Sample placed in Nephelometer which detects light scattering	Values can vary from device to device	[28,31]
Biochemical oxygen demand (BOD)	Determine amount of biodegradable materials present and the amount of oxygen needed to degrade said material	Introduce seed bacteria to waste water then determine amount of dissolved oxygen remaining after 5 days	Requires extended period for testing	[31,32]
Chemical oxygen demand (COD)	Measures the amount of material capable of being oxidized	Introduce an excess of strong oxidizer to waste water then titrate to see how much oxidizer remains	Does not reflect actual reaction rates	[30,31]
Total dissolved solids (TDS)	Determines the dissolved amount of organic and inorganic compounds	Sample is filtered then the filtrates change of mass determined with a scale, after complete evaporation is reported as TDS	Incomplete evaporation, due to crusting, can result in inflated values	[31]
Conductivity	Measure of electrical current to pass through substance	Conductivity probe	Affected by initial water quality and temperature	[31,35]
Oxidation-Reduction Potential (ORP)	Measure of intensity of electron activity in a sample	ORP probe	Results affected by probe material, pH of solution, temperature, and electrode poisoning	[26,31]

Table 1: Analytical measurements to quantify organic load. The table is described as follows: "Method" describes the name of the technique, "Purpose" is the goal of the method, "Approach" is a brief summary of how to method is carried out, "Limitations" are possible drawbacks.

Produce	Time of measurement	COD (mg/L)	pH	Turbidity (NTU)	ORP (mV)	Source
Lettuce	2 hr	218.6	7.2	87.4		[25]
Escarole	2 hr	173.6	7.3	95.7		
Chicory	2 hr	33	7.8	42.4		
Carrot	2 hr	18	7.6	0.6		
Onion	2 hr	747.3	7.1	5040.4		
Spinach	2 hr	68	7.5	88.9		
Sugar snap peas	Approximately 1 hr	30± 5	8.0 ± 0.1	5.2 ± 1.1		[18]
Iceberg Lettuce	2-3 hr	119				[42]
Lettuce	3 hr	2550	5.6		868	[44]
Iceberg lettuce (Company 1)	2 hr	465±2	7.34±0.01	13.8±0.9		[45]
Iceberg lettuce (Company 2)	2 hr	1,405±57	7.2±0.1	72.6±6.6		
Spinach (Facility A)	5-8 hr		7.33±2.19	0.058±0.053	N/A	[24]
Spinach (Facility B)	4-8 hr		7.53±0.11	0.036±0.036	383±127	
Spinach (Facility C)	30 hr		7.47 ±0.26	0.123 ± 0.27	598 ±152	
Tomato (Facility A Primary Tank)	4 hr	390	7.0–7.5	38	950	[46]
Tomato (Facility B Primary Tank)	8 hr	732	5.5-6.5	74.90	1100	
Tomato (Facility C Primary Tank)	4 hr	519.5	6.5–7.0	107.0	870	

Table 2: A summary of approaches used to measure physiochemical properties of water quality surveyed from industrial wash water application.

in commodities such as spinach and most lettuce varieties, there is no outer layer to remove so any potential chlorine residue on the surface will be present in the wash water solution. In order to design a standard model, we must first understand the physiochemical properties of the wash water; such properties as seen in Table 2. By understanding the physiochemical properties of wash water that are seen in industry settings it will enable researchers to bridge the gap between laboratory settings and industry reality, allowing for research findings to be more applicable outside of the laboratory. The majority of the research presented here used prepared dilutions of artificial water using either percent weight (% wt/vol) or COD. Examples of current methods used in the generation of artificial wash water and the efficacy of different sanitizers in post-harvest conditions are summarized in Table 3: "*Summary of Postharvest Artificial Wash Water Research*".

Different researchers have used different methods to generate and measure organic load in research applications. Some use % wt/vol to generate organic load like Davidson, Kaminski and Ryser [44], while others use COD such as Chen and Hung [27]. These two different methods, summarized in Table 3, can render varying results in the physiochemical properties of wash water, making the development of a standard model more challenging. Understanding the effects on organic load on sanitizer efficacy is key to establishing a standard method of organic load replication for further postharvest research, keeping in mind that when generating artificial wash water, the introduction of microbes may change the physiochemical properties, such as an increase in COD values, which need consideration when investigating sanitizer efficacy [45-48].

