

Hydrogen Peroxide Disinfection of Minimally Processed Fruits and Vegetables

Hydrogen peroxide treatments are as effective as chlorine in reducing microbial load and extending shelf life of fresh-cut fruits and vegetables

GERALD M.
SAPERS AND
GILBERT F.
SIMMONS

Author Sapers, a Professional Member of IFT, and author Simmons are respectively, Research Food Technologist at the Eastern Regional Research Center, Wyndmoor, PA 19038, and Research Associate at the Horticultural Crops Research Laboratory, Fresno, CA 93727, both laboratories of the Agricultural Research Service, U.S. Dept. of Agriculture. Send reprint requests to author Sapers.

Fresh-cut fruits and vegetables, a large and rapidly growing segment of the fresh produce industry, are subject to rapid spoilage by microorganisms (Nguyen-the and Carlin, 1994) and may be a potential source of infection by foodborne pathogens such as *Salmonella* and *Listeria* (Brackett, 1992; Fain, 1996).

Chlorine is widely used to sanitize fresh-cut fruits and vegetables. However, its effectiveness is limited with some products, e.g., suppressing growth of *Listeria monocytogenes* in shredded lettuce (Beuchat and Brackett, 1990) or completely eliminating *Salmonella montevideo* from inoculated tomatoes (Zhuang et al., 1995; Wei et al., 1995). Furthermore, some food constituents may react with chlorine to form potentially toxic reaction products (Richardson, 1994). Consequently, the safety of chlorine use for food or water treatment has been questioned, and future regulatory restrictions may require the development of alternatives (Hurst, 1995). Thus, improved means of sanitizing fresh-cut fruits and vegetables are needed.

Other researchers have looked at treatments with chlorine dioxide (Roberts and Reymond, 1994), ozone (Hampson and Fiori, 1994; Montecalvo et al., 1995), or trisodium phosphate (Zhuang and Beuchat, 1996) as alternatives to use of chlorine. We have investigated uses of hydrogen peroxide (H_2O_2) as a vapor treatment or wash for various fresh-cut fruits and vegetables.

Food Applications of H_2O_2

The antimicrobial properties of H_2O_2 have long been recognized (Block, 1991). Dilute H_2O_2 is used as a topical disinfectant and is available as a consumer product. H_2O_2 vapor shows promise as a sterilizing agent for medical equipment and supplies (Klapes and Vesley, 1990) and for aseptic packaging systems and packaging materials (Wang and Toledo, 1986).

H_2O_2 is classified as generally recognized as safe

(GRAS) for use in food products as a bleaching agent, oxidizing and reducing agent, and antimicrobial agent. Three antimicrobial applications are approved by the Food and Drug Administration: treatment of milk for use in cheese, preparation of modified whey, and preparation of thermophile-free starch. For these and other food applications, the FDA regulation specifies use levels and requires that residual H_2O_2 be removed by appropriate physical and chemical means during processing.

Various experimental antimicrobial applications of H_2O_2 for foods have been described, including preservation of fresh vegetables and fruits (Honnay, 1988), control of postharvest decay in table grapes (Forney et al., 1991; Rij and Forney, 1995), washing of fresh mushrooms (McConnell, 1991; Sapers et al., 1994), and preservation of salad vegetables, berries, and fresh-cut melons (Sapers et al., 1995). As discussed below, H_2O_2 vapor treatments have reduced microbial populations in cantaloupe, raisins, prunes, and walnut meat.

H_2O_2 Vapor Treatments

In studies conducted at the USDA/ARS Horticultural Crops Research Laboratory in Fresno, Calif., table grapes that had been inoculated with *Botrytis cinerea* spores were exposed to the vapor in equilibrium with 30–35% H_2O_2 at 40°C for 10 min. This treatment significantly reduced the number of germinable spores and also reduced the incidence of decay (Forney et al., 1991). In a subsequent study, Rij and Forney (1995) reported that exposure to vapor-phase H_2O_2 at a concentration of 0.27 mg/L was effective in killing *Botrytis* spores without causing visible injury to treated grapes. Higher H_2O_2 vapor concentrations induced browning.

Simmons and coworkers at the Fresno laboratory carried out comprehensive studies of H_2O_2 vapor treatments for various fresh commodities (Simmons, 1996). They used an AMSCO VHP 1000 Biodecontamination System (American Sterilizer Co., Apex, N.C.) as the H_2O_2 vapor source and a 30-ft³ treatment chamber at ambient temperature (Fig. 1). Cantaloupe, intended for fresh-cut,

should be given a thorough surface disinfection treatment to reduce the population of potential spoilage organisms and pathogens. This is done conventionally by washing with hypochlorite solution. As an

used as part of hurdle technology if prunes were to be packed aseptically.

