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review

Efficacy of Washing and Sanitizing Methods for Disinfection of Fresh Fruit and Vegetable Products

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Summary

In recent years, as a consequence of the increasing number of produce-related outbreaks of food-borne illness, greater attention has been given to interventions that kill or remove human pathogens on fresh produce. A key goal of washing and sanitizing treatments, therefore, is removal or inactivation of such pathogens. However, published information suggests that conventional washing and sanitizing methods, even using newer sanitizing agents, are not capable of reducing microbial populations by more than 90 or 99 %, although greater efficacy is required to assure product safety. The response of microorganisms to washing and sanitizing treatments will depend in part on the conditions of contamination that affect attachment and survival on produce surfaces. Major factors limiting decontamination efficacy include strength and rapidity of microbial attachment, inaccessibility of attachment sites, attachment and growth in cuts and punctures, internalization of microbial contaminants within plant tissues, and biofilm formation. The performance of conventional produce washing equipment and washing and sanitizing agents in reducing microbial loads is examined. Recent improvements in washing and sanitizing technology are described. New approaches to washing and sanitizing produce that overcome the barriers limiting human pathogen detachment and inactivation are examined.

Key words: fruit and vegetable products, washing and sanitizing treatments, pathogene detachment and inactivation

Introduction

In recent years, producers, regulatory agencies, and the public have become increasingly concerned about the microbiological safety of fruits and vegetables. Produce-related outbreaks of foodborne illness are more numerous. Outbreaks have been attributed to sprouted seeds, leafy vegetables, tomatoes, melons, berries, and unpasteurized juices (*1*). *Escherichia coli* O157:H7, *Salmonella* species, *Listeria monocytogenes*, *Shigella* species, *Cyclospora cayetanensis*, Hepatitis A virus, and Norwalk-like virus have been the causative organisms (*1,2*). Increasing attention is being given to the need for better methods to disinfect fresh produce containing human pathogens.

Most processors and consumers have assumed that washing and sanitizing fresh fruits and vegetables will reduce the microbial load. However, published efficacy

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data indicate that these conventional, time-honored methods are not capable of reducing microbial populations on produce by more than 90 to 99 % (*3,4*). While such population reductions are useful and not to be minimized, they are insufficient to assure microbiological safety. It must be realized that conventional washing technology was developed primarily to remove soil from produce, not microorganisms, and even with newer sanitizing agents such as chlorine dioxide, ozone, and peroxyacetic acid, improvements in efficacy have been incremental. Because of these limitations, it is preferable, wherever possible, to avoid microbial contamination of fruits and vegetables by following good agricultural and manufacturing practices rather than depend on decontamination technologies.

Microbiologists generally express population reduction data as logarithms rather than as percentages to avoid calculations with very large numbers, such as are usually encountered with microbial populations in the environment and on foods. In this article, population reduction data will be expressed as $\log_{10}$(CFU/g) values where log reductions of 1, 2, 3, 4 and 5 are equivalent to percentage reductions of 90, 99, 99.9, 99.99, and 99.999 %, respectively.

In order to improve the efficacy of pathogen reduction by washing and sanitizing produce, one needs to understand and overcome the mechanisms by which attached bacteria resist detachment or inactivation. This requires knowledge of the physiological state of attached bacteria, their attachment sites, their interactions with the plant surface and other microorganisms, and their sensitivity to antimicrobial agents. In developing new or improved washing and sanitizing treatments for fruits and vegetables, one must also take into account compatibility of treatments with commercial practices, treatment cost, absence of treatment-induced adverse effects on product quality, the need for regulatory approval, and the need for consumer acceptance. In this paper, these issues will be examined, and several promising new approaches to produce decontamination will be identified.

Factors Limiting the Efficacy of Washing

Contamination conditions

Contamination of produce with human pathogens may occur at any point during production, harvesting, packing, processing, distribution, or marketing where exposure to human or animal feces is possible. Generally, the earlier in this sequence of events contamination occurs, the more difficult it is to disinfect the product. This is a consequence of the increasing likelihood that the contaminating bacteria have become firmly attached in inaccessible locations, incorporated into biofilms, or even internalized within the fruit or vegetable interior. Of particular concern is exposure of produce to contaminated irrigation or spray make-up water (*5*), windblown dust from a nearby pasture or feedlot (*6*), and insects (*7–9*) or birds (*10*) that may be vectors of human pathogens. These conditions favor microbial internalization or attachment in inaccessible sites such as the calyx or stem areas of apples and skin punctures. The moist environment and nutrient availability prevailing in these sites also might favor biofilm formation. Inaccessibility, internalization and biofilm formation would impede or preclude disinfection of contaminated produce by application of washing or sanitizing agents.

