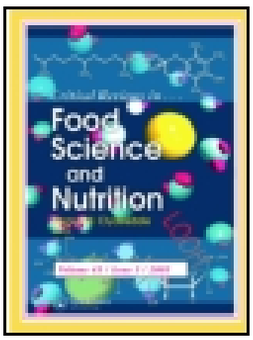

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Aqueous chlorine dioxide treatment of horticultural produce: Effects on microbial safety and produce quality -- A review

Ulrike Praeger[1,*], Werner B. Herppich[2], Karin Hassenberg[3]

[1]Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V., Max-Eyth-Allee 100, D-14469 Potsdam, Tel.: ++49 (0)331 5699-917, Fax.: ++49 (0)331 5699-849,

[2]Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V., Max-Eyth-Allee 100, D-14469 Potsdam, Tel.: ++49 (0)331 5699-620, Fax.: ++49 (0)331 5699-849, e-mail: wherppich@atb-potsdam.de

[3]Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V., Max-Eyth-Allee 100, D-14469, otsdam, Tel.: ++49 (0)331 5699-911, Fax.: ++49 (0)331 5699-849, e-mail: khassenberg@atb-potsdam.de

[*]E-mail: upraeger@atb-potsdam.de

Abstract

Microbial load on fresh fruit and vegetables causes decay and losses after harvest and may lead to foodborne illness in case of contamination with human pathogens on raw consumed produces. Washing with tap water only marginally reduces microorganisms attached to produce surfaces. Chlorine is widely used for decontamination on fresh horticultural produces. However, due to harmful by-products and the questionable efficacy it has become increasingly challenged. During the last 20 years, the interest to study ClO_2 treatments as an alternative sanitation agent for industrially prepared fresh produce has largely increased. For

a wide range of commodities, the application of gaseous ClO_2 has meanwhile been investigated. In addition, since several years, the interest in aqueous ClO_2 treatments has further risen because of the better manageability in postharvest processing lines compared to gaseous application. This article critically evaluated the effects of postharvest application of aqueous ClO_2, either alone or in combination with other treatments, on microbial loads for various horticultural produces. In laboratory investigations, application of aqueous ClO_2 at concentrations between 3 and 100 ppm effectively reduced counts of natural or inoculated microorganisms (bacteria, yeasts and mold) in the range of 1 and 5 log. However, various effects of ClO_2 treatments on produce quality have been described. These mainly comprise implication on sensory and visual attributes. In this context, there is increasing focus on the potential impacts of aqueous ClO_2 on relevant nutritional components of produces such as organic acids or phenolic substances.

Keywords

chlorine dioxide, aqueous treatment, sanitizer, human pathogen, microbial load, fresh produce

INTRODUCTION

Most horticultural produces are highly perishable after harvest due to physiological changes, resulting in loss of water and nutrients. Additionally microbial spoilage causes decay and produce losses along the postharvest supply chain. Human pathogenic microorganisms on fresh fruit and vegetables (F&V) consumed uncooked may lead to serious outbreaks of foodborne illnesses and public health scares. Between 1996 and 2006, 72 outbreaks of foodborne diseases were associated with the consumption of fresh F&V, only in the USA. Furthermore, 25% of these outbreaks were related to the consumption of fresh-cut salads (CFSAN, 2008). In Europe, the frequency of produce-associated outbreaks seems to be similar to that in the USA. For example, F&V consumption caused 4.3% of the total number of outbreaks of food-borne disease between 1992 and 1999. Epidemiologically, outbreaks of illness due to bacterial, viral and parasitic contaminations have been linked to the consumption of a wide range of vegetables, while fruits seem to be responsible to a lesser extent (SCF, 2002). Nevertheless, the rates of foodborne outbreaks are relatively low (SCF, 2002) if compared to the total consumption of F&V in the EU (110 million tons in 2007) (OECD 2010). However, when they occur, their severity can be high, as illustrated by an *Escherichia coli* outbreak in Germany in 2011. In this case, sprouted seeds were identified as outbreak vehicles (Goodburn and Wallace, 2013). Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae (SCF, 2002). Bacterial pathogens isolated from raw vegetables or fruits include Aeromonas, *Bacillus cereus*, Campylobacter, *Clostridium* ssp., *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* ssp., *Shigella* ssp., *Staphylococcus aureus*, *Vibrio* ssp., *Yersinia enterocolitica* and others (Beuchat, 1998).

Fruit and vegetables as well as seeds destined for sprouting are grown usually in a natural environment. During production and distribution, contamination of these produces might occur from various sources such as soil, irrigation water, animals and personnel. They may also spread from equipment for harvest and postharvest processes, during sorting or along the washing lines and in transport containers (Suslow et al., 2003; Goodburn and Wallace, 2013). Probability of cross-contamination of fresh produce is higher during washing, when re-circulated water systems are used due to the accumulation of organic matter (Allende et al., 2008; Lopez-Velasco et al., 2012). For fresh-cut escarole contaminated with *E. coli* at a high inoculum density, Allende et al. (2008) showed that cross-contaminations were more frequent when applying recirculated water compared to potable water or diluted recirculated water.

The ready-to-eat vegetable industry grows by about 10% per year due to an increasing demand for fresh and convenience produce (Rico et al., 2007). The strict control of hygiene and an accurate cool chain management are important especially for ready-to-eat products as they are consumed raw. Fresh-cut F&V with wounded tissue surfaces are more prone to support survival and growth of pathogenic bacteria than intact produce (Thomas-Callejas et al., 2011). In case of pathogen presence, cleaning effect of wash water without disinfectant might be insufficient. Several authors reported that washing without sanitizer only marginally affects microbial population counts on F&V surfaces independent of water quality, potable tap water or reuse water (Reina et al., 1995; Gonzalez et al., 2004; Wu and Kim, 2007; Lee and Baek, 2008).

Globally, chlorine (Cl_2) is commonly used for disinfection of fresh F&V and it is either applied as calcium hypochlorite ($Ca(OCl)_2$) or sodium hypochlorite (NaOCl). However, the application of Cl_2 as disinfectant for fresh produce is increasingly challenged. Most of all, Cl_2

may react with natural organic matter resulting in the formation of carcinogenic halogenated by-products such as trihalomethanes or haloacetic acids (HAAs) (Hua and Reckhow, 2007; Cardador and Gallego, 2012; Nikolaou and Lekkas, 2001). In addition, the safety of workers may be affected by release of Cl_2 vapours (Ölmez and Kretschmar, 2009; Ramos et al., 2013).

Furthermore, the efficacy of Cl_2 as a decontaminant for produce has also been questioned in general. Especially in the presence of organic matter, its effectiveness has been shown to be lower and the antimicrobial efficacy is strongly pH dependent (Ölmez and Kretschmer, 2009; Tomas-Callejas et al., 2012; Parish et al., 2003). Consequently, alternative sanitation agents and technologies for application on fresh F&V are urgently needed. This concomitantly results in an increasing need for research investigating potential sanitation agents' effectiveness against pathogens and their practical applicability (Ramos et al., 2013; Joshi et al., 2013; Goodburn and Wallace, 2013; Ölmez and Kretschmar, 2009).

The major requirements for a potent disinfection method are its effectiveness against pathogens, the absence of human toxic by-products and residues, and no environmental impact. On the other hand, shelf-life and nutritional quality of the produce must not be affected (Gomez-Lopez et al., 2009; Joshi et al., 2013). One of the alternatives for chlorine that has gained attention as potentially non-hazardous sanitation agent for fresh produce within the last decade is chlorine dioxide (ClO_2). Chlorine dioxide was used primarily for the treatment of water supplies to control taste and odors (Benarde et al., 1965). Today it is used for various applications such as drinking water disinfection, sanitation of industrial wastewaters, medical treatment and sanitation, or as bleaching agent in paper manufacturing plants (Gomez-Lopez et al., 2009; Pillai et al., 2009). Due to the high oxidative capacity of ClO_2 (2.5-fold that of chlorine in HOCl)

(Benarde et al., 1965; Beuchat et al., 2005), the required amount of ClO_2 is lower and the required contact time is shorter to obtain the same bactericidal effect as chlorine (Huang et al., 1997). ClO_2 has a strong bactericidal and virucidal effect even at concentrations as low as 0.1 ppm (EPA, 1999; Artes et al., 2009) in a wide pH range (pH 3-8 against bacteria) (Huang et al., 1997; Aieta and Berg, 1986).

Additionally, ClO_2 does not react with organic matter to form carcinogenic by-products such as trihalomethanes (Werdehoff and Singer 1987; Artes et al., 2009) nor does it form chloramines in the presence of ammonia (Beuchat, 1998). In contrast to chlorine and bromine, it does not ionize to generate weak acids (Artes et al., 2009). Interestingly, the carcinogenicity of ClO_2 for human cannot be adequately identified due to a limited data base of relevant investigations on humans or animals. Those available, however, do not indicate a particular cancer concern (EPA, 2000; ATSDR, 2004).