Conventionally packed raisins may have mold counts as high as 72,000 cfu/g. Simmons et al. (1995) found that exposure of raisins to H_2O_2 vapor for 60 min reduced mold counts to 0 in 17 of 20 cases. Thus, H_2O_2 vapor treatment may be a promising technology for disinfection of raisins.

Walnut nut meats are currently fumigated with propylene oxide to reduce microbial populations to acceptable levels. However, the future use of this treatment is in doubt because of recent regulatory action. H_2O_2 vapor treatments show promise as a possible substitute for propylene oxide in disinfecting walnut meat. The vapor treatments reduced total plate counts by as much as 95% (Simmons, 1996).

Sapers et al. (1995) explored use of H_2O_2 vapor to extend the shelf life of various other fresh and fresh-cut commodities using the AMSCO Biodecontamination System. Our primary goal in these preliminary trials was to determine whether spoilage could be suppressed by H_2O_2 exposure without damage to product quality. Samples were exposed to H_2O_2 vapor for 2–15 min at injection rates of 2.5 or 5 g of H_2O_2 /min. Following treatment, samples were packaged in plastic boxes

with a perforated polyvinyl chloride film overwrap and observed for indications of spoilage or adverse reactions to treatment during storage at 7.5°C.

H_2O_2 vapor treatments appeared to delay or diminish the severity of bacterial soft rot in fresh-cut cucumber, green bell pepper, and zucchini but had no effect on spoilage of fresh-cut broccoli, carrot, cauliflower, or celery or fresh raspberries and strawberries. The only indications of treatment-induced injury were severe brown-

ing of mushrooms and bleaching of anthocyanins in some raspberry drupelets and strawberries. Bleaching was more severe with longer exposure times and at the higher H_2O_2 injection rate.

Use in Mushroom Washing

Washing predisposes mushrooms to premature spoilage due to the development of a brown or purple blotch during storage, caused by growth of *Pseudomonas tolaasii* (Guthrie and Beelman, 1989;

Rainey et al., 1992). This defect can be controlled but not eliminated by addition of chlorine or sulfite to wash water (Beelman et al., 1989; Guthrie and Beelman, 1989), and exposure of mushrooms to bactericidal concentrations of chlorine may induce darkening (Sapers et al., 1994; Choi and Sapers, 1994). Mushrooms might be washed successfully if the bacterial population could be lowered by a surface pasteurization treatment with H_2O_2 prior to washing.

Our first approach was to apply an H_2O_2 vapor treatment, similar to that developed at the Fresno laboratory for treating grapes (Rij and Forney, 1995). Exposure of mushrooms, previously inoculated with *P. tolaasii*, to H_2O_2 vapor suppressed lesion formation for 4–6 days at 4°C, even at inoculum levels as high as 10^9 bacterial cells/mushroom. However, with this system, an exposure time of 45–60 min was required, and prolonged

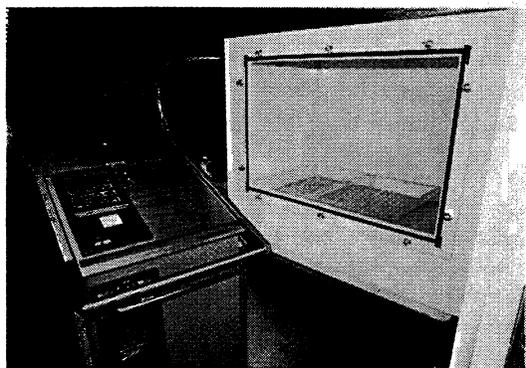


Fig. 1—AMSCO VHP 1000 Biodecontamination System and 30-ft³ treatment chamber (ARS design)

alternative treatment, exposure of whole cantaloupes to H_2O_2 vapor at a concentration of 3 mg/L of air for 60 min was effective in reducing microbial counts and preventing decay during storage at 2°C for 4 weeks without injuring the melons (Fig. 2) (Simmons, 1996).

