63rd JECFA - Chemical and Technical Assessment (CTA), 2004

HYDROGEN PEROXIDE, PEROXYACETIC ACID, OCTANOIC ACID, PEROXYOCTANOIC ACID, AND 1-HYDROXYETHYLIDENE-1,1-DIPHOSPHONIC ACID (HEDP) AS COMPONENTS OF ANTIMICROBIAL WASHING SOLUTION

CHEMICAL AND TECHNICAL ASSESSMENT (CTA)

Prepared by Ma. Patricia V. Azanza

1 Summary

Hydrogen peroxide, peroxyacetic acid (POAA), octanoic acid, peroxyoctanoic acid (POOA) and 1-hydroxyethylidene-1,1-diphosphonic (HEDP) acid as components of antimicrobial washing treatments were recommended for evaluation at the 63rd Joint FAO/WHO Expert Committee for Food Additives (JECFA). The aqueous mixtures of the components as antimicrobial washing solutions, along with acetic acid as additional constituent, have not been subject of previous evaluation of the Committee. Commercial formulations of the mixtures, however, have already been subject of numerous governmental reviews and authorizations, and are approved for use in the United States, Canada and Australia. Drafting of the CTA was made based on the information provided through JECFA and review of some open literature.

2 Description

The components in mixtures intended to be used as antimicrobial wash treatments were described as clear, colorless liquids with sharp, pungent vinegar-like odor and water-soluble. Mixtures of the components as antimicrobial washing solutions are manufactured using acetic acid, hydrogen peroxide, octanoic acid and HEDP, following prescribed relative proportions and order of addition at 13-27 °C. Mixtures of components are allowed to equilibrate for about 7-13 days. The mixtures are intended for washing of fruits, vegetables, meat, and poultry. Governmental assessments and authorizations for the use of the mixtures of the five components as antimicrobial agents of food surfaces and process water treatments have been done in some countries.

3 Methods of analyses

For measurements of concentrations of components, either in commercial mixtures, residues in treated food surfaces or in rinsates, analytical methods as well as estimates have been cited by a commercial manufacturer. Analytical methods for determination of the concentrations of peroxyacids and hydrogen peroxide; peroxyoctanoic acid and octanoic acid; and HEDP in mixtures of the components are presented. Estimates to determine concentrations of peroxyacids (as POAA), HEDP and octanoic acid in residues of treated food surface or in food rinsates were likewise described.

3.1 Analytical methods

Hydrogen peroxide (and peroxyacid as POAA)

Hydrogen peroxide content (and peracid as peroxyacetic acid) is determined by an oxidation-reduction titration with ceric sulfate. After the endpoint of this titration has been reached, an excess of potassium iodide is added to the solution. The potassium iodide reacts with peroxyacids to liberate iodine, which is titrated with a standard solution of sodium thiosulfate.

POOA and octanoic acid

Peroxyoctanoic and octanoic acid are determined by high performance liquid chromatography. Reversed phase liquid chromatography is used by ultraviolet detection and comparison of peaks using an external standard.

HEDP

The content of HEDP in solution is determined by titration with a solution of thorium nitrate. The thorium forms a stable colorless complex with HEDP. Chrome Azurol S is used as an indicator. Chrome Azurol S forms a complex with thorium that has a purple colour, which does not form until all the HEDP has been complexed.

3.2 Estimates for concentration of components

Total peroxyacid concentration (as POAA)

The concentration of hydrogen peroxide can be easily determined, but analytical measurements to differentiate between POAA and POOA are relatively complex, time consuming and expensive (EC 2003). For practical purposes, the concentration of the peroxyacids is measured as the sum of both peroxyacids (POAA and POOA), corrected

for the different molecular weights of POAA and POOA, and expressed as POAA. The calculation is as follows:

$$\textbf{total peroxyacid concentration (as POAA)} = [\text{weight \% POAA}_{\text{solution}}] + [(\text{weight \% POOA}_{\text{solution}} \div 160) \times 76]$$

Where: 160 = molecular weight of POOA
76 = molecular weight of POAA

In order not to exceed the maximally peroxyacetic acid concentration of 220 mg/l, the peroxyacetic acid concentration in poultry process water is generally aimed at 200 mg/l, thus allowing for 10% variation in target peroxyacid composition. Over a period of 6 months the total peroxyacid composition will decrease by about 4%; peroxyacids containing process water has a shelf-life of 12 months (USDA 2002).