The limitations of ClO_2 for practical applications include its pronounced explosiveness at higher concentration, at partial pressures above 0.1 bar. ClO_2 can be toxic to humans at concentrations greater than 1000 ppm (Pillai et al., 2009). Furthermore, ClO_2 has to be generated on-site, because it cannot be compressed and stored or transported under pressure (EPA, 1999; Gomez-Lopez et al., 2009). Another relevant aspect is that ClO_2 is very unstable and readily decomposes when exposed to sunlight (Tomas-Callejas, 2012).

Numerous studies have reported on the application of ClO_2 gas for fresh F&V decontamination (Han et al., 2001; Sy et al., 2005; Gomez-Lopez et al., 2009; Ölmez and Kretzschmar, 2009). Gaseous application of ClO_2 has been described as more effective against pathogens than aqueous ClO_2 treatment because of the higher penetrability of gas into

microorganism protecting sites at the produce surface (Han et al., 2001). However, the application of gaseous ClO_2 is laborious because of the technical requirements and numerous steps for preparation as well as for the treatment of fresh produce with precise gas concentrations (Wu and Kim, 2007). Aqueous ClO_2 offers several advantages for fresh F&V sanitation. ClO_2 is strongly water soluble (8 g L^{-1} at 20°C) especially in cold water. It is also approximately 10 times more soluble than Cl_2 (above its boiling point of 11°C) and remains in solution as a dissolved gas without being hydrolyzed (EPA, 1999; Aieta and Berg, 1986). These advantages are further augment by the fact that spraying, washing or immersion in water are common practices in postharvest processing of fresh F&V for removal of soil and cooling. Hence, treatments with ClO_2 solutions can be easily adapted in existing washing lines without the need for extensive modifications (Wu and Kim, 2007). In this context, aqueous ClO_2 treatments are also generally safer for operators as no gastight chamber is required. Nevertheless, efficient ventilation of the washing line will be necessary because ClO_2 is volatile and it degases from the washing water. This was indicated by Reina et al. (1995) who found excessive odor after treatment of hydro cooled pickling cucumbers at 2.8 ppm and 5.1 ppm ClO_2 in the water. The odour problem and the explosiveness of ClO_2 at high concentrations may demand some enclosure of the washing line and an effective ClO_2 gas control system to prevent any hazardous situation. In addition, the benefit of the application with ClO_2 in an industrial washing line has been questioned because successful sanitation of fresh produce requires long treatment times (≥ 10 min) and temperatures of approx. 22°C (Goodburn and Wallace, 2013).

The legal framework for the use of sanitation agents of fresh produce is non-uniform in different countries. In the USA aqueous ClO_2 treatment of F&V is permitted using maximum

concentration of 3 ppm residual ClO_2. After the treatment produce have to be rinsed with potable water (FDA, title 21, part 173.300). In the European Union the Regulation EC 852/2004 (EU, 2004) describes that potable water or clean water may be used whenever necessary to prevent contamination of primary food products. The application of ClO_2 for surface decontamination is not regulated explicitly.

Despite all this, application of aqueous ClO_2 for disinfection of F&V has been widely investigated in an ever increasing number of studies during the last decade. In these studies, the sanitation effects of ClO_2 have been evaluated for a variety of F&V, on intact and fresh-cut products as well as sprouts and seeds. In a large number of reports, the effects of the treatments on various microorganisms have been analysed; main focus, however, was laid on *E. coli*, *L. monocytogenes* and *Salmonella enterica*.

The present paper aims to outline the antimicrobial effectivity of aqueous ClO_2 treatment against pathogens attached to the produce surface and in the wash water. In addition, studies of the hygienic effectiveness of ClO_2 treatment when used in combination with other sanitation techniques or agents are evaluated. Special emphasis is also laid on the potential influence of ClO_2 treatments on produce quality, and on the targeted use of ClO_2 to improve quality maintenance, e.g. as anti-browning agent. Investigations on ClO_2 residues in the produce and on effects of ClO_2 against residues of pesticides are also included.

MODE OF ACTION OF CLO_2 AGAINST MICROORGANISMS

ClO_2 is known to be effective as bactericide, virucide and fungicide. The antimicrobial activity of ClO_2 is primarily due to its destabilizing effects on cell membranes. In addition, ClO_2

oxidizes cell-surface proteins (EPA, 1999; Vandekinderen et al., 2009). Berg et al. (1986) observed an enhanced potassium efflux due to a disturbed control of membrane permeability and, consequently, a deterioration of the trans-membrane ion gradients in *E. coli*. In addition, ClO_2 may also enhance membrane permeability by altering structure and function of proteins and lipids of the outer membrane (Olivieri et al., 1985; Ghanbari et al., 1983). Young and Setlow (2003) found that an intact spore coat of *Bacillus subtilis* is important for resistance against ClO_2 treatment. ClO_2 treatment did not inhibit initial steps in spore germination of *B. subtilis* but further development of the germinated spores was blocked probably due to membrane damage. Lee et al. (2004) reported of reduced viability of spores of the bacterium *Alicyclobacillus acidoterrestris* after ClO_2 treatment in an aqueous solution.

Another mode of action for pathogen inactivation is the impact of ClO_2 on amino acids in the cells and the suppression of protein synthesis (Artes et al., 2009). However, only a few amino acids have been reported to be responsive to ClO_2 following in the order of reactivity this include cysteine > tyrosine > tryptophan > histidine > proline (Sharma and Sohn, 2012). The inhibition of enzymatic browning of plant tissues by ClO_2 treatments might, thus, result from structural changes of polyphenol oxidase (PPO) due to ClO_2 effects on functional amino acid residues necessary for PPO activity (Chen et al., 2010). In this context, viruses might be inhibited by an impact of ClO_2 on the viral capsid proteins (EPA, 1999), while ClO_2 does not affect viral ribonucleic acid (RNA).

Unfortunately, ClO_2 treatment may not necessarily irreversibly damage microbes. Lindsay et al. (2002) observed that cells of *B. cereus* and *Pseudomonas fluorescens* increased in length after

ClO_2 treatment. Cell elongation has been reported as indicator for reversible cell injury in several studies on the action of other oxidizing sanitizers (Lindsay et al., 2002).

SENSITIVITY OF MICROORGANISMS TO CLO_2 AND OTHER SANITATION AGENTS

Sensitivity of microorganisms to sanitation treatments may be influenced by various factors such as the respective produce type and the degree of overall microbial load as well as the intrinsic properties of microorganisms and their possible internalization. In particular, protective sites for the microbes such as injuries of the produce, biofilms at the surface and suspended solids or aggregates of organic matter in the washing water may largely affect sanitation efficacy (Francis and O'Beirne, 2002; Goodburn and Wallace, 2013; Mahajan et al., 2014).

In general, the antimicrobial effects of ClO_2 can pronouncedly vary with the sensitivity of the respective microorganisms. Vandekinderen et al. (2009) found that ClO_2 was more effective against gram-negative bacteria compared to gram-positive bacteria, except for *P. fluorescens*. Yeasts showed an intermediate resistance to ClO_2 treatment, while the spores of both mold and *B. cereus* were rather robust. For instance, the resistance of the gram-positive *S. aureus* against aqueous ClO_2 is higher than that of gram-negative *E. coli*. This was attributed to the ability of *S. aureus* to form 3-dimensional network structures with high mechanical strength, while *E. coli* is limited to single layered scattered structures (Huang et al., 1997). The age of the bacteria also plays a role for their resistance against disinfectants. The formation of a polysaccharide shell (glycocalyx) surrounding the cell envelope seems to be more effective to protect against disinfectants in older bacteria (Huang et al., 1997; Sutherland, 2001; Ayyildiz et al., 2009). Noszticzius et al. (2013) found that ClO_2 is a size-selective antimicrobial agent. The authors

measured ClO_2 penetration depths and estimated bacterial killing time. The study showed that the time needed to destroy the microbes after ClO_2 application was proportional to the diameter of the microorganism.

The antimicrobial effect of ClO_2 as well as that of other sanitation agents is much reduced if microorganisms are attached to a surface and not floating in the washing water (Costilow et al., 1984; Reina et al., 1995; Lee et al., 2004). Shredded lettuce and other leafy vegetables easily provide diverse protective sites for microorganisms. This has been convincingly shown by scanning electron microscopy for cut edges, stomata or epidermal folds and cracks (Singh et al., 2002b; Huang et al., 2006; Lopez-Galvez et al., 2010b). ClO_2 treatment of lightly processed cut produce such as apple or lettuce slices is less effective against *E. coli* and *L. monocytogenes* than when use with whole apples or lettuce leaves (Rodgers et al., 2004). Injuries on surfaces of whole produce may also protect bacteria against sanitation treatments. As reported by Han et al. (2001), the log reductions of *L. monocytogenes* by aqueous ClO_2 treatment were significantly higher on uninjured than on surface-injured green pepper fruit. Due to the physicochemical properties of cuticle and waxy layers, pockets in leaf epidermis are hydrophobic and, thus, may serve as protective pockets for microbes. Here, the aqueous ClO_2 cannot penetrate and the bacteria could stay undisturbed (Adams et al., 1989). Costilow et al. (1984) assumed that many microorganisms, closely associated with the cucumber fruit, are completely protected from labile compounds such as ClO_2. In contrast fruit and leafy vegetables with smooth surface like apples or baby carrots enhances the contact of ClO_2 with bacteria thus amplifying the antimicrobial effect of ClO_2 (Huang et al., 2006; Singh et al., 2002b). ClO_2 treatment of oranges against *E. coli*

was more effective on the smooth non-stem-scar surface than on the rough stem-scar area, where microorganisms may be shielded by entrapped air or debris (Pao and Davis, 1999).