Prunes may be hydrated to a moisture content exceeding 30% to improve palatability, and potassium sorbate may be added to retard spoilage. The domestic prune industry would like to have an

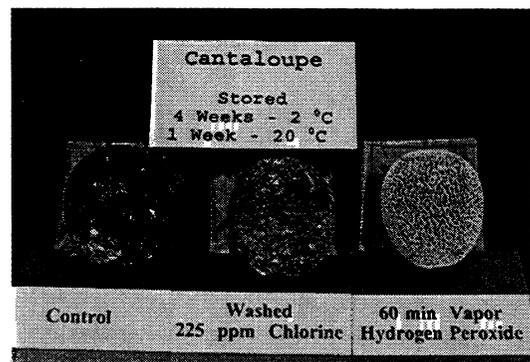


Fig. 2—Control of decay in cantaloupe by H_2O_2 vapor treatment

alternative to potassium sorbate for use with prunes intended for export. Simmons et al. (1997) found that exposure of dry prunes to H_2O_2 vapor at a concentration of 3 mg/L of air for 10 min greatly reduced the microbial population. H_2O_2 residues could not be detected after 20 days of storage, except when longer exposure times were used. However, some bleaching and blistering were observed in prunes treated for 20 min or longer. The vapor treatment might be



Fig. 3—Washing mushrooms in 5% H_2O_2

exposure to H_2O_2 vapor resulted in browning of the treated mushrooms. Browning could be controlled by application of a browning inhibitor dip containing sodium erythorbate immediately following exposure. The dip also served as a wash to remove adhering soil from mushroom surfaces. Further experiments were carried out in Fresno with the AMSCO Biodecontamination System. Treatment times needed for suppression of lesion formation were still long, and these treatments induced severe browning, even when browning inhibitor dips were used.

Washing of mushrooms with dilute H_2O_2 solutions (Fig. 3) was investigated as an alternative to H_2O_2 vapor treatments (Sapers et al., 1994). Since mushrooms were exposed to 5% H_2O_2 for only 30 sec, this treatment would be more compatible with a commercial mushroom washing operation than the slower vapor exposure treatment. The H_2O_2 wash was followed

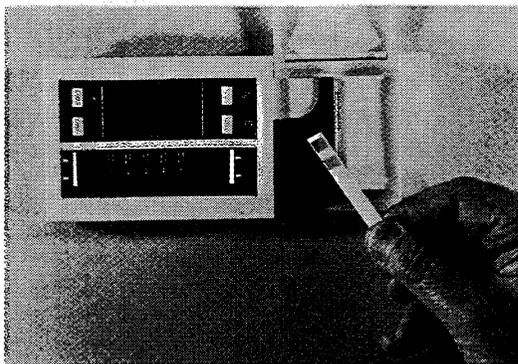


Fig. 4—Detection of H₂O₂ residues with the Reflectoquant system

guard to product quality and compliance with FDA regulations), and occurrence of treatment-induced injury to the product such as that seen with H₂O₂ vapor treatments.

Vigorous gas evolution was observed when shredded cabbage, carrot sticks, celery sticks, diced green bell pepper, shredded lettuce, peeled potato, and sliced zucchini were dipped in H₂O₂ solution. However, little or no gas

by a dip in erythorbate solution to control browning. The H₂O₂ wash treatment was found to be highly effective in suppressing bacterial blotch development (Table 1). Good control of bacterial blotch and browning was achieved with a mushroom wash formulation containing 1% H₂O₂ and 1,000 ppm of EDTA (McConnell, 1991).

Because H₂O₂ residues would have to be removed from treated mushrooms to comply with FDA regulations, we tested for the presence of residues by two sensitive methods: the Reflectoquant System (EM Science, Gibbstown, N.J.), which measures color development on a paper indicator strip applied to a solution or product surface (Fig. 4), and the TOOS-4AA qualitative color test of Miyamoto et al. (1993). The limits of detection are 0.2 ppm of H₂O₂ for the paper strip and 0.1 ppm of H₂O₂ for the TOOS-4AA test. Within a few minutes after treatment, we were unable to detect significant H₂O₂ residues in the treated mushrooms by either test procedure. This is a consequence of two reactions: the rapid decomposition of residual H₂O₂ by endogenous catalase, and the reduction of H₂O₂ by erythorbate in the browning inhibitor dip.

The effectiveness of the H₂O₂ dip treatment depends on two factors. One is the lethality of H₂O₂ to bacteria on mushroom surfaces or washed into the

H₂O₂ solution. The other is enhancement of soil removal from mushroom surfaces by the mechanical action of many small oxygen bubbles, produced at mushroom surfaces by the catalase-H₂O₂ reaction, which dislodges both soil particles and loosely attached microorganisms from mushroom surfaces.