Interval between contamination and washing

We have found that the effectiveness of washing may depend on the time interval between the contamination event and washing. Data obtained with apples, artificially inoculated with *E. coli* and then held for various times before washing with water, indicate that an interval of 30 min between inoculation and washing resulted in a 1 log population reduction. However, after 24 hours, essentially all of the bacteria were firmly attached and could not be removed by washing (*11*). When cantaloupes, inoculated with a non-pathogenic *E. coli* or *Salmonella stanley*, were washed with 1000 ppm chlorine (added as sodium hypochlorite), or 5 % hydrogen peroxide immediately after inoculation, population reductions exceeding 3 logs were obtained. Washing with these anti-microbial agents 72 hours after inoculation was much less effective in reducing the bacterial populations, resulting in population reductions less than 1 log unit for *S. stanley* (*12,13*). Liao and Cooke (*14*) showed that the initial attachment of *Salmonella* to cut surfaces of pepper disks was very rapid.

Attachment in inaccessible sites

When bacteria attach to the surfaces of fruits and vegetables, they tend to locate in pores, indentations or other natural irregularities on the intact surface where there are protected binding sites (*15*). Bacteria also attach at cut surfaces (*14,16*) or in punctures and cracks in the commodity surface (*17*). We found greater attachment of *E. coli* in the calyx and stem areas of inoculated apples than elsewhere on the fruit, and greater survival after washing in these areas than elsewhere on the apple surface (Table 1) (*11*). Bacteria in these locations escape contact with washing or sanitizing agents. Riordan *et al.* (*5*) reported high levels of bacteria in the calyx and stem areas of naturally contaminated apples.

Salmonella chester survived washing to a much greater extent when attached at cut surfaces of apple and green pepper disks than on unbroken external surfaces (*14,18*). These results are a matter of concern to the fresh-cut industry since their products provide extensive cut sur-

Table 1. Distribution of *E. coli* (ATCC 25922) on surface of inoculated apples before and after washing with 5 % H_2O_2 at 50 °C[a]

Location	*E. coli* log(CFU/cm^2)[b]	
	Inoculated	Washed[c]
Skin at calyx end of core	6.79[d]	4.46[d]
Skin on stem end of core	5.61[d]	4.89[d]
Skin except removed calyx and stem portions	4.37[e]	1.63[e]

[a] Adapted from Sapers *et al.* (*11*).
[b] Based on calculated surface area of skin.
[c] Washed 72 hours after inoculation; washed for 1 min in 5 % H_2O_2 at 50 °C.
[d–e] Within the same column, means with no letter in common are significantly different ($p<0.05$) by Bonferroni LSD.

faces for bacterial attachment, making them especially vulnerable to contamination.

A number of commodities (*i.e.*, apples, pears, cherries, grapes, zucchini squash, potatoes, carrots, and lettuce) often have punctures, cuts or splits that could be sites for bacterial attachment. Growth of *E. coli* within punctures was demonstrated in artificially inoculated apples in spite of the fruit's high acidity (*11*). Apparently, the bacteria could create a more hospitable microenvironment within the puncture. Janisiewicz *et al.* (*7,19*) also reported growth of *E. coli* in wounds on apples. Our data (Table 2) indicate that when the bacteria have become established within a puncture, they are very difficult to kill (*11*).

Table 2. Efficacy of H_2O_2-based washes for decontamination of punctured Golden Delicious apples inoculated with *E. coli* (ATCC 25922)[a]

Treatment[b]	log(CFU/g reduction)[c]	
	No puncture	Punctured[d]
5 % H_2O_2	2.34[f]	0.58[g]
1 % APL-Kleen® 245; 5 % H_2O_2[e]	2.83[f]	1.62[f]

[a] From Sapers *et al.* (*11*).
[b] 1 min wash at 50 °C.
[c] Means of duplicate trials; based on control populations of 4.88 log(CFU/g).
[d] 1-cm deep puncture made with 3.7 mm diam. sterile nail on top surface of apple 2–3 cm from stem.
[e] Two-stage treatment: 1 % APL-Kleen® 245 followed by 5 % H_2O_2.
[f–g] Within the same column, means with no letters in common are significantly different ($p<0.05$) by Bonferroni LSD.