For example, comparing the works of Chen and Hung [27] and Weng et al. [49], both conducted experiments looking at ways to assess chlorine demand in a wash water system. Chen and Hung [27] used various produce and measured 50 g of sample and homogenized it with equal parts water in a stomacher, using COD as the base measurement of organic load. From this base measurement, different physiochemical measurements were taken throughout the experiment to determine which measurement (COD, pH, turbidity, UV254, protein content) best correlated to chlorine demand in a washing system. Weng et al. [59] was also assessing a parameter to determine chlorine demand. The samples for this experiment were prepared by putting the produce through a household juicer and straining the solution over cheesecloth. Water samples were evaluated throughout the experiments to measure nitrogen composition, COD, TOC, and UV254 as a means to determine the best indicator for chlorine demand. Between both of these experiments, there were two different conclusions. Chen and Hung [27] determined that even with similar COD among the various crops, the chlorine demand was different depending on the type of crops used to generate the organic load, leading to the ultimate conclusion that COD was not a good indicator of chlorine demand, and showed that UV254 was the best measurement to determine this. However, Weng et al. [59] concluded that COD was a good indicator for determining chlorine demand in a wash system, regardless of the produce present. Comparing these two experiments the biggest difference in the preparation of organic load was the use of a stomacher versus the use of a juicer, and varying results were observed. Comparing the COD values of these two studies to the industrial values listed in Table 2, Weng et al. [59] used values that are not comparable to observed values. Both studies used iceberg lettuce, romaine lettuce, and spinach, COD values reported by Weng et al. [59] were between 2,000 and 36,000 mg/L, but the industry values for these commodities ranged from 120-220 mg/L. Chen and Hung [27] remained in the range of 50 and 750 mg/L, which is more comparable to the reported values in Table 2.

Davidson et al. [44] and Van Haute et al. [21] have both studied the effects of chlorine efficacy in *Escherichia coli* inactivation, more specifically the influence of organic load on chlorine efficacy in *E. coli* inactivation. Both conducted experiments using lettuce as the commodity of choice. Davidson et al. [44] inoculated lettuce leaves with *E. coli* O157:H7 to determine the efficacy against the pathogen in pilot-scale processing water. Lettuce was homogenized with an "Urschel TransSlicer" and then combined in the recirculation tank with tap water. Three different sanitizer treatments were used and Davidson et al. [44] concluded that increasing organic load resulted in *E. coli* O157H:7 persistence throughout the processing line due to sanitizer depletion. Van Haute et al. [21] also used lettuce as the commodity of choice, but a stomacher was used to homogenize the organic materials for mimicking processed wash water and tap water was used to dilute the sample to the desired COD before inoculating the water. While Van Haute et al. [21] experiment was conducted using a research scale approach, the water flow in the washing bath was maintained by manual stirring to mimic conditions similar to process flumes. Just as Davidson et al. [44] the experiment showed that increased organic load by measure of COD leads to a higher sanitizer consumption, which in this case was chlorine. Statistical analysis also showed that COD was the predominant predictor of sanitizer efficacy in fresh-cut lettuce.

On the other hand, Gil, Marin, Andujar and Allende [58] and Zhou, Luo, Nou, Lyu and Wang [57] have both conducted research on chlorine depletion. Gil et al. [58] completed his experiments by homogenizing lettuce samples in a stomacher and using COD as the primary measurement of organic load, he also compared the COD to ORP readings of the water sample. Zhou et al. [57] created organic load by putting iceberg lettuce through a household juicer and filtering through cheese cloth, he also used COD as measurement of organic load, and compared it to ORP of water samples. Both researchers concluded that an increase in organic matter correlated with an increase in chlorine demand. They both also concluded that ORP measurements correlated with the amount of free chlorine in the system not with the amount of organic load. ORP is often used in chlorine systems as a measurement of water quality, however this does not give an accurate depiction of the organic load present, because it will only measure the presence of a strong oxidizer, sanitizer, present in the solution, giving it limited applications.