With support from a consortium of mushroom packers, we have scaled up the H₂O₂ washing process for fresh mushrooms and made it continuous. This process is now undergoing evaluation to determine operating costs under conditions simulating commercial washing.

Treatment of Fresh-Cut Produce with H₂O₂ Solution

Because of the effectiveness of H₂O₂ washing treatments for fresh mushrooms, we investigated use of H₂O₂ solutions as an alternative to chlorine for disinfection of fresh-cut fruits and vegetables. Such treatments would be faster, easier to apply commercially, and easier to control than exposure to H₂O₂ vapor.

Various commodities, both whole and pre-cut, were screened to determine their response to immersion in 5 or 10% H₂O₂

evolution occurred with broccoli and cauliflower florets or with whole cherry tomatoes. The latter vegetables might be expected to contain significant H₂O₂ after exposure if not treated to remove residual H₂O₂. In this experiment, exposure to H₂O₂ had little effect on the appearance of most vegetables. Only shredded lettuce showed severe browning, the same H₂O₂-induced injury seen with mushrooms. However, use of a browning inhibitor treatment probably would not be feasible with lettuce. Unpeeled potatoes showed slight bleaching of the skin, a potential benefit.

Apple and pear wedges, sweet cherries, raspberries, and strawberries showed little or no gas evolution, indicating low catalase activity and the possibility of an H₂O₂ residue problem. Bleaching of anthocyanins was seen in H₂O₂-treated raspberries and strawberries, the extent of bleaching being greater at higher H₂O₂ concentrations and with longer exposure times. Such bleaching, as well as mechanical injury sustained during treatment and dewatering, may make H₂O₂ treatments infeasible for these fruits.

H₂O₂ Residues

Residual H₂O₂ in treated fruits and vegetables might be eliminated passively by the action of endogenous catalase, given sufficient time for reaction, or actively by rinsing immediately after treatment to avoid reactions between H₂O₂ and food constituents that might affect product quality or safety. We compared the passive approach with a water rinse or 1-min dip in 1% sodium erythorbate, determining residual H₂O₂ with EM test strips and by the TOOS-4AA color test (Table 2).

Mushrooms given the H₂O₂ treatment and browning inhibitor rinse contained less than 0.1 ppm of H₂O₂ within 5 min of treatment. Cucumber cross cuts, given no rinse, showed large H₂O₂ residues even 1 or 2 hr after treatment. However, H₂O₂ residues could not be de-

Table 1 Effect of H₂O₂ dip on lesion development in mushrooms inoculated with *Pseudomonas tolaasii*

Inoculum level ^a	Treatment	No. of mushrooms with bacterial blotch lesion ^b		
		Day 3	Day 6	Day 10
0	Control	0/3	0/3	0/3
	H ₂ O ₂ ^c	0/3	0/3	0/3
10 ⁶	Control	0/3	0/3	3/3
	H ₂ O ₂	0/3	0/3	3±/3
3×10 ⁶	Control	3/3	3/3	3/3
	H ₂ O ₂	0/3	0/3	3/3
10 ⁷	Control	3/3	3/3	3/3
	H ₂ O ₂	2±/3	3±/3	3/3

^aNo. of *P. tolaasii* cells/mushroom; inoculated with 10 L of suspensions containing 10⁶, 3×10⁶, or 10⁷ cells/mL

^bDipped in 3% H₂O₂ for 30 sec; held 2 min; dipped in 4.5% sodium erythorbate (pH 5.5) for 20 sec

^cSets of 3 inoculated mushrooms incubated at 4°C for 10 days. ± = atypical lesion, i.e., brown but not sunken

solutions for 0.5–5 min. Observations included the extent of gas evolution from treated surfaces, an indication of the location and level of endogenous catalase activity (catalase would break down residual H₂O₂ in treated products, an important consideration with re-

Table 2 H₂O₂ residues in treated fresh-cut vegetables and melons

Commodity	Treatment ^a	Storage time (min)	H ₂ O ₂ residue	
			Test strips	TOOS-4AA
Mushrooms, whole	5% H ₂ O ₂ + browning inhibitor	5	none	none
Cucumber, cross-cuts	5% H ₂ O ₂	120	>25 ppm	nd ^{b,c}
	5% H ₂ O ₂ + H ₂ O, rinse	5	none	none
	5% H ₂ O ₂ + erythorbate	5	none	none
Cantaloupe, cubes	5% H ₂ O ₂	20	>25 ppm	nd ^d
	5% H ₂ O ₂ + H ₂ O, rinse	20	none	none
	5% H ₂ O ₂ + erythorbate	10	none	none

^aWhole cucumber treated with H₂O₂ and then cut into cross-cuts; cantaloupe cubes dewatered after treatment

^bnd=not determined

^cNegative at 48 hr

^dNegative at 2 hr

ected in the rinsed samples within 5 min after rinsing. Fresh-cut cantaloupe given no rinse after treatment contained significant residues for as long as 24 hr, and H₂O₂ could be detected on the sample container overwrap for at least 5 days. With rinsing and dewatering, H₂O₂ residues were less than 0.1 ppm within 20 min after treatment.