Besides the protective sites on plant surfaces also the formation of biofilms reduces the effectiveness of sanitation treatments against microorganisms (Nguyen-the and Carlin, 1994; Keskinen et al., 2009; Poulsen, 1999). In biofilms, accumulations of microorganisms are embedded in a matrix of exopolymers and adherent to plant or to container surfaces during food processing (Annous et al., 2006). Therefore, the presence of various species may enhance the resistance of bacteria to aqueous ClO_2 treatment. The presence of *B. cereus* DL5 in binary biofilms promotes the survival of *P. fluorescens* M2 cells after exposure to ClO_2. The formation of micro-colonies and their associated extracellular polymeric substance (EPS) might be one reason for the reduced efficiency of sanitizer treatments against *P. fluorescens* (Lindsay et al., 2002). The existence of biofilms is probably also the reason for the less effective reduction of populations of microorganisms pre-existing on vegetables, fruits and sprouts by aqueous ClO_2 treatments if compared to artificially inoculated produce samples (Chun et al., 2013a; Jin and Lee, 2007).

Resistance of microorganisms against sanitation agents is also increased by suspended solids and organic matter in the treatment solution. Narkis et al. (1995) found that some microorganisms entrapped in suspended solids or adsorbed to their surface can survive disinfection with ClO_2. Therefore, the removal of any dirt particles and pollution by coagulation, sedimentation and filtration is necessary for successful wastewater disinfection.

EFFECTS OF AQUEOUS CLO_2 ON THE MICROBIAL LOAD ON FRESH PRODUCE

Varying effectiveness of aqueous ClO_2 treatments for various F&V have been reported. The success of sanitation depends on numerous factors; in particular, the effective concentration of ClO_2, the duration of and the temperature during treatment and the presence of organic matter in the water. In addition, the effectiveness of aqueous ClO_2 treatments differs for each fresh produce and target microorganisms. Some of these aspects will be subject in the following part of this review.

Produce and pathogens

Table 1 presents a summary on the application of aqueous ClO_2 for fresh produce, which are washed after harvest. Several studies focussed on fresh-cut salads due to their increasing importance as ready-to-eat produces and high sensitivity for microbial spoilage (Zhang and Farber, 1996; Singh et al., 2002b; Kim et al., 2007; Keskinen et al., 2009; Lopez-Galvez et al., 2010a). Also, aqueous ClO_2 treatments of seeds and sprouts aroused particular interest because the consumption of raw sprouted vegetable seeds (e.g. radish, alfalfa and broccoli) has largely increased during recent years. The microbial contamination of sprouts usually resulted from propagation and cross-contaminations of microorganisms on seeds (Kim et al., 2013; Jin and Lee, 2007). This increases the demand for effective but gentle sanitation techniques such as aqueous ClO_2.

In most of the studies, produce had been artificially inoculated with various bacteria after harvest for testing the effectiveness of aqueous ClO_2 treatment. Yet another important aspect of aqueous ClO_2 treatments is the application for extending the shelf-life of F&V by inhibition of decay from native microbial growth such as bacteria, yeasts and molds (Table 1). Aqueous ClO_2

treatments were reported to be effective against *E. coli* O157:H7, *L. monocytogenes*, *S. enterica*, *S. typhimurium* on lettuce, apples, strawberries, cantaloupe, green pepper (Han et al., 2001; Rodgers et al., 2004; Kim et al., 2008). Wu and Kim (2007) found that ClO_2 (15 ppm, 2 h treatment time) was more effective in reducing *L. monocytogenes* (4.88 log) compared to other inoculated pathogens *Pseudomonas aeruginosa*, *S. typhimurium*, *S. aureus* and *Y. enterocolitica* with reductions of 2.16, 3.32, 4.56 and 3.49 log respectively. For these latter pathogens, the authors reported highly varying combinations of disinfectant concentrations (5 to 15 ppm) and exposure times (5 to 120 min) to yield maximum reduction. Microbial population naturally occupying F&V decrease even slower and to lower extent in response to an aqueous ClO_2 treatment than those inoculated on produce samples (Chun et al., 2013a).

Different sanitation effects of ClO_2 treatment have been reported for different produce probably due to varying surface structure (see above). After treatment with ClO_2concentrations between 20 and 200 ppm higher log-reductions of *E. coli* were found on the surface of cut iceberg lettuce (1.06-1.45 log) than on romaine lettuce (0.38-0.44 log) (Keskinen et al., 2009). Aqueous ClO_2 treatment (20 mg L^{-1}) did not reduce aerobic plate count of minimally processed lettuce and cabbage but yielded > 1 log reduction (20 mg L^{-1} for 1 min or 5 mg L^{-1} for 5 min) in carrots (Gomez-Lopez et al., 2008).

ClO_2 concentration

Many investigations have indicated that washing of fresh produce with pure water without any additional sanitizing treatment reduces both pre-existing as well as inoculated microorganisms on F&V surfaces by less than 1 log. These minor effects were observed for a

large variety of produce such as pickling cucumbers (Reina et al., 1995), apples, strawberries and cantaloupe (Rodgers et al., 2004; Kim et al., 2010) and blueberries (Wu and Kim, 2007, Chun et al., 2013a), carrots and cabbage (Singh et al., 2002a; Gomez-Lopez et al., 2008; Lopez-Galvez et al., 2010b), spinach (Lee and Baek, 2008) or iceberg and romaine lettuce, and alfalfa (Kim et al., 2009b) and buckwheat sprouts (Chun and Song, 2013). With relatively longer washing times of 10 min or more, higher reductions up to 1.4 log were achieved for *E. coli* on lettuce or baby carrots (Singh et al., 2002a) and *L. monocytogenes* on green pepper surface (Han et al., 2001).

Similar to water washing without sanitizer, aqueous ClO_2 treatments often show insufficient disinfection effects (maximum reduction < 1 log) against surface-attached microorganisms for fresh produce, if applied at concentrations of 5 ppm and less (Costilow et al., 1984; Zhang and Farber, 1996; Kim et al., 2007; Lopez-Galvez, 2010a; Lopez-Galvez, 2010b; Tomas-Callejas et al., 2011; Tomas-Callejas et al., 2012). In contrast, Rodgers et al. (2004) found 5 log reductions of *E. coli* and *L. monocytogenes* after 3 and 5 mg L^{-1} ClO_2 treatments (5 min) of apples, shredded lettuce, strawberries and cantaloupe. In addition, Han et al. (2001) reported about 3.7 log reduction of *L. monocytogenes* on green pepper after 10 min aqueous ClO_2 treatment (3 mg L^{-1}).

After 1 min washing of tomato in 10 and 20 ppm ClO_2 solutions, however, a 4-5 log reduction of inoculated *S. enterica* and *E. carotovora* was achieved (Pao et al., 2007). Aqueous ClO_2 treatment of cut iceberg lettuce with 50 ppm during 10 min increased the log reduction of *E. coli*, *S. typhimurium* and *L. monocytogenes* by 0.92, 1.77, 1.03 log compared to 5 ppm ClO_2 treatment (Kim et al. 2008). Sanitation effects of aqueous ClO_2 (log reduction of 1-2) was significant against natural and inoculated microorganisms in most cases when treatment

concentrations were 50 ppm or higher. This observation was reported for iceberg and romaine lettuce (Kim et al., 2007; Keskinen, 2009), spinach (Lee and Baek, 2008) and blueberries (Chun et al., 2013a). ClO_2 solutions of 100 ppm may achieve reduction of microbial contaminations of more than 3 log. This was, for example, shown for natural microbial load on fresh-cut asparagus (Chen et al., 2010) and for populations of *Cronobacter* spp. on radish seeds (Kim et al., 2013). *E. coli* O157:H7 on alfalfa seeds was significantly reduced after treatment with 100 ppm and 500 ppm acidified ClO_2 (Taormina and Beuchat, 1999). ClO_2 treatment (100 ppm) of mungbean sprouts resulted in a reduction of inoculated *S. enterica* and *L. monocytogenes* of 1-3 log, while total mesophilic microorganism were reduced only by 0.7 log (Jin and Lee, 2007). For the development of industrial sanitation procedures it has to be considered that high ClO_2 concentrations tested in these studies exceed largely the legalized range in some countries and maybe hazardous for consumers or personal during industrial application.