A comparison of multiple studies seen in Table 3 has shown that the source of the interferent used in the artificial wash water and the dilution method were primary differences between experiments. The source of water used also varied, where some researchers used municipal water, while others used deionized or dechlorinated water. Different water sources may render different results, due to their different mineral composition, which can cause interference in certain analytical methods and may not reflect in field conditions. Filtration provides a difference in the quality and size of particles present in solution. A major factor that influences the research is that not all researchers have organic matter present in their experiments. A lack of organic matter in the experimental design does not allow for a translation of the information into real world settings, creating a gap between laboratory settings and real-world applications. However, many different methods are used ranging from glass fiber filters to cheesecloth. There was some variation of produce used to generate artificial wash water, but the majority of experiments used leafy greens, as seen in Table 2. However, the type of produce used can result in different physiochemical property profiles, which can in turn can have affect sanitizer efficacy and response [25]. One common varying condition is the use of diverse commodities. Table 2 shows that there is a large difference in the physiochemical properties of wash water not only depending on the produce washed but the length of time that the produce is exposed to the wash water. When the produce breaks or cracks during processing these commodities may potentially introduce cellular components into the sample, changing conditions of the wash water replication system affecting the experimental results. If these qualities are not considered in experimental design, an experiment will lack the ability to be real-world applicable. It is important to think about the aim of the study when choosing a commodity in efforts to ensure that the organic load concentration created best represent in farm conditions and properties, for example using leafy greens verses melons where there is a protective outer layer [23].

Comparisons between studies is challenging due to limited and inconsistent data collection, as well as the difference in produce selection used. However, data from these studies can provide some guidance for future development of artificial organic load that accurately reflects the wash water found in an industrial setting. Studies have shown that with an increase in produce washing, a greater level of BOD, COD, and conductivity were observed, while TDS increased at a slower rate [33]. ORP values may vary by the type and amount of sanitizer present, as

Title	Goal	Produce	Wash Water Creation Method	Method of measuring organic load	Sanitizer	Physiochemical properties measured	Outcome	Reference
Efficacy of sanitizers to inactivate *Escherchia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions	Evaluate chlorine, citric acid, peracetic acid, and acidified sodium chlorite efficacy to reduce pathogens in tap water and simulated washing conditions.	Carrots	Shreds added directly to water	COD	1. Acidified sodium chlorite 2. Citric acid & peracetic acid 3. Sodium hypochlorite	Microbial Analysis only	The efficacy of all sanitizers was affected by the presence of organic load, with chlorine being the most effected and acidified sodium chlorite being the lease effected.	[21]
Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce.	Investigate the effects from reusing wash water on water quality, and its effect on product-to-water quality and microbial growth.	Romaine Lettuce	Cut and added directly to water	N/A	Sodium Hypochlorite	1. COD 2. pH 3. Conductivity, 4. brix 5. BOD 6. TDS	Reuse rapidly decreased water quality by increasing COD, BOD, TDS, and decreased chlorine levels.	[33]
Impact of Wash Water Quality on Sensory and Microbial Quality, Including *Escherichia coli* Cross-Contamination, of Fresh-Cut Escarole	Evaluate the effects of different water qualities on sensory and microbial properties of fresh cut escarole and examine the role of cross contamination.	Escarole	Cut and added directly to water	N/A	N/A	1. COD	High contamination affects wash water ability to contain pathogenic contamination, but no effect on sensory properties	[13]
Heterogeneous photo catalytic disinfection of wash waters from the fresh-cut vegetable industry	Evaluate efficacy of photocatalytic disinfection in wash water for pathogen control and the effect of wash water physiochemical properties on disinfection.	Iceberg lettuce, escarole, chicory, carrot, onion, spinach	Passed produce through wash tanks	N/A	1. TiO_2 2. Photocataly-tic system	1. COD 2. pH 3. Turbidity	Heterogeneous photocatalytic systems are able to effectively reduce pathogens in wash waters but the rate depends on the produce type.	[25]

Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157: H7	Investigate the transfer of *E. coli* O157:H7 during washing, determine efficacy of PAA mixed with peracid and chlorine to reduce transfer and presence in wash water with high organic load	iceberg Lettuce	blender	Percentage	1. Peroxyacetic acid + peracid 2. Chlorine	N/A	PAA mixed with peracid was most effective at reducing pathogens in wash water with or without organic load and on inoculated leaves.	[11]
Cross-contamination of fresh-cut lettuce after a short-term exposure during pre-washing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite	Determine the prevention of cross-contamination by *E. coli* in fresh cut lettuce using Sodium Hypochlorite or Chlorine Dioxide	Romaine Lettuce	Cut and added directly to water	N/A	1. Sodium Hypochlorite 2. Chlorine Dioxide	N/A	Both Chlorine Dioxide and Sodium Hypochlorite inhibited *E. coli* transfer to processing water.	[47]
Efficacy of a Novel Sanitizer Composed of Lactic Acid and Peroxyacetic Acid against Single Strains of Nonpathogenic *Escherichia coli* K-12, *Listeria innocua*, and *Lactobacillus plantarum* in Aqueous Solution and on Surfaces of Romaine Lettuce and Spinach	Compare the antimicrobial properties of lactic acid combined with peroxyacetic acid versus chlorinated water	Romaine Lettuce and Spinach	Cut and added directly to water	N/A	1. Sodium Hypochlorite 2. Lactic Acid 3. Peroxyacetic Acid	Microbial Analysis only	Lactic acid in combination with Peroxyacetic acid showed to be a potential alternative to Chlorinated water for frsh produce procesing	[48]
Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross contamination during fresh-cut produce wash	Investigate free chlorine concentrations in wash water on pathogen reduction, survival and cross contamination during washing.	Romaine Lettuce	Cut and added directly to water	N/A	Sodium Hypochlorite	1. TDS 2. pH 3. Turbidity	Maintaining sufficient free chlorine levels is needed to prevent cross contamination during washing.	[16]
Enhanced chlorine efficacy against bacterial pathogens in wash solution with high organic loads	Investigate the effect of T128 and chlorine on *E. coli* and determine modes of antimicrobial action	Iceberg Lettuce	Juicer	N/A	1. Sodium Hypochlorite 2. T-128	N/A	Free chlorine in combination with T-128 proved to be effective at reducing the presence of *E. coli*.	[49]
Electrochemical disinfection: An efficient treatment to inactivate *Escherichia coli* O157:H7 in process wash water containing organic matter	Evaluate the efficacy of electrochemical treatments to inactivate *E. coli* and to reduce organic matter levels in was water.	iceberg Lettuce	stomacher	N/A	Boron-doped Diamond Electrode treatment	1. COD 2. pH 3. ORP 4. Temperature 5. Conductivity	Treatment reduced levels of *E. coli* and showed potential to be used as a disinfectant and to reduce COD levels in wash water.	[50]
Chlorine dioxide dose, water quality and temperature affect the oxidative status of tomato processing water and its ability to inactivate *Salmonella*	Investigate the effect of chlorine dioxide on *S. enterica* survival in processing water and evaluate the effect of ClO_2 concentration, temperature and turbidity on ORP levels in various water qualities	tomato	Plants with dirt agitated in water	Turbidity	Chlorine Dioxide	1. ORP 2. pH 3. Temperature 4. Turbidity	Turbidity, temperature and ClO2 concentration all had interacting effects and correlated well with pathogen survival and the associated ORP of the wash water.	[51]
Dynamic effects of free chlorine concentration, organic load, and exposure time on inactivation of *Salomonella*, *Escherchia coli* O157:H7 and Non-0157 Shiga toxin-producing *E. coli*	Study the effects of free chlorine concentration, contact time and organic load on pathogen inactivation.	lettuce and tomato	juicer	COD	Sodium Hypochlorite	N/A	Pathogen inactivation is dependent on free chlorine concentrations in wash water.	[19]
Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with *E. coli* O157:H7	The effect of Boron-Doped Diamond Electrode in inactivating microorganisms and decreasing COD	Ice Berg Lettuce	Stomacher	COD	Boron doped Diamond Electrode treatment	1. COD 2. pH 3. Temperature 4. ORP 5. Free and Total chlorine	At specific conditions of flow density, flow rate and doping level provided disinfection efficacy to decrease possible cross-contamination	[52]
Physiochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing	Assess chlorine as a disinfectant to maintain water microbial loads in wash water by using chlorine in a reconditioning treatment and as a wash water disinfectant.	Butter head lettuce	Stomacher	COD	Chlorine	1. COD, 2. Turbidity, 3. UV254	Chlorine is an effective reconditioning agent to eliminate pathogens in wash water with high COD levels, Models also established in order estimate chlorine dosage.	[53]