Estimate for HEDP

The concentration of HEDP is estimated based on the weight change of the treated food before and after application of antimicrobial wash treatment. Adjustment to HEDP concentration estimate is done by assuming possible 10% variations that may occur in the used treatment mixtures as a result of automatic dispensing equipment dosing configuration and/or variation in measurement of peroxyacid concentration. Calculation of the estimate is as follows:

$$\textbf{HEDP estimate} = [\text{food weight}_{\text{final}} - \text{food weight}_{\text{initial}}] \times \left(\frac{\text{weight \% HEDP}_{\text{solution}}}{\text{weight solution}} \right)$$

$$\textbf{Corrected HEDP estimate} = [\text{HEDP estimate}] \times 10\% \text{ variation}$$

Estimate for octanoic acid

Estimates for concentration of octanoic acid residues were determined using a bridging calculation based on corrected HEDP estimate multiplied by the predetermined ratio of octanoic acid to HEDP in the mixtures to be used. Calculation of the estimate is as follows:

$$\textbf{Octanoic acid estimate} = [\text{corrected HEDP estimate}] \times \left(\frac{\text{weight \% octanoic acid}_{\text{solution}}}{\text{weight \% HEDP}} \right)$$

4 Functional use

4.1 Technological purpose for use

The antimicrobial action of the mixtures of the components was primarily attributed to POAA and octanoic acid constituents by the commercial laboratory promoting the use of antimicrobial treatments. Although hydrogen peroxide, acetic acid and POOA are known to have antimicrobial properties, these components in the mixtures were considered as either intended mainly to participate in the formulation of POAA or is an unavoidable reaction product. These components were reported to provide insignificant antimicrobial activities at its intended use concentrations. The HEDP has been cited to have no antimicrobial efficacy and is primarily used as a stabilizer that prevents certain metals in the mixtures from catalyzing the degradation of POAA and hydrogen peroxide. Octanoic acid was likewise reported to reduce surface tension needed in wetting hydrophobic surfaces such as those in meat.

4.2 Levels of use in food commodities

The recommended uses of the components, as aqueous antimicrobial treatments, are for spraying, washing, rinsing, dipping, chilling, and scalding operations of poultry, meat, fruits and vegetables. Directions for use of the mixtures indicate dilution in water to achieve a specified POAA concentration. Under intended conditions of use, the commercial product formulations were recommended to be added to process water such that levels of hydrogen peroxide, POAA and HEDP will not typically exceed legal limits for use in antimicrobial applications in jurisdiction in which these components are approved for use as components of antimicrobial treatments.

4.3 Antimicrobial efficacy of mixtures and individual active components

Review of open literature validated claims on the possible efficacy of POAA as an antimicrobial agent in the commercial wash treatments at its target use concentration. Oxidation has been cited to be the primary mode of antimicrobial action of POAA (Cords and Dychdala, 1993). At reported target concentrations 40-200 ppm POAA commercial, review of published literature indicated that POAA, alone or in combination with other components, could effect 2 to 9 fold microbial reductions based on analyses of total microbial contamination and on some species of pathogenic microorganisms including Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella spp. on fruits and vegetables or in chemically defined media (Orth and Mrozek, 1989; Masson, 1990; Winniczuk, 1994; Parish et al., 2003; Cords and Dychadala, 1993).