Test procedure and treatment time

A stepwise procedure is necessary for testing the effectiveness of a sanitizer in aqueous solution for decontamination of fresh produce which are inoculated with pathogenic microorganisms: preparation of an inoculum suspension, inoculation of the produce, drying, preparation of the sanitizer and produce (whole or cut), storage before treatment, sanitizer exposure. The method is not standardized and different procedures influence the test results. The decontamination effect decreases with increasing time interval between inoculation of the pathogen and washing with a sanitation agent (Sapers, 2001). In addition, the hydration state of the inoculum may pronouncedly affect the disinfection efficacy of the ClO_2 treatment. This was

investigated for fresh wet and dried inocula of *S. enterica* and *E. carotovora*, respectively, on the surface of tomatoes at 24°C for 24 h (Pao et al., 2007). For the dried inoculum, almost no sanitation effect was found at ClO_2 concentrations of up to 20 ppm. In this context, the observation of Singh et al. (2002b) is also relevant that also inoculation method, i.e. whether dip, drop or sprinkle inoculation is used, may affect the efficacy of the treatment. The authors found that washing of romaine lettuce with ClO_2 (10 mg L^{-1}, 10 min) was most effective for the inactivation of *E. coli* after 6 and 24 h of incubation at 5 +/- 1°C after drop inoculation maybe due to less adherence of the bacteria than after the dip or sprinkle method. Similarly, Lang et al. (2004) found that a larger number of pathogen cells adhered to tomato surface after dip inoculation than after spot or spray inoculation The choice of inoculation method depends also on the produce and weather it is cut or not as indicated in Table 2.

Another important factor for sanitation effectiveness is the treatment time. For ClO_2 disinfection of water, major bacterial reductions occur within the first minute of contact as stated by Benarde et al. (1965). The authors concluded that residuals of ClO_2 in the water have little disinfectant value. Similarly, Pao et al. (2007) described a rapid reduction of *S. enterica* and *E. carotovora* in water of 7 log to the minimum detection level (10 cfu ml^{-1}) after 10, 6, 4 s treatment with 5, 10 and 20 ppm ClO_2, respectively. However, with microorganism attached to the produce surface, maximum reduction was not achieved with aqueous ClO_2 within some seconds.

Wu and Kim (2007), studied the effect of various contact times (10 s; 1, 5, 10, 20, 30 min; 1, 2 h) on the efficacy of aqueous ClO_2 treatment at different concentrations (1, 3, 5, 10, and 15 ppm) and against different pathogens on blueberries. Although the greatest reduction of *L.*

monocytogenes (4.88 log) was achieved at the longest treatment time (2 h) and with the highest ClO_2 concentration (15 ppm), microbial reduction was not significantly different when the duration of the ClO_2 application was shortened (60 and 30 min) and the disinfectant concentrations reduced (10 or 5 ppm). The authors concluded that application of high ClO_2 concentrations at short treatment durations is more effective to reduce *L. monocytogenes* than long treatment times at low ClO_2 concentration.

In this context, increasing the duration of washing with 5 mg L^{-1} ClO_2 from 1 to 15 min showed no significant reduction of the load of *E. coli* on romaine lettuce but on baby carrots. It has been assumed that the low effect on lettuce was due to the penetration of microorganisms through cut edges to sites in leaves virtually inaccessible for the disinfectant (Singh et al., 2002a). Further, increasing the duration of treatment with ClO_2 (25 or 50 ppm) from 5 to 10 min had no effect on the reduction of *E. coli* on alfalfa seeds (Singh et al., 2003). Similarly, Kim et al. (2009b) found no significant difference between 5 and 10 min ClO_2 treatment (50 mg L^{-1}) against *E. coli* or *L. monocytogenes*, although the sanitation effect was clearly better at these longer durations than after 1 min of treatment.

Washing systems

Besides disinfectant concentration and duration of treatment, the type of washing system may also influence the efficacy of ClO_2 treatment. Tomas-Callejas et al. (2012) explicitly investigated the potential effect of washing types on fresh-cut red chard leaves, inoculated with *E. coli* or *S. enterica*. The authors found that the log reduction of *E. coli* was greater after ClO_2 treatment in an aerated agitation bath than after immersion. In contrast, log reduction of *S.*

enterica was higher after the immersion treatment. The reason for this effect was not explained. Furthermore, the effect of repeated application of ClO_2 solutions against pathogen contaminations on the overall success of the treatments has been studied by Singh et al. (2002b). A second washing with aqueous ClO_2 (10 mg L^{-1}) during 5 min significantly reduced *E. coli* contamination on shredded romaine lettuce. A third washing, however, did not further enhance the effect on the population of the pathogen probably due to its penetration into inaccessible sites of the leaves.

Effects of packaging and storage

Lightly processed convenient products such as fresh-cut fruit or leafy vegetables are highly perishable. To generally facilitate handling and, most of all, to increase their shelf-life, they are commonly distributed packed in film-covered plastic trays. Most of these packaging allow the formation of a modified atmosphere (MAP), with a high CO_2 level at a concomitantly low O_2 content. MAP is known to reduce overall metabolic activity of the produce but also of potentially adherent pathogens. Hence, it is also important to know whether ClO_2 treatment during processing may persistently affect the development of pathogenic microorganism during storage in MAP.

On tap water washed fresh-cut produce, the amount of microbes naturally adherent on the produce surface frequently increases even if stored under MAP at cold temperatures (Kim et al., 2007; Chen et al., 2010; Chun et al., 2013b). After sanitation with aqueous ClO_2, however, most investigations indicated a sustained effect of the treatment on both natural and inoculated

microbial contaminants during MAP storage (Kim et al., 2007, Jin et al., 2007, Chen et al., 2010).

In cut iceberg lettuce, the inhibitory effect of ClO_2 treatment (50 ppm) on total aerobic bacteria, yeasts and molds, and coliforms counts sustained during storage at 4 C for 7 d (Kim et al., 2007). After the initial direct reduction of *E. coli, S. typhimurium* and *L. monocytogenes* counts by ClO_2 treatment (5 and 50 ppm), *E. coli* and *S. typhimurium* load further decreased during 4 d cold storage. In contrast, the populations of *L. monocytogenes* increased again (Kim et al., 2008). Also on strawberries, the reducing effect of ClO_2 washing (50 ppm), compared to tap water-washed fruit, on total aerobic bacteria, yeasts and molds was maintained during 1 week storage (Jin et al., 2007). Similar results have been reported for red chicory and pak choi (Kim et al., 2011) and cut yam (Chun et al., 2013b). Washing asparagus lettuce with ClO_2 (100 mg L^{-1}) for 20 min prolonged its shelf-life at 4°C by 10 d if compared to that of controls (4 d). With this treatment, the initial log reductions of the main microbial groups (ranging between 1.2 to 3.3 log) significantly delayed their increase during storage (Chen et al., 2010).

Lopez-Galvez et al. (2010a) found that the natural microbiota of fresh-cut lettuce in active MAP (addition of N_2) was almost equally affected by washing in plain water or in ClO_2 solution (3 mg L^{-1}), both directly after washing and after storage (3 d at 4°C following 7d at 7°C). Growth of yeasts, however, was pronouncedly high in ClO_2-washed samples after 10 d of storage. Even at high temperature of 20°C, Chun et al. (2013) found only a slight increase of total aerobic bacteria (< 1 log) on blueberries during 12 d storage, independent of the treatment (water washing or 100 pm ClO_2 treatment). While, at 4°C, the bacterial population as well as

yeast and molds remained almost stable during storage after both treatments. This was assumed to be due to the fact that the produce were not cut.

The composition of the atmosphere in MAP has an influence on microorganism growth after ClO_2 treatment. On mungbean sprouts, populations of *S. typhimurium* and *L. monocytogenes,* which were reduced after ClO_2 treatment (100 ppm), increased again during storage in MAP (air) but the reduced pathogen levels were maintained or their growth was delayed when samples were packed under vacuum, 100% N_2 or CO_2 gas (Jin and Lee, 2007). Kim et al. (2013) investigated the inactivation of *Cronobacter ssp*. on radish seeds, treated with aqueous ClO_2 (50, 100 µl mL^{-1}, 5 min), dried at 25°C for 2h and stored under MA afterwards. After washing the seeds with sterile water and 4 d storage in ambient air, the bacterial population was lower than after MA storage. After ClO_2 treatment, the bacterial population decreased similarly when the seeds were stored in air or under MA conditions during the 4 d storage period. The viability of the seeds was not affected even at ClO_2 concentrations of up to 100 µl mL^{-1}.