Shelf-Life Extension

The primary purpose of H₂O₂ treatment is to extend shelf life by reducing the population of spoilage organisms on product surfaces. In these experiments, we evaluated product shelf-life subjectively, based on the onset of visible spoilage. In trials with cucumbers, green bell peppers, and zucchini, we found that dipping the whole vegetables in 5 or 10% H₂O₂ for no more than 2 min, prior to slicing, was highly effective in delaying soft rot. Improvements in shelf life also were observed with fresh-cut cantaloupe and honeydew melon cubes treated with 5% H₂O₂ after cutting.

In direct comparisons of H₂O₂ and chlorine treatments, we found that the former were more effective in delaying the onset of spoilage with fresh-cut zucchini

to the oxidation of some endogenous antimicrobial compounds or release of nutrient-rich cellular fluids as a result of H₂O₂-induced tissue damage.

Immersion of mushrooms and melon cubes in H₂O₂ solutions produced copious amounts of foam, due to oxygen generated by the catalase reaction. The presence of foam might interfere with product treatment or rinsing to remove residual H₂O₂. Therefore, antifoam agents might have to be added to the H₂O₂ solution.

We observed no adverse effects of H₂O₂ on the aroma, flavor, or appearance of treated products. However, some flavor loss may have occurred in fresh-cut melon from rinsing. Whether this represents a significant problem or not will require more exact evaluation by a trained taste panel.

Effects on Microflora

Preliminary data indicate that H₂O₂ treatment reduced the load of fluorescent pseudomonads on mushrooms, zucchini, and cantaloupe by about 90% and was similar in effectiveness to chlorine with

and cantaloupe (Table 3, Fig. 5). In these experiments, prolonged exposure or use of higher H₂O₂ concentrations had an adverse effect on shelf life. We speculate that this could be due

were similar for treated and control mushroom and cantaloupe.

Shelf-life extension is due in part to the lethal effect of H₂O₂ on bacteria in the washing medium that were removed from product surfaces during treatment and would be redistributed on uninfected product surfaces if the washing medium were water. When dirty mushrooms were washed for 20 sec, in either water or 0.2 or 0.5% H₂O₂ solution, bacterial counts in the washing medium were reduced by 90% at the lower H₂O₂ concentration and by 99% at the higher concentration (Table 5). When other dirty mushrooms were washed in 5% H₂O₂ for 30 sec and the decanted H₂O₂ solution was held for 1 min before being diluted for plating, no viable bacteria could be recovered from the H₂O₂ solution. When other dirty

mushrooms were washed in water, the bacterial count of the used wash water was almost 10⁶ cfu/mL. Similarly, no viable bacteria were recovered from an H₂O₂ solution after soil, previously used for mushroom

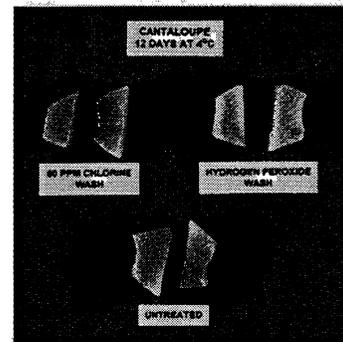


Fig. 5—H₂O₂ vs chlorine-treated fresh-cut cantaloupe after 12 days at 4°C

production, had been suspended in 5% H₂O₂ for 30 sec and then removed by straining through glass wool. The H₂O₂/soil filtrate was held for 1 min before being diluted for plating. With water as the washing medium, the used wash water contained more than 10⁶ cfu/mL. Thus, bacteria adhering to soil particles washed from surfaces of fresh-cut fruits or vegetables during H₂O₂ treatment are likely to be killed before they could infect incoming product.

Research Needs

Use of H₂O₂ as an alternative to chlorine for disinfecting fresh-cut fruits and vegetables shows promise. Treatments appear to reduce microbial populations on fresh-cut products and extend shelf life without leaving significant residues or causing loss of quality. However, more definitive data are required to establish the technical and economic feasibility of the treatment.