Biofilms

Once attached, bacteria might become incorporated into a biofilm, an extracellular polysaccharide matrix that holds the cells together and glues them to the commodity surface (*20–22*). In this state, the bacteria are more resistant to detachment or inactivation by washing treatments. Human pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* as well as other bacteria such as *Pseudomonas* and *Erwinia* spp. are capable of forming biofilms (*23*). The presence of human pathogens in biofilms on fruits and vegetables and on processing equipment would greatly limit the ability to disinfect such produce (*20,22,24*).

Internalization of bacteria within produce

Internalization of bacteria within certain fruits and vegetables can occur during packing or processing (*25–27*). When a warm commodity that has internal air spaces is placed in colder water, perhaps in a packing plant dump tank or flume, the internal gas cools and contracts. This creates a partial vacuum that will allow water and any microorganisms contained therein to be drawn in or »infiltrated« through pores, channels, or punctures into the commodity (*25,27*). Infiltration of *Erwinia carotovora* subsp. *carotovora*, a spoilage organism, and *Salmonella montevideo*, a human pathogen, has been demonstrated in tomatoes (*25,28*). Internalization also can occur naturally due to contamination during flowering or fruit development (*29*). Internalization of *E. coli* O157:H7 has been reported in lettuce (*15,16*) and radish sprouts (*30*), while other bacterial species have been detected within cucumbers and tomatoes (*29,31*). We have detected coliform bacteria but no human pathogens internalized within the core of apples that were exposed to dust from an adjacent pasture (*6*). As stated previously, internalization of human pathogens within produce would preclude effective disinfection by washing and sanitizing treatments.

Efficacy of Conventional Washing Technology

Washing equipment

Various types of washers are available for commodities such as apples and potatoes, leafy vegetables, broccoli, root vegetables, corn, *etc.* These include brush washers, reel washers, pressure washers, hydro air agitation wash tanks, and immersion pipeline washers. These units were designed to remove soils from produce, and not much is known about their ability to remove or inactivate bacterial contaminants.

To obtain such information, we conducted a series of washing trials with artificially contaminated apples using commercial U-bed and flat-bed brush washers. In these trials the fruit was washed with hot or cold water, chlorine solutions, acidic detergent solutions, trisodium phosphate solutions, and dilute hydrogen peroxide, an experimental sanitizing agent (Table 3). In the laboratory, some of these treatments were capable of reducing bacterial populations on inoculated apples by 2–3 log units. However, when the same treatments were applied using commercial washers, population reductions were less than 1 log unit (*32,33*). This lack of efficacy was due in part to deficiencies in equipment design resulting in insufficient exposure of inoculated apple surfaces to the washing agents, especially in the inaccessible calyx and stem areas where contact with brushes was minimal. Bacterial adherence to apple surfaces, biofilm formation and internalization also might have contributed to the poor results, as discussed previously.

Table 3. Decontamination of apples inoculated with *E. coli* (Strain K12) with sanitizing washes applied in a flat-bed brush washer [a]

Wash treatment	Temp. °C	*E. coli* log(CFU/g)[b]		
		Before dump tank	After dump tank	After brush water
Water	20	5.49 ± 0.09	4.92 ± 0.37	4.81 ± 0.26
	50	5.40 ± 0.09	5.04 ± 0.16	4.59 ± 0.08
200 ppm Cl_2	20	5.87 ± 0.07	5.45 ± 0.05	5.64 ± 0.23
8 % Na_3PO_4	20	5.49 ± 0.09	5.02 ± 0.43	4.98 ± 0.02
	50	5.49 ± 0.09	5.02 ± 0.08	4.75 ± 0.45
1 % acidic deterg.[c]	50	5.87 ± 0.07	5.49 ± 0.03	5.42 ± 0.50
5 % H_2O_2	20	5.87 ± 0.07	5.46 ± 0.40	5.27 ± 0.09
	50	5.87 ± 0.07	5.54 ± 0.31	5.49 ± 0.10

[a] Adapted from Annous *et al.* (*33*).
[b] Mean of 4 determinations ± standard deviation.
[c] APL Keen 245 (Elf Atochem North America, Inc., Decco Dept., Monrovia, Calif., U.S.A.).