Beside the effects of ClO_2-treatment on storage behaviour of fresh vegetables, fruit or seeds in MAP, Other studies have investigated storage life of produce, which are commonly not stored under modified atmosphere. For instance, the application of 100 ppm CIO_2 in the washing water did not delay the growth of mold during storage of intact cucumber fruit in glass jars (Costilow et al., 1984). Similarly, Reina et al. (1995) did not find differences in storage life of cucumbers during 6 d after hydrocooling without and with 5 ppm ClO_2 in the water. In lettuce (whole and shredded) and apple (whole and slices), strawberry and cantaloupe fruit, treated with 5 mg ClO_2, population of *E. coli* and *L. monocytogenes* remained almost unchanged at less than 1.3 log cfu g^{-1}, whereas loads of mesophilic bacteria, yeasts and molds increased during 9 d of storage,

though at lower rates than in water washed samples (Rodgers et al., 2004). Hence, in this study, ClO_2 treatment could not fully prevent the decrease of produce shelf-life. Aqueous ClO_2 treatment (10 ppm, 3 min) tended to reduce the load of chestnuts with shell mold after storage at 4°C for 60 d, but no significant differences were observed (Donis-Gonzales, 2008).

Effect against pathogens in the washing water / Inhibition of cross-contamination

Each washing process may certainly help to reduce the microbial loads of processed and unprocessed fresh produce. However, it also bears some risks for cross-contaminations, i.e. produce might be contaminated by microorganisms flushed off infected surfaces during the washing process. This clearly emphasizes the high need for controlling the hygienic status of the washing water. In this context, various studies have reported on the efficacy of aqueous ClO_2 to reduce the microbial loads in wash waters (Lopez-Galvez et al., 2010b; Lopez-Velasco et al., 2012; Pao et al., 2007; Pao et al., 2009; Hassenberg et al., 2014).

Allende et al. (2008) demonstrated the occurrence of cross-contamination with *E. coli* on washed fresh-cut escarole. At a high contamination level, the water quality directly influenced the degree of cross-contamination. For example, the possibility of cross-contamination was increased in recirculated water with high chemical oxygen demand compared to potable water. Lopez-Galvez et al. (2010b) found that ClO_2 (3 mg L^{-1}) inactivated most *E. coli* cells that were washed off inoculated fresh-cut lettuce samples. Therefore, treatment of washing water with ClO_2 might help to prevent cross-contamination between clean and contaminated product during this processing step. To avoid microbial cross-contamination in tomato washing lines, both the immersion of fruit in ClO_2 solutions (Pao et al., 2007) and the spray washing of tomatoes (Pao et

al., 2009) were tested. Pao et al. (2007) indicated that immersion of tomatoes in 5 ppm ClO_2 solution completely prevented cross-contamination by *S. enterica* and *E. carotovora*. The transfer of *Salmonella* from contaminated brushes to fruit surfaces was reduced by 4.7 log cycles after spray washing with 5 ppm ClO_2 at flow rates of 5 ml s^{-1} for 10 s. In tomato processing water, a 7 log reduction of *S. enterica* was achieved with 3 or 5 mg ClO_2 in a temperature range of 25-40°C and 0-40 NTU turbidity of the water within 30 s (Lopez-Velasco et al., 2012). Tomas-Callejas et al. (2012) found that a treatment with 3 mg L^{-1} ClO_2 prevented *E. coli* cross-contamination on fresh-cut red chard but not the release of *S. enterica* from inoculated leaves to the processing water.

ClO_2 treatment was also tested for the inactivation of microorganisms in washing water of a cucumber handling line (Costilow et al., 1984). A 1-log reduction of total microbial counts was found after treatment with 2.5 ppm ClO_2 in the wash water and 5 log in water with 25 ppm ClO_2. Reina et al. (1995) studied the sanitation effect of ClO_2 in water for hydrocooling of pickling cucumbers. This water is usually recycled and may, thus, be easily contaminated with spoilage microorganisms. In the hydrocooling water, a ClO_2 concentration of 1.3 ppm was found to optimally control the numbers of bacteria, reducing total aerobic microflora by 2-6 log.

APPLICATION OF CLO_2 IN COMBINATION WITH OTHER AGENTS -- EFFECTS ON MICROBIAL POPULATIONS

For water sanitation treatments, enhancements of the efficiency of sanitation techniques by sequential treatments or by combination with oxidants such as chlorine, resulting in synergistic effects have been reported (Driedger et al., 2000; Son et al., 2005). Consequently, application of

such additive and synergistic antimicrobial effects of combined preservation techniques following the so-called hurdle technology are also described for food disinfection (Leistner, 2000; Lee et al., 2014). For sanitation of fresh horticultural produce, aqueous ClO_2 has been combined with other sanitation agents or techniques, in particular with UV-C irradiation, ultrasonication or treatment with acids (Huang et al., 2006; Kim et al., 2009a,b; Kim et al., 2011). Furthermore, some investigations analyzed the effects of combining aqueous ClO_2 with two additional sanitation treatments (UV-C irradiation and fumaric acid) on buckwheat sprouts (Chun and Song, 2013).

These investigations showed that the additive application of various sanitizing techniques increased the disinfection effectiveness of aqueous ClO_2 treatment. On romaine lettuce and baby carrots or alfalfa seeds, higher log-reductions of *E. coli* were achieved by a combined treatment of aqueous ClO_2, ozonated water and thyme oil than when applying each technique individually (Singh, 2002a; Singh et al., 2003). Singh et al. (2002a) assumed that thyme oil may increase the permeability of cell membrane, thereby enhancing the efficacy of ClO_2 or other sanitizers.

For apples, ClO_2 treatment showed a better effect against *Salmonella* and *E. coli* when applied simultaneously with ultrasonic treatment (Huang et al., 2006). The fact that the success of such combination was only marginally for lettuce leaves maybe due to the pronounced structural differences between apple and lettuce surfaces. Furthermore, the simultaneous application of aqueous ClO_2 and ultrasonication resulted in a higher log-reduction of the initial loads of bacteria, yeasts and molds than the separate two-step treatment with these techniques (Chen and Zhu, 2011).

Similar additive effects have been reported for ClO_2 and fumaric acid (Kim et al., 2009a). The combined treatment of broccoli sprouts with ClO_2 and fumaric acid resulted in a significantly better effect against *S. typhimurium* and *L. monocytogenes* that the separate application of either ClO_2 or fumaric acid. However, for other microorganisms such as total aerobic bacteria, yeasts and molds, coliforms and *E. coli*, the efficacy of the combined application of these techniques was not significantly better than using only fumaric acid. On alfalfa sprouts, on the other hand, the combined treatment of ClO_2 (50 g L^{-1}, 10 min.) and fumaric acid (5 g L^{-1}) was more effective against loads of total aerobic bacteria, *E. coli*, *S. typhimurium* and *L. monocytogenes* than each agent singly (Kim et al., 2009b).

Also, the combination of ClO_2 and UV-C irradiation more effectively decreased the initial populations of total aerobic bacteria on strawberries than the treatment with either ClO_2 or UV-C (Kim et al., 2010). Similar positive effects of combining aqueous ClO_2 and UV-C irradiation on the reduction of total aerobic bacteria, yeasts and molds, adherent to produce surface, were observed for strawberries (Shin et al., 2012), red chicory and pak choi (Kim et al., 2011), and yam tubers (Chun et al., 2013b). It was assumed that the lower effectiveness in reducing microbial counts of UV-C treatment alone in comparison to the combination with aqueous ClO_2 and UV-C may be due to photoreactivation, a DNA repair mechanism, after exposition to visible light (Chun et al., 2013b).

EFFECT OF CLO_2 TREATMENT ON PRODUCE QUALITY

Sensory quality

Various studies have shown that some effective sanitation techniques could render fresh produce useless, because they negatively affect produce quality (Vandekinderen et al., 2008; Martínez-Sánchez et al., 2006; Hassenberg et al., 2011). In case of aqueous ClO_2, however, the influence of treatments on the sensory quality of fresh produce has been considered in fewer studies than the effects on microbial populations.

Investigations by Kim et al. (2007; 2011) indicated that the sensory quality of shredded iceberg lettuce, pak choi and red chicory was not affected after 10 min treatment with ClO_2 at concentrations up to 50 ppm and during storage for 1 week. Similarly, ClO_2 treatment with 3 or 5 mg L^{-1} (1 or 5 min) had no influence on the sensory quality of fresh-cut iceberg or green leaf lettuce, subsequently stored in MAP (Rodgers et al., 2004; Lopez-Galvez et al., 2010a). In contrast, washings with 20 mg L^{-1} ClO_2 affected sensorial quality of iceberg lettuce, while cabbage and carrots were unaffected (Gomez-Lopez et al., 2008). In these latter experiments, treatment time (5 min) was even shorter than in the study of Kim et al. (2007).