The octanoic acid component in the commercial wash treatments has been claimed as major antimicrobial agent at target concentrations of 37-180 ppm. However, based on limited literature assessing efficacy of octanoic acid as antimicrobial agent, concentrations ranging from >1,000 to 36,000 ppm were required to attain minimum inhibitory concentrations for some spore formers, pathogenic bacteria and yeasts (Kabara et al., 1972; Conley and Kabara, 1972, Doores 1993). One reference reported minimum inhibitory concentration (about 1 log reduction) at 100 ppm concentration against Vibrio parahaemolyticus in tryptic soy broth medium (Beuchat 1980). Unfortunately, most of available literature that evaluated the antimicrobial efficacy of octanoic acid was done in chemically defined media. Traditionally, higher concentrations of antmicrobial agents would be required for the inactivation of microorganisms on complex food systems to parallel efficacies in chemically defined media.

Based on the above technical assessment on the efficacy of octanoic acid at target concentration during typical use of the mixtures, it could be suggested that perhaps the antimicrobial action of octanoic acid be attributed more to its surface-active properties. As a surfactant, octanoic acid may possibly modify surface properties of the mixtures to aid in wetting of hydrophobic food surfaces such as meat and thus enhance spreading and encourage retention of other more active antimicrobial agents at effective concentration (Clark 2003).

Hydrogen peroxide is known to be a very powerful oxidizing agent that is in general effective against a wide spectrum of microorganisms including bacteria, yeasts, molds, viruses and spore-forming organisms (Cords and Dychdala, 1993). However, the reported concentrations of hydrogen peroxide at which it was reported to elicit significant microbial reduction ranges were way above the target concentrations of hydrogen peroxide (50-100 ppm) in the commercial mixtures (Beuchat and Ryu, 1997; Park and Beuchat, 1999; Taormina and

Beuchat, 1999; Sapers et al. 1999). Independent research showed that hydrogen peroxide at 10,000-50,000 ppm, applied alone or in combination with other organic acids could only effect ≤5 log reductions of microbial contamination of fruits and vegetables (Peters 1995,Beuchat and Ryu, 1997; Park and Beuchat, 1999; Taormina and Beuchat, 1999; Sapers et al. 1999). Typically, a 5 log reduction in microbial count is used as a benchmark for measuring efficacy of disinfectants (Taylor et al. 1999).

At target concentrations in the commercial mixtures of the components, POOA was reported to have no antimicrobial function. Also, during the drafting of the CTA, the reviewer did not come across any published literature reporting functional use of POOA in antimicrobial washing treatments. The HEDP has also not been reported to have antimicrobial use; it was reported to function as stabilizer or sequestrant in the mixtures of the components, immobilizing metal ions.

At this point, it may be concluded that the antimicrobial efficacy of the antimicrobial washing treatments can primarily be ascribed to POAA component as a powerful oxidizing agent as previously claimed by a commercial manufacturer. Although both hydrogen peroxide and octanoic acid are known to be antimicrobial agents, these two components at target concentrations in commercial mixtures of the components theoretically could not effect significant reductions of microbial populations. In terms of overall efficacy of the antimicrobial wash treatments, perhaps it is wise to consider acidification of treated food surfaces as a possible secondary antimicrobial mechanism of action. This assumption is actually in congruence with the assessment of SCVPH (EC 2003) on its evaluation of peroxyacids mixture as antimicrobial agent in poultry processing. Generally, acidification of food surface limits microbiological activity due to pH change (Smulders 1995).

Results of commissioned work to measure the efficacy of antimicrobial treatments were based on corrected values using water control treatments as control. Reductions of natural microbial flora of poultry and beef carcasses measured in terms of aerobic plate count, total coliform and generic E. coli were shown to be in the order 0.24 – 1.0 log reduction. Generally, higher reductions were reported for pathogens artificially inoculated on some food samples. It may be inappropriate to assume, however, that treatments have greater efficacy against pathogens relative to indicator organisms since the experimental designs of commissioned work were not the same. Generally, microorganisms that are newly associated with a substrate are less resistant against any physical or chemical treatment because proper colonization of the inoculated organisms are yet to be established. Perhaps, a better measure of the efficacy of the wash treatments against microorganisms should be based on results reported for studies conducted on natural flora of food.