In particular, additional research is needed to optimize H₂O₂ treatments with respect to efficacy in delaying spoilage. The presence of persistent H₂O₂ residues

Table 3 Shelf-life extension of fresh-cut products by hydrogen peroxide vs chlorine

Sample	Treatment	No. of days at 4°C	
		Onset of spoilage ^a	Advanced spoilage ^b
Zucchini cross-cuts ^c	None	7	8
	200 ppm Cl ₂ for 2 min	7	8
	5% H ₂ O ₂ for 2 min	8	11
Cantaloupe cubes ^d	None	7	11
	50 ppm Cl ₂ for 2 min	9	11
	5% H ₂ O ₂ for 2 min	14	>15

^aTreated prior to slicing

^bWhole melons sanitized in 200 ppm of Cl₂ before cutting; cubes treated after cutting, then rinsed in H₂O

^cSmall colonies of wet spots

^dColonies, mold, liquifaction, off-odor

the latter two commodities (Table 4). These reductions persisted for 5 days of storage at 4°C, but by the 7th or 8th day, bacterial counts

would rule out the possibility that pathogens resistant to H₂O₂, perhaps as a consequence of sufficient bacterial catalase activity, might fill a niche created by a reduction in the population of spoilage organisms.

The applicability of H₂O₂ treatments to a broad range of fresh-cut commodities should be determined, especially with commodities that are subject to rapid spoilage and/or may be key components of salad bars, salad mixes, or important new product lines. Treatment costs should be determined for commodities that show significant benefits from H₂O₂ treatment.

in some treated commodities that contain endogenous catalase demonstrates the need to understand the site and kinetics

Table 4 Effects of H₂O₂ on fluorescent pseudomonads in fresh-cut vegetables and melons

Product	Treatment	Mean cfu/g of tissue		
		0 days at 4°C	5 days	7/8 days*
Mushrooms	Control	2.1×10 ⁸	3.6×10 ⁹	7.5×10 ⁹
	Water wash	1.9×10 ⁸	7.8×10 ⁹	6.6×10 ⁹
	5% H ₂ O ₂ for 30 sec	1.6×10 ⁷	7.2×10 ⁸	9.0×10 ⁸
Zucchini	Control	9.0×10 ⁵	6.3×10 ⁷	4.2×10 ⁹
	Cl ₂ wash	3.0×10 ⁴	9.0×10 ⁵	3.0×10 ⁷
	5% H ₂ O ₂ for 1 min	7.5×10 ⁴	6.0×10 ⁵	3.0×10 ⁶
Cantaloupe	Control	1.3×10 ⁶	9.0×10 ⁸	2.4×10 ¹⁰
	Cl ₂ wash	9.9×10 ⁴	3.3×10 ⁷	3.0×10 ⁹
	5% H ₂ O ₂ for 1 min	2.7×10 ⁵	2.3×10 ⁶	1.4×10 ⁹

*Mushrooms sampled on day 7, other products on day 8

of the catalase reaction. Potential effects of treatments on product quality, especially color, flavor, and nutrient composition, must be examined more rigorously. The reaction between H₂O₂ and erythorbate used to eliminate residual H₂O₂ indicates a need to investigate the potential loss of ascorbic acid and other labile nutrients in fresh-cut products as a consequence of H₂O₂ treatment.

The effects of H₂O₂ treatments on specific microorganisms responsible for spoilage of fresh-cut products must be established. At the same time, the sensitivity to H₂O₂ treatment of human pathogens that may be present as contaminants of fresh-cut products must be established. This

Table 5 Effect of H₂O₂ on bacteria removed from mushrooms or soil during washing

Sample	Bacteria in washing medium	
	Washing medium	(cfu/mL)
Dirty mushrooms ^a (75g/L)	Water	5.5×10 ^{6d}
	0.2% H ₂ O ₂	3.8×10 ^{5c}
	0.5% H ₂ O ₂	4.9×10 ^{4c}
Dirty mushrooms ^b (75g/L)	Water	7.5×10 ⁵
	5% H ₂ O ₂	0
Mushroom soil ^c (2.5g/L)	Water	1.5×10 ⁶
	5% H ₂ O ₂	0

^aWashed 20 sec, drained, immediately diluted for plating

^bWashed 30 sec, drained, held 60 sec before diluting

^cStirred for 30 sec, decanted through glass wool, held 60 sec before diluting

^dMean of 3 replicate treatments

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