Impact of organic load on *Escherichia coli* O157:H7 survival during pilot-scale processing of iceberg lettuce with acidified sodium hypochlorite	Determine the efficacy of chlorine treatments in pilot-scale processing against *E. coli* in the presence of organic load, and investigate the relationship between water quality and inactivation	Iceberg Lettuce	Blender	N/A	1. Chlorine 2. Chlorine + Citric Acid 3. Chlorine plus T128	1. COD 2. Turbidity, 3. pH, 4. TDS, 5. Max- Filterable Volume 6. ORP	Regardless of chlorine treatment or organic load present there was minimal *E. coli* reduction. It was also observed that TDS, COD, and turbidity were inversely related to linear trends of *E. coli* inactivation.	[44]
Quantitative study of cross-contamination with *Escherichia coli*, *E. coli* O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process	Produce useful quantitative data on microbial transfers between water and lettuce, and understand how water quality impact pathogen distribution.	Lettuce	Stomacher	COD	N/A	Microbial Analysis only	Water alone to clean contaminated crops is not sufficient for pathogen removal.	[54]
Laboratory and Pilot-Scale Dead-End Ultrafiltration Concentration of Sanitizer-Free and Chlorinated Lettuce Wash Water for Improved Detection of *Escherichia coli* O157:H7	Evaluate Portable Multi-use Automated Concentration System for *E. coli* detection in wash water.	Ice Berg Lettuce	Blender	N/A	N/A	Microbial Analysis only	The Multi-use Automated Concentration System allowed for the detection of pathogens at lower levels than non-chlorinated wash water.	[55]
Development of an algorithm for feed-forward chlorine dosing of lettuce wash operations and correlation of chlorine profile with *Escherichia coli* O157:H7 inactivation	Investigate correlations between changes in water chemistry during chlorination with various organic loads, and develop a feed forward system to control chlorine concentration.	iceberg Lettuce	Blender	COD	Sodium Hypochlorite	1. COD 2. pH 3. ORP	An algorithm was developed for feed-forward control of chlorine levels with increasing organic load.	[56]
Inactivation dynamics of *Salmonella enterica, Listeria monocytogenes,* and *Escherichia coli* O157:H7 on wash water during simulated chlorine depletion and replenishment processes	Identify factors relating to pathogen survival and inactivation in produce washing with organic load during chlorine depletion and replenishment.	Iceberg Lettuce	Juicer	N/A	Sodium Hypochlorite	1. COD 2. pH 3. ORP	Organic load and chlorine concentration have an indirect effect on pathogen inactivation, rather the longer the contact time the less chlorine needed, and vise versa.	[57]
Predicting chlorine demand of fresh-cut produce based on produce wash water properties	Develop a model to predict chlorine demand in different fresh and fresh-cut wash waters.	Iceberg and romaine lettuce spinach, celery, mushroom, broccoli, strawberry, grape, cantaloupe, tomato	stomacher	COD	Sodium Hypochlorite	1. pH, 2. ORP, 3. UV254 4. COD 5. Turbidity, 6. Total phenolics, 7. Total protein, 8. Color	Equations based on UV254 had good prediction accuracy on chlorine demand, because UV254 had the highest correlation with chlorine demand of all parameters measured.	[27]
Should chlorate residues be of concern in fresh-cut salads?	Evaluate chlorate accumulation in process water and the residues in fresh cut lettuce when using sodium hypochlorite.	Iceberg lettuce	blender	COD	Sodium Hypochlorite	1. pH 2. ORP, 3. Conductivity, 4. Temperature	The higher the organic load in wash water, the more sodium hypochlorite is needed to act as a washing aid, leading to an increased chlorate accumulation	[58]
Assessment and speciation of chlorine demand in fresh-cut produce wash water	Evaluate the organic input from produce by measuring chemical properties of wash water to determine the origins of chemical demand and decay.	Romaine and iceberg lettuce, carrots, baby spinach	Juicer	N/A	Chlorine	1. COD, 2. Nitrogen, 3. TOC, 4. UV254 5. IC	There is a rapid reaction between elements in the water and chlorine, also TOC and COD are good measures to estimate chlorine means regardless of the type of produce.	[59]