On the other hand, ClO_2 treatment may also enhance sensory quality of fruit during storage. This was proven for strawberries, blueberries and mulberries, which had better sensory scores after ClO_2 washing than untreated controls (Jin et al., 2007; Wu and Kim, 2007; Chun et al., 2013a; Chen et al., 2011). Also, sensory quality of strawberries and shredded yams was preserved by the combined treatment with ClO_2 and UV-C (Kim et al., 2010; Chun et al., 2013b), and by the combination of ClO_2 and UV-C and film packaging made from rice bran protein (Shin et al., 2012). For plums, Chen and Zhu (2011) reported that after combined treatment of aqueous ClO_2 and ultrasonic, sensory quality of fruit was better maintained during storage than in untreated controls. One reason for better preservation of sensory quality by ClO_2

treatment might be the retarded growth of microorganisms (Chen and Zhu, 2011, Chen et al., 2011).

Visual quality

Different observations have been made regarding the effect of aqueous ClO_2 treatment on the visual quality, in particular the color of horticultural produce. During storage, changes in the Hunter color L, a, and b values of iceberg lettuce was negligible after 10 min treatment with ClO_2 at concentrations up to 50 ppm (Kim et al., 2007; Hassenberg et al., 2014). Also, the color of red chicory and pak choi (Kim et al., 2011) or strawberries (Shin et al., 2012) was not affected by treatments with 50 ppm ClO_2 (5 min for strawberries) combined with UV-C irradiation (10 kJ m^{-2} and 5 kJ m^{-2} respectively). Similarly, the color (Hunter L, a, b) of stored buckwheat sprouts was not affected after ClO_2 treatment with 100 mg L^{-1} (5 min) in combination with 0.3% fumaric acid followed by UV-C irradiation (12 W m^{-2}) (Chun and Song, 2013). Aqueous treatment with 15 ppm ClO_2 for up to 2 h did not negatively influence the visual quality of blueberries (Wu and Kim, 2007).

Treatments with high ClO_2 concentrations, however, may negatively affect the visual quality of various horticultural produce. Treatments of both romaine and iceberg lettuce with high concentration of ClO_2 such as 200 ppm chlorite ion (Tri Nova) for 2 min resulted in noticeable discoloration of leaf samples (Keskinen et al., 2009). The reasons for the color changes are not clear. In addition, white blushing of mulberries after treating fruit with 80 mg L^{-1} ClO_2 for 15 min was more pronounced than after ClO_2 treatment at 60 mg L^{-1} for 15 min (Chen et al., 2011).

The authors assumed that ClO_2 may enhance white blushing by oxidation of different oligosaccharides such as cellulose and hemicellulose.

Treatment of strawberries with ClO_2 at 6 mg L^{-1} (5 min) alone or combined with ultrasonication (20 kHz, 30 W) caused better maintenance of L^* and a^* values during 4 week storage indicating an increased anthocyanin stability (Aday et al., 2013; Aday and Caner, 2014). A combined treatment of 5 kJ m^{-2} UV-C and 50 ppm aqueous ClO_2 (10 min) as well as UV-C treatment alone prevented the loss of lightness (L-value) and delayed the reddish-brown discoloration of shredded yams during storage for 10 d (Chun et al., 2013b). This effect is assumed to be due to the inhibition of microbial growth and of the enzymes responsible for browning. After treatment with 100 ppm ClO_2 (5 min), the visual quality of mungbean sprouts, stored in different atmospheres, was better than after washing in water (Jin and Lee 2007).

ClO_2 was also shown to inhibit enzymatic browning of fresh-cut products due to reduced activity of PPO. Browning and PPO activities were strongly inhibited in fresh-cut lotus root by treating them with 100 mg L^{-1} ClO_2 for 10 min (Du et al., 2009). The authors supposed that amino acids and/or disulfide bonds that are involved in the active site in the PPO are oxidized by ClO_2. Decreased PPO activities and, thus, reduced browning was also found after aqueous ClO_2 treatments of minimally processed lettuce (Youm et al., 2004), fresh-cut asparagus lettuce (Chen et al., 2010), fresh-cut few-flower wildrice (Liu et al., 2010) and apples (Fu et al., 2007).

Mass loss and firmness

Application of aqueous ClO_2 may also influence (either positively or otherwise) the preservation of other important parameters of fresh food quality such as mass loss and firmness.

In strawberries, mass losses during 7 d of storage were delayed after 50 ppm ClO_2 treatment (Jin et al., 2007). On the other hand, Chun et al. (2013b) did not find any effects of 50 ppm ClO_2 treatment, alone or in combination with 5 kJ m^2 UV-C on the mass of shredded yam during 10 d storage.

Similar to mass, ClO_2 treatment also retarded the loss of firmness of fresh strawberries throughout MAP storage, regardless of the difference in the tested concentrations of 3, 6 or 9 ppm. Again, the combined treatment of ClO_2 (6 mg L^{-1}, 5 min) and ultrasound (20 kHz, 30 W) was even more beneficial. ClO_2 may inhibit the pectin-degrading enzymes (Aday et al., 2013; Aday and Caner, 2014). Retardation of fruit softening was also found after aqueous ClO_2 treatment of plums (Chen and Zhu, 2011). In contrast, aqueous treatment with 100 mg L^{-1} ClO_2 inhibited the increases of toughness and the contents of lignin and cellulose during storage of fresh-cut few-flower wildrice (Liu et al., 2010). Delayed lignification of few-flower wildrice was attributed to changed enzyme activities, mainly the inhibition of phenylalanine ammonia lyase (PAL). Still unknown, however, is the mode of action of sanitizers such as ClO_2 on the activities of relevant enzyme.

Respiration rate

Treating strawberries with aqueous ClO_2 in a concentration range of 3 to 9 ppm for 5 min reduced the respiration rates of the fruit during MAP storage (Aday et al., 2013). The effect was further enhanced, when the treatment with 6 mg L^{-1} ClO_2 (5 min) and ultrasound (20 kHz, 30 W) were combined (Aday and Caner, 2014). Also in fruit of plum cultivars, which were characterized by climacteric respiration patterns, ClO_2 treatment (20 or 40 mg L^{-1}, 5 and 10 min)

reduced respiratory activity during storage (Chen and Zhu, 2011). In contrast, treatment of fresh-cut iceberg lettuce with aqueous ClO_2 of 3 mg L^{-1} (1 min) did not alter respiration rates in MAP storage as reflected by similar gas composition inside the packages compared to water-washed lettuce (Lopez-Galvez et al., 2010a).

Nutritional compounds

ClO_2 is an effective oxidant; hence, nutritional compounds such as ascorbic acid and phenolics e.g. flavonoids may readily be oxidized by ClO_2 (Gomez-Lopez et al., 2009). Nevertheless, several authors report about higher stability of compounds such as acids after ClO_2 treatment than after washing in tap water. An aqueous ClO_2 treatment of 3 to 9 ppm (5 min) slowed down the decrease of titratable acidity of strawberries stored in MA packages (Aday et al., 2013). Similar finding were reported for mulberries washed with 60 or 80 mg L^{-1} ClO_2 solution up to 15 min (Chen et al., 2011) and for plums after aqueous ClO_2 treatment at 40 mg L^{-1}, 10 min (Chen and Zhu 2011). In contrast, Shin et al. (2012) and Jin et al. (2007) did not observe a retarded decrease of titratable acidity in strawberries after ClO_2 treatment at 50 ppm (up to 5 min).

The vitamin C content of fresh-cut iceberg lettuce decreased after washing. However, this occurred irrespective washing the samples in tap water or in sanitation solution with ClO_2 at 3 mg L^{-1} for 1 min (Lopez-Galvez et al., 2010a). Directly after treatment with 60 or 80 mg L^{-1} ClO_2 (5, 10,15 min) and during following 6 d, the ascorbic acid content of mulberries was lower than in tap water-washed fruit. At the same time, the ClO_2 treated fruit better retained ascorbic acid content during 14 d of storage (Chen et al., 2011). In fresh-cut romaine samples, the vitamin

C content was not at all affected by ClO_2 treatments (up to 30 mg L^{-1}, 2 min) (Hassenberg et al., 2014).

In strawberries, decrease in total soluble solids (TSS) was small during 4 week storage in MAP; TSS was, however, even better maintained when fruits were treated with aqueous ClO_2 (3 to 9 ppm) for 5 min (Aday et al., 2013) and after combined treatment with ClO_2 (6 mg L^{-1}, 5 min) and ultrasound (20 kHz, 30 W) (Aday and Caner, 2014). These authors attributed this effect to the retardation of respiration activity frequently observed after ClO_2 treatments (see above). Changes in TSS closely reflect variations in content of free sugars. Washing of produce with aqueous ClO_2 should, consequently, retard the metabolic consumption of reducing sugars. This was, indeed, reported for plums treated with ClO_2 solutions at a concentration of 40 mg L^{-1} for10 min (Chen and Zhu, 2011). Again, this effect of ClO_2 washing was more pronounced when combined with ultrasonication. Similarly, retention of reducing sugars was found in stored mulberry after treatment with 60 or 80 mg L^{-1} ClO_2 solutions (5-15 min), relative to untreated controls (Chen et al., 2011).