Washing and sanitizing agents for fruits and vegetables

A number of washing and sanitizing agents have been approved for fruits and vegetables, and some of these have been evaluated in laboratory-scale investigations. These studies generally apply such treatments by immersion of an artificially contaminated commodity in an aqueous solution for a prescribed time. The results of such studies vary widely, depending on the method of sample inoculation, the choice of test organism, the time interval between inoculation and treatment, treatment conditions (*i.e.*, temperature, degree of agitation), and the method of recovering and enumerating the test organisms. However, some insights into means of improving treatment efficacy can be gleaned from these studies.

Chlorine. Chlorine is the most widely used sanitizing agent for fresh produce (*3,4*). Published data indicate that at permitted concentrations, population reductions on produce surfaces will be within the range of 1–2 log units (*28,34–38*). This is due in part to the rapid breakdown of chlorine in the presence of organic matter in soil and on product surfaces. Some improvement in efficacy can be obtained by adding a wetting agent (*39*). Another means of improving treatment efficacy is to monitor the oxidation-reduction potential or ORP (target value of 650 mV) and pH (about 6.5) of the process water and to use these values to control hypochlorite addition and pH adjustment (*40*).

Use of electrolyzed water as a sanitizing agent for produce has received a lot of recent attention. This is a special case of chlorination (*41*). Electrolysis of water containing a small amount of sodium chloride generates a highly acidic hypochlorous acid solution containing 10–100 ppm available chlorine. The results of electrolyzed water treatments have been mixed. Park *et al.* (*42*) reported population reductions on lettuce leaves exceeding 2.49 log units for *E. coli* O157:H7 and *L. monocytogenes*. Horton *et al.* (*43*) reported population reductions of *E. coli* O157:H7 on apples of 3.7–4.6 log units, but Izumi (*42*) could demonstrate only a 1 log unit reduction in the microbial population on fresh-cut vegetables.

The reaction of chlorine with organic residues can result in the formation of potentially mutagenic or carcinogenic reaction products (*44,45*). This is a cause for concern since some restrictions in the use of chlorine might eventually be implemented by regulatory agencies. Therefore, a number of alternatives to chlorine have been examined, and some are in commercial use.

Detergent formulations and other commercial produce washes. Numerous commercial washing formulations for fresh produce are available, including surfactant solutions, combinations of surfactants with organic or mineral acids, and alkaline washes. In tests with artificially inoculated apples, we found that these formulations were generally similar to chlorine, achieving a 1–2 log units reduction in the *E. coli* population (*38*). Population reductions were about 0.5 log unit greater when treatments were applied at 50 °C instead of at ambient temperature. Wright *et al.* (*46*) reported similar population reductions in apples inoculated with *E. coli* O157:H7 with a commercial phosphoric acid fruit wash and with a 200 ppm hypochlorite wash.

Alternative sanitizing agents. Ozone is one of several new sanitizing agents for produce introduced in recent years and approved by the U.S. Food and Drug Administration as alternatives to chlorine (*47–51*). Ozone is effective in reducing microbial populations in wash and flume (*52–53*). However, ozone treatment was ineffective in reducing decay of pears (*54*), and Kim *et al.* (*50*) obtained less than a 1 log unit reduction in lettuce inoculated with *Pseudomonas fluorescens*.

Chlorine dioxide can reduce microbial populations in dump tank and wash water. However, tests with cucumbers resulted in less than a 90 % (1 log unit) population reduction on product surfaces (*55*). A chlorine dioxide product, Oxine, applied at the recommended concentration, was ineffective in reducing the population of *E. coli* O157:H7 on inoculated apples (*56*).