Influence of temperature and organic matter load on chlorine dioxide efficacy on *Escherichia coli* inactivation	Evaluate the effects of temperature and organic load on the effectiveness of chlorine dioxide on pathogens.	Iceberg lettuce	blender	COD	Chlorine dioxide	pH	efficacy is influenced by concentration, time, pH, temperature and organic load. Increased organic load with a decreased temperature require more ClO_2 demand in the system.	[60]

Table 3: Summary of postharvest artificial wash water research. The table is described as follows: "Goal" is the purpose of the study, "Wash Water Creation Method" is the method of generating artificial wash water, "Sanitizers" are the sanitizers examined, "Outcomes" are the conclusions of the study, and "Physiochemical Properties Measured" are qualities examined.

Note: This table is a comparison of industrial wash water to compare future research to. It also can be used as a reference to show similarities and differences between water quality measurements seen in research and those in industrial settings. As well as act as a guide for future research on what measurements to aim for in research to tie it to industry settings.

well as the water flow in the system. While in turn, turbidity and ORP may also vary based on the type of produce used [56]. For example, carrots have natural antimicrobial properties, which could interfere with studies investigating a sanitizer's ability to eliminate pathogens [61] (as noted in Table 2), where varying physiochemical properties between produce and experimental conditions were seen.

Conclusion

Sanitizer research becomes more important with the enactment of the FSMA "Produce Rule" and its implementation in produce handling and washing. While chlorine is the most studied sanitizer, its potential health detriments combined with its rapid depletion in the presence of organic matter shows the need for other options. We know that the addition of sanitizer to a produce wash water system is a crucial step in preventing pathogen cross-contamination, as well as reducing *some pathogen* concentrations, which is why understanding the relationships between these physiochemical parameters and their effects on sanitizer efficacy is of great importance. The implementation of the "Produce Rule" pushes for a need to establish a standard method of preparing and measuring organic load to help build a wider scope of research to provide validated methods to manage processing water on-farm to help improve food safety. Standardizing a method to replicate the organic load conditions from the produce industry helps to provide the appropriate environment for validation research. More research is needed in order to evaluate the relationship between different commodities and their physiochemical properties; and how these can affect sanitizer efficacy in a wash water system.

Postharvest wash water research has not yet shown a clear relationship between key physiochemical factors and the effect that organic load can play with sanitizer depletion, making the selection of physiochemical properties for industrial application and analysis challenging to implement. COD, total dissolved solids, and turbidity have shown the greatest degree of correlation with sanitizer depletion [44], however each measure has limitations. COD, TDS and turbidity have shown a linear relationship to the amount of produce washed, where ORP correlates with the amount of sanitizer, in the case of chlorine, present in solution [46]. Although ORP has demonstrated a correlation with antimicrobial properties of wash water, based on research conducted thus far, COD and TDS seem to be the most promising aspects in quantifying organic load in relation to sanitizer depletion because they show a linear relationship to the amount of produce washed.

Future Work

Differences in the preparation of the produce such as the cut, size, and washing conditions need to be examined and how these are handled can directly affect physiochemical properties. With such a variety of methods used for the creation of artificial wash water for research conducted in the laboratory, it is currently difficult to create and provide recommendations for best practices for improved produce safety. More research is needed to better understand how organic matter, produce characteristics, and other properties present in wash water affect different commercial sanitizer's efficacy and demand. Research could also be expanded in the area of measuring physiochemical properties in efforts of establishing a rapid method to quantify organic load in field. This will allow for additional guidance to growers on when to best change wash water throughout post-harvest processing.

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