Additionally, phenolic compounds are generally seen as important nutritional and potentially health promoting components in F&V (Schreiner and Huyskens-Keil, 2006; Eichholz et al., 2012). Phenolic substances are often involved in stress responses of plants (Dixon and Paiva, 1995). Hence, it is reasonable to assume that ClO_2 treatments may have some effects on these compounds. However, Lopez-Galvez et al. (2010a), investigating the influence of aqueous ClO_2 treatment at 3 mg L^{-1} for1 min on fresh-cut iceberg lettuce, found no significant changes in the contents of total phenolics, phenolic acids, flavonoles and flavones after washing or during MAP storage for up to 10 d. A lack of effects of ClO_2 washing (10 min), even at high concentrations

(100 ppm), on the anthocyanin content was also reported for blueberries, stored at 20°C after treatments (Chun et al., 2013a). Nevertheless, anthocyanins content of treated blueberries was better maintained when stored at low temperatures (4°C). For strawberries, Aday et al. (2013) attributed delayed changes of a* color values during storage after ClO_2 washing (3 to 9 ppm) to a treatment-related increase in anthocyanin stability. Furthermore, the decrease of total flavonoids during storage of plums was retarded after aqueous ClO_2 treatment (40 mg L^{-1} ClO_2, 10 min). This positive effect was further amplified in the combination of ClO_2 and ultrasonic treatment (100 W, 10 min) (Chen and Zhu 2011). Also the decrease of flavonoids in mulberry fruit treated with ClO_2 (60 or 80 mg L^{-1}, 10- 15 min) during 14 d storage was significantly slowed down compared to fruit washed in tap water (Chen et al. 2011).

As a result of the application of a mixture of 100 mg L^{-1} ClO_2 and 0.3 % fumaric acid for 5 min combined with UV-C irradiation (12 W m^{-2}), the rutin content of buckwheat sprouts increased (Chun and Song, 2013). However, there was no significant variation in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity between samples of the different treatments. The mechanisms behind the treatment effects are still not fully clear. However, it is probable that the increase in rutin content might be related to a stress response mostly to UV-C irradiation (Chun and Song, 2013).

Summarizing the available results it seems highly probable that potential negative effects of ClO_2 on nutrients may be limited to fresh-cut products rather than to whole intact produce. In lightly processed produce, the resistance against penetration of ClO_2 molecules into plant tissues might be much lower as a result of the more cutting-induced wounding, this in turn largely facilitates the reactions of ClO_2 with the various nutritional substances (Chen and Zhu, 2011).

Other quality criteria

A general stabilizing effect of ClO_2 treatment on the overall integrity of plant tissue and, concomitant, on plant metabolism has been indicated by the investigations of Aday and Caner (2014) on strawberries. The authors reported a much less pronounce increase in electrical conductivity during storage in the tissue of fruit treated with ClO_2 (6 mg L^{-1}, 5 min) alone or in combination with ultrasound (20 kHz, 30 W) than in untreated controls. According to Aday and Caner (2014), this might be due to a treatment related stabilization of cell membrane integrity and the prevention of structural changes of cellular components because of reduced respiration and metabolism activity compared to the untreated control. In this context, Fourier transform near-infrared (FT-NIR) spectroscopy non-destructively provided comprehensive information about water and sugar contents of the strawberries (Aday et al., 2013). In similar experiments with the same material, Aday and Caner (2014) found a good correlation between FT-NIR spectra and textural properties of stored strawberries. Spectra of fruits treated with ClO_2 (6 mg L^{-1}, 5 min) and ultrasound (20 kHz, 30 W) were more similar to spectra of fresh fruit than to untreated stored fruit. This effect was attributed to reduced carbohydrate metabolism or inactivation of enzymes responsible for depolymerization of cell walls after the treatment.

Aqueous ClO_2 treatment has also been tested for the removal of pesticides from the surface of fresh produce and in aqueous solution. Added in tap water, ClO_2 (20 mg L^{-1}, 5-20 min) washing significantly enhanced the removal of phorate and diazinon from lettuce leaves if compared to use of only tap water (Chen et al., 2014). In addition, ClO_2 treatment (10 ppm, 15 min) of whole and sliced apples strongly decreased there content on mancozeb and ethylenethiourea residues (Hwang et al., 2002).

Although only few reports are available, it seems reasonable to assume that aqueous ClO_2 treatment does not result in lasting chemical residues in fresh produce. Scientific evidence for this hypothesis was provided by Chen and Zhu (2011). In their study, these authors found no residues of ClO_2, ClO_2^-, and ClO_3^- in plums after combined treatment with ClO_2 (40 mg L^{-1} ClO_2, 10 min) and ultrasound (100 W, 10 min). In addition, in fresh-cut iceberg lettuce, the trihalomethane concentration was well below detection limits of 5 µg L^{-1} after treatment for 30 min with ClO_2 (3.7 mg L^{-1}) solved in process water with a chemical oxygen demand of 700 mg L^{-1} (Lopez-Galvez et al., 2010a). Also in samples of mulberries, no ClO_2, ClO_2^-, or ClO_3^- residues could be detected after treatment of fruit with 60 mg L^{-1} ClO_2 for 15 min (Chen et al. 2011).

CONCLUSIONS

During the last 20 years, application of aqueous ClO_2 in a wide range of concentrations has been studied as alternative sanitation agent for chlorine in various commodities, based on the fact that ClO_2 is effective as bactericide, virucide and fungicide. Under laboratory conditions, log reductions up to 3 to 4 of native or inoculated microorganisms on produce surfaces have been achieved. However, this effect could often only be obtained with high ($\geq$ 50 ppm) ClO_2 concentrations. Higher reductions were found by increasing treatment time. Though, contact times up to one or two hours seems not to be of practical relevance. Also, for practical application the respective regulations for the use of sanitation agents must be taken into consideration.

The reason for some inconsistency in the results published about the true ClO_2 efficacy in different publications might be due to numerous factors potentially influencing sanitizing effectiveness, as the surface texture and the processing status (whole or cut) of produce. In addition to ClO_2 concentrations and time of treatment, these factors may also include the specific resistance of the respective microorganisms, the method of inoculation used, and the residence time of the inoculum on the produce. Other important influencing parameters are treatment temperature and organic matter in the wash water. High organic matter content resulted in an increased demand of ClO_2, since ClO_2 reacts first with organic matter and afterwards with microorganisms. Therefore, the assessment of the increased ClO_2 demand is very important for a successful decontamination in practice. However, most studies evaluate the sanitation effects of ClO_2 under laboratory conditions and based on tap water systems. Hence, more investigations are desirable, which analyze the effects of ClO_2 treatments under practical conditions with common cooling temperature and practically relevant contents of organic matter in the water.

The combination of aqueous ClO_2 treatment with other sanitation techniques frequently increased the disinfection efficacy due to additive effects. Nevertheless, a consecutive application of additional agents may also enhance the effect of the ClO_2 treatment.

Investigations with focus on the impact of ClO_2 treatment on produce quality evaluated primary visual and sensory quality by standard color measurements and sensory test panels, respectively. Most authors did not find negative effects on produce like pak choi, red chicory, cabbage and carrots after ClO_2 treatment and during storage. However, results of iceberg lettuce treatment were inconsistent. Produce quality may be influenced by ClO_2 treatments in very different, direct and also indirect manners. An obvious indirect effect is that the reduction of

microbial loads by ClO_2 treatment decreases the probability of decay and increase shelf life of the produce. On the other hand, ClO_2 may directly influence produce quality through oxidation of relevant compounds such as organic acids or by its impact on activity of various enzymes. This include the inhibition of PPO resulting in the inhibition of enzymatic browning of fresh-cut products. Though, much more research is needed to evaluate the mode of ClO_2 action(s) at the cellular level.

In summary, aqueous ClO_2 application is a suitable method for horticultural produce treatment to maintain product quality and increase shelf life. However, the effect of ClO_2 on microorganisms on the produce surface is limited, but ClO_2 application is very effective in solution and prevents cross-contamination in washing processes. The optimal ClO_2 concentration and treatment time depend primary on the type of produce, the processing status (whole or cut) and the load of organic matter in washing water. For a successful application of ClO_2 in washing processes it is essential to determine the optimal process parameters, so that the best disinfection will result without negative effects on produce quality.