Peroxyacetic acid (or peracetic acid, actually an equilibrium mixture of the peroxy compound, hydrogen peroxide, and acetic acid) has been recommended for treatment of process water (*57,58*). However, population reductions for aerobic bacteria, coliforms, and yeasts and molds on fresh-cut celery, cabbage and potatoes, treated with 80 ppm peroxyacetic acid (the recommended concentration), were less than 1.5 log units (*59*). Wright *et al.* (*46*) obtained a 2 log units reduction in apples inoculated with *E. coli* O157:H7 using 80 ppm peroxyacetic acid, but the interval between inoculation and treatment was only 30 min. In contrast, in a similar apple study, Wisniewsky *et al.* (*56*) obtained less than a 1 log unit reduction at the same peracetic acid concentration, but the interval between inoculation and treatment was 24 hours. We obtained comparable results at this concentration using apples inoculated with a non-pathogenic *E. coli* and had to increase the concentration to 1000 ppm in order to obtain a 2 log units reduction (*38*).

These reports clearly show that the commercially available alternatives to chlorine, like chlorine, are limited in their ability to kill bacteria attached to produce surfaces when realistic inoculation and treatment conditions are used. In order to exceed the apparent population reduction »ceiling« of 1–2 log units, more effective sanitizing agents and application methods must be developed that provide better contact between the sanitizing agent and microbial attachment sites on produce surfaces. It should be noted however, that chlorine and the approved chlorine alternatives are highly effective against microorganisms suspended in water, and so are added to hydrocooling, flume and wash water to reduce microbial populations in recirculating water systems. Thus, these sanitizers play an important role in preventing or reducing the risk of produce cross-contamination.

New Technology for Disinfection of Produce

Hydrogen peroxide as a sanitizing agent

We have had extensive experience with hydrogen peroxide and believe that it shows potential as a highly effective sanitizing agent for produce. Hydrogen peroxide vapor treatments have been investigated for control of post-harvest decay in grapes (*59*), melons (*60*) and other commodities, and to disinfect prunes (*61*). However, such treatments require lengthy application times

(*i.e.*, 15–60 min) and can cause injury to some commodities such as mushrooms, raspberries and strawberries (*62*). Dilute hydrogen peroxide solutions were shown to be effective in washing mushrooms (*63–65*), controlling postharvest decay of vegetables (*65*), extending the shelf-life of fresh-cut vegetables and melons (*62*), and decontaminating apples containing *E. coli* (*11,38*). Recent studies in our laboratory with inoculated apples and cantaloupes have shown that 5 % hydrogen peroxide solutions can achieve log units reductions of 3 or higher when applied by full immersion of the commodity in the solution with vigorous agitation and at a temperature of 50–60 °C for apples and 70–80 °C for cantaloupe (*67*).

Hydrogen peroxide is Generally Recognized as Safe (GRAS) for some food applications but has not yet been approved as an anti-microbial wash for produce. Hydrogen peroxide produces no residue since it is rapidly decomposed by catalase, an enzyme found throughout the plant kingdom, to water and oxygen. However, hydrogen peroxide is injurious to some commodities, causing browning of apple skin at temperatures greater than 60 °C and bleaching of anthocyanins in mechanically damaged berries (*62*).

Novel means of applying sanitizing agents

Vacuum infiltration. Previously, we investigated vacuum infiltration as a means of increasing uptake of browning inhibitors by cut apples, thereby improving treatment efficacy (*68*). The same technology was applied to treatment of whole apples with hydrogen peroxide or chlorine to improve contact between the sanitizing agent and bacteria attached in inaccessible sites on the apple surface by removing gas or liquid barriers that block penetration of the sanitizing agent. Application of a 5 % hydrogen peroxide solution to inoculated apples under vacuum resulted in a 4–5 log units reduction in the *E. coli* population in the calyx area, and a 4 log units reduction, based on the total weight of the treated apples (*69*). Vacuum infiltration of hydrogen peroxide appears to be non-injurious to apples, leaves no peroxide residue, and might be suitable for fresh market apples or prior to fresh-cut processing.

Vapor-phase treatments. Application of anti-microbial agents in the vapor phase might be another means of reaching microbial contaminants attached in inaccessible sites. Chlorine dioxide vapor-phase disinfection of cut green pepper, inoculated with *E. coli* O157:H7 achieved a 6.45 log units population reduction (*70*). Acetic acid vapor treatment of cabbage, mung bean seeds, and grapes reduced microbial populations and prevented decay (*71–74*). In our laboratory, an *E. coli* population reduction exceeding 3 log units was obtained in inoculated apples treated with hot acetic acid vapor, applied in multiple vacuum/pressure cycles (*69*). However, the treated apples showed browning, indicative of injury during storage following treatment. Our research on the vapor-phase application of volatile anti-microbial agents is continuing.