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Table1 Summary of reports on the short- and long-term effectiveness of sanitation of various horticultural products with aqueous ClO_2 at different concentrations

Produce	**Microorganism**	**Inoculation**	**Drying**	**ClO_2**	**Treatment**		**Storage**	**log-Reduction**	**References**
		Method	Time, Temp.	Concentration	Time	Temp.	Time, Temp.	**(**highest vs. initial conc.)	
fresh-cut lettuce	*L. monocytogenes*	sprinkle	-	1, 2, 3, 5 ppm	10 min	4°C22°C	-	1.10.8 (compared to tap water)	Zhang and Farber, 1996
fresh-cut cabbage	*L. monocytogenes*	sprinkle	-	1, 2, 3, 5 ppm	10 min	4°C22°C	-	0.40.8 (compared to tap water)	
fresh-cut iceberg lettuce	total aerobic bact., yeasts and molds, coliforms			0, 5, 10, 50 ppm	10 min		8d, 4°C	1.77 1.34 1.10	Kim et al., 2007
fresh-cut	*E. coli*	-	30	0, 5, 10,	10		4d,	2.9	Kim et

iceberg lettuce	O157:H7 *L. monocytogenes* *S. typhimurium*		min	50 ppm	min		4°C	2.3 3.4	al., 2008
fresh-cut iceberg lettuce	*E. coli* O157:H7	dip, 5 min	2h, 22° C	20, 100, 200 ppm	2 min	22°C	-	1.06 1.11 1.45	Keskinen et al., 2009
fresh cut romaine lettuce				20, 100, 200 ppm	2 min	22°C	-	0.44 0.55 0.38	
fresh-cut romaine lettuce	*E. coli* O157:H7	drop	1h, 22° C	10 mg L^{-1}	10 min	22°C	-	3.9	Singh et al., 2002b
fresh-cut iceberg lettuce	mesophiles, psychrophiles, *Pseudomonas* spp.,			3 mg L^{-1}	1 min	4°C	3 d, 4°C + 7d, 7°C	1-2	Lopez-Galvez et al., 2010a

	Enterobacteriaceae, lactic acid bacteria, yeasts and molds								
fresh-cut asparagus lettuce	aerobic mesophilic, aerobic psychro-troph., lactic acid bacteria, yeasts and molds			10, 40, and 100 mg L^{-1}	5, 10, 20 min	-	14d, 4°C	3.3 3.3 1.2 1.6	Chen et al., 2010
fresh-cut iceberg lettuce	*E. coli*	immersed, 15 min	12h, 4°C	3 mg L^{-1}	1 min	4°C	3d, 4°C + 4d, 7°C	0.5	Lopez-Galvez et al., 2010b
carroticeberg	total aerobic			5, 10, 20 mg L^{-1}	1, 5,	26°C		>1 (carrots) no reduction	Gomez-Lopez

lettucecabbage	plate count				10, 20 min			(iceberg lettuce/ cabbage)	et al., 2008
Cucumber (for brining)	lactic acid bacteria,yeast and molds,total microb. counts			25, 105 ppm	15 min	5°C	4d, 10° C 4d, 22° C	marginal effect on the numbers of microorganismsabout 1.5	Costilow et al., 1984
pickling cucumber	total aerobic bact.,total Enterobacteriaceae			0.95, 5.1 ppm	17 min	6°C	6d, 10-12° C	about 1.5	Reina et al., 1995
green pepper	*L. monocytogenes*	drop	2h 22° C	0.3, 3 mg L^{-1}	10 min	20°C		0.44 (injur. pepper)3.67 (uninjured pepper)	Han et al., 2001
Mizuna, Tatsoi and red	*E. coli* 0157:H7	spray		3 mg L^{-1}	90 s	-	7d, 5°C	< 1	Tomas-Calleja

chard baby leaves									s et al., 2011
fresh-cut red chard	*E. coli* 0157:H7, *S. enterica*	submerged, 1 min	12h 15°C	3 mg L^{-1}	1 min	20°C		< 1 1.5	Tomas-Callejas et al., 2012
spinach	*E. coli* O157:H7			100 ppm	5 min	22°C	7d, 7°C	2.6	Lee and Baek, 2008
tomatoes	*S. enterica*, *E. carotovora*	spot	0h or 2h, 25°C and 22h, 23°C	5,10, 20 ppm,	1 min	ambient temp.		1-2 3-5 5	Pao et al., 2007
apples,	*E. coli*	dip, 20	18-	3, 5	5	21-	9d,	5-6	Rodge

lettuce, strawberries, cantaloupe	O157:H7, *L. monocytogenes*	min	24h, 24°C	ppm	min	23°C	4°C		rs et al., 2004
strawberries	total aerobic bact., yeasts, molds			5, 10, 50 ppm	2 min	-	7d, 4°C	1.35 1.45	Jin et al., 2007
blueberries	*L. monocytogenes*, *P. aeruginosa*, . *typhimurium*, *S. aureus*, *Y. enterocolitica*, yeasts and molds	spot	2h	1, 3, 5, 10, 15 ppm	10 s; 1 min - 2h	21°C	-	4.88(15 ppm 2h) 2.16(15 ppm 15 min) 3.32(15 ppm 20 min) 4.56(15 ppm 30 min) 3.49(5 ppm 2h) 2.82(15 ppm 1h)	Wu and Kim, 2007

blueberries	total aerobic bact., yeasts, molds			100 ppm	10 min	-	12 d4°C, 20°C	1.4-1.59 0.8-0.9	Chun et al., 2013a
oranges	*E. coli*	immersion, 15 min	2h, 20°C	100 ppm	8 min	30°C		3 (non stem scar area)1 (stem scar area)	Pao and Davis, 1999
mulberry fruit	aerobic mesophilic, aerobic psychro-trophic,lactic acid bacteria yeasts and molds			20, 60, 80 mg L^{-1}	5, 10, 15 min	22°C	14 d, -1°C	2.4- 2.8 2.4 - 2.5 1.4-1.5 1.0-1.1	Chen et al., 2011
chestnut	mold, decay			10 ppm	3 min	-	60 d, 4°C	visual mold evaluation	Donis-Gonzales, 2008

mungbean sprouts	total mesophilic microorganisms,*S. typhimurium*, *L. monocytogenes*	immersion, 5 min	1h	100 ppm	5 min	22°C	7d, 5°C	0.73 1.5	Jin and Lee, 2007
alfalfa seeds	*E. coli* O157:H7	seeds placed in suspension, 1 min	48h, 23°C	20, 50, 100, 200, 500 ppm	3, 10 min	23°C	1 week, 5, 25,37°C 38 weeks, 5°C	2.4	Taormina and Beuchat, 1999
radish seeds	*Cronobacter* spp.	seeds placed in suspension, 5	2h	50, 100 µL mL^{-1}	5 min	20°C	4d, 25°C	3.6	Kim et al., 2013

		min							

Table 2 Combined postharvest treatments with aqueous ClO_2 of horticultural products

Produce	Microorganism	Inoculation method	drying	ClO_2 conc.,Treatment time	Additional treatment	log reduction combined method	References
fresh-cut romaine lettuce baby carrots	*E. coli*	sprinkle, 3min	1h, 22°C	10 mg 10 min	ozonated water, thyme oil	3-4	Singh et al., 2002a
apple	*Salmonella*, *E. coli*	spreading the suspension	40 min, 20°C	5, - 40 ppm 3, 6, 10 min	ultrasonication	3.12 to 4.25 2.24 to 3.87	Huang et al., 2006
lettuce		dip, 2min	1h, 20°C			2.26 to 2.97 1.36 to 2.26	
red chicory	total aerobic bacteria yeast, fungi			50 ppm	UV-C	2.64 2.41	Kim et al., 2011
pak choi						2.55 2.00	
plum	aerobic mesoph. bact. aerobic psychrotrophic bacteria yeasts and molds			40 mg L^{-1} 10 min	ultrasonic waves	2.4-3.1 2.3-3 1.7-2.3	Chen and Zhu, 2011
strawberry	total aerobic bacteria, yeast, molds			50 mg L^{-1} 5 min	UV-C irradiation	2.15 1.85	Kim et al., 2010
strawberry	total aerobic bacteria yeast and molds			50 ppm 5 min	UV-C, packed with rice bran protein (RBP) film with GSE	1.51 1.89	Shin et al., 2012
broccoli sprouts	total aerobic bact. yeasts and molds, coliforms, *E.*	dip, 5 min	30 min	50 ppm 5 min	fumaric acid	2.70 2.46 1.71 2.39	Kim et al., 2009a

	coli, S. typhimurium, L. monocytogenes					2.74 2.65	
alfalfa sprouts	total aerobic bact. *E. coli*, *S. typhimurium*, *L. monocytogenes*	immersion, 5 min	30 min	50 mg L^{-1} 10 min	fumaric acid	3.18 4.06 3.57 3.69	Kim et al., 2009b
buckwheat sprouts	*E. coli*, *S. typhimurium*, aerobic mesoph. bact., yeasts and molds coliforms			100 mg L^{-1}5 min	UV-C, fumaric acid	3.0 2.3 1.8 1.9 1.4	Chun and Song, 2013
alfalfa seeds	*E. coli*			25 mg L^{-1} 5 min	ozonated water, thyme oil	3.4-4.2	Singh et al., 2003
yam	total aerobic bacteria, yeast and mold coliform bacteria			50 ppm 10 min	UV-C	3.2 3.4 3.8	Chun et al., 2013b