Surface pasteurization. Application of hot water or stream to the surface of fresh fruits and vegetables might be used to pasteurize product surfaces provided that heat transfer into subsurface or inaccessible microbial attachment sites was sufficient. However, the feasibility of surface pasteurization would depend on the absence of heat injury (altered flavor, color, texture or storage stability) at the required exposure times and temperatures. We carried out studies with cantaloupe to determine whether immersion in hot water could pasteurize the product surface without causing injury. The cantaloupes tolerated exposure to water or 5 % hydrogen peroxide at 80 °C for 3 min with no indication of injury initially or after storage at 4 °C for 26 days (*75*). Immersion of cantaloupe, inoculated with *E. coli* or *Salmonella stanley*, in 5 % hydrogen peroxide at 80 °C for 3 minutes resulted in at least a 4 log units population reduction. These results indicate that surface pasteurization of cantaloupes with hydrogen peroxide solutions is possible and that this treatment would preclude transfer of human pathogens from the rind to the flesh during fresh-cut processing that would result in contamination of the product (*75*).

Conclusions

Washing and sanitizing treatment can play an important role in reducing microbial populations on fresh fruits and vegetables intended for fresh market or fresh-cut processing, thereby improving product quality and safety. Conventional washing and sanitizing agents typically achieve 1–2 log units reductions in microbial populations under laboratory conditions; reductions can be substantially smaller with some commercial produce washing systems. Such reductions are not sufficient to assure microbiological safety. Among the factors limiting efficacy of conventional washing and sanitizing treatments are bacterial adherence to produce surfaces, bacterial attachment in inaccessible sites, formation of resistant biofilms, and internalization of microorganisms within commodities. Additionally, conventional washing equipment may not permit sufficient contact between attached bacteria on produce surfaces and the washing and sanitizing agents and/or brushes. While incremental improvements can be made in sanitizer formulations and washer design, these are unlikely to greatly increase efficacy of washing and sanitizing treatments in decontaminating produce.

New washing technologies using sanitizing agents of greater lethality are needed to contact and kill microorganisms that survive conventional washing and sanitizing methods. Such technologies must not only be superior in efficacy; they must also be approved by regulatory agencies, safe to apply, compatible with existing industry practices, and affordable. Favorable results have been obtained with hydrogen peroxide, applied as a washes or by vacuum infiltration or as a medium for surface pasteurization. Vapor-phase application of anti-microbial agents also shows promise. Commercialization of such innovations might bring about large improvements in the microbiological quality and safety of fresh and fresh-cut fruits and vegetables.

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Učinkovitost pranja i sanitacijski postupci za dezinfekciju svježeg voća i povrća

Sažetak

Posljednjih godina, kao posljedica povećanog broja oboljenja prouzročenih namirnicama, sve se veća pažnja pridaje postupcima za uništavanje ili uklanjanje ljudskih patogena sa svježih proizvoda. Stoga je glavna svrha postupka pranja i sanitacije uklanjanje ili inaktivacija patogena. Do sada objavljeni radovi navode da uobičajeni postupci pranja i sanitacije, koristeći čak i najnovija sredstva za sanitaciju, ne mogu sniziti populaciju mikroorganizama za više od 90 ili 99 %. Da bi se osigurala potpuna sigurnost proizvoda, potrebna je još veća učinkovitost. Uklanjanje mikroorganizama pranjem i postupcima sanitacije ovisit će dijelom o uvjetima kontaminacije što obuhvaćaju učvršćivanje i preživljavanje na površini proizvoda. Glavni su činitelji koji ograničavaju uspješnost kontaminacije jakost i brzina mikrobnog prijanjanja, nedostupnost mjestima pričvršćivanja, rast u zasjecima i rupicama, ulazak mikrobnih onečišćenja unutar tkiva biljaka i stvaranje biofilma. Ispitana je djelotvornost konvencionalnih uređaja za pranje proizvoda te sredstava za pranje i sanitaciju i ustanovljen je postotak uništenja mikroorganizama. Opisana su najnovija poboljšanja u postupcima pranja i sanitacije. Također su opisani novi pristupi pranju i sanitaciji proizvoda koji prelaze granice što su ograničavale uklanjanje i inaktivaciju ljudskih patogena.