5 Reaction and fate in foods

5.1 General reactions

The mixtures of the components intended to be used as antimicrobial treatments may include the following: POAA, octanoic acid, hydrogen peroxide, acetic acid, POOA and HEDP. Its active ingredients rapidly break down to non-toxic products upon contact with food. Breakdown of POAA may take place based on two mechanisms to form acetic acid and hydrogen peroxide or acetic acid and oxygen. The first mechanism (1) shows that POAA can also be formed as an equilibrium product from the reaction of acetic acid and hydrogen peroxide.

$$CH_3COOOH + H_2O \rightleftharpoons CH_3COOH + H_2O_2 \text{ (hydrolysis)} \quad (1)$$

$$CH_3COOOH \longrightarrow CH_3COOH + \tfrac{1}{2} O_2 \quad (2)$$

Amounts of acetic acid from the breakdown of POAA that remain in food after treatments with mixtures of the components present no safety concern since it would be at levels, which are considered acceptable for use as antimicrobial, based on previous review of JECFA (Seventeenth report of JECFA, WHO Technical Report Series, No. 539, 1973).

Another product of the breakdown of POAA is hydrogen peroxide. Upon contact with food, hydrogen peroxide rapidly beaks down into water and oxygen, as described by the reaction below (3). Hydrogen peroxide also oxidizes acetic acid and octanoic acid to form POAA and POOA, respectively (1,4).

$$HOOH \longrightarrow H_2O + \tfrac{1}{2} O_2 \qquad (3)$$

POOA, on the other hand, rapidly breaks down to form octanoic acid and hydrogen peroxide or octanoic acid and oxygen:

$$CH_3(CH)_6COOOH + H_2O \rightleftharpoons CH_3(CH)_6COOH + H_2O_2 \text{ (hydrolysis)} \quad (4)$$

$$CH_3(CH)_6COOOH \rightleftharpoons CH_3(CH)_6COOH + \tfrac{1}{2} O_2 \qquad (5)$$

Octanoic acid in the commercial mixtures of components may come from two sources: the octanoic acid that may result from the breakdown of POOA (4,5), and the octanoic acid intentionally added to the mixture. Although small quantities of octanoic acid will remain in food after treatment with the mixtures of the components, octanoic acid is relatively stable, non-toxic and a normal component of food.

HEDP provides long-term storage stability to the antimicrobial mixtures of the components by preventing certain metal ion contaminants, which may come from the water, from catalyzing the degradation of POAA and hydrogen peroxide. Small residues of HEDP will remain on the surface of treated food systems.

5.2 Reactivity Studies and Nutrient Testings

Results of commissioned tests by commercial manufacturer of the mixtures of the components as antimicrobials to validate its impact to quality, nutritional value and other properties of food were summarized in this CTA.

1. The TBA values and fatty acid profiles of raw and cooked red meat samples treated using 200 ppm POAA-based spray solution at 5-min contact time were not significantly different at 5% level of significance with the untreated controls. The same results were observed in the TBA values and fatty acid profiles of raw and cooked poultry samples.

2. The concentration of POAA and hydrogen peroxide on tomatoes, broccoli and potatoes washed in a solution containing 80 ppm POAA and 59 ppm hydrogen peroxide, with moderate agitation, 5 min contact time, 70-75ºF (21-24ºC), were not significantly different before and after treatment ($p>0.01$). These results were interpreted as non-reactivity of the active agents with the components of fruit and vegetable samples analyzed. The Vitamin C content of potatoes and broccoli and the β-carotene content of tomatoes, and broccoli were not significantly affected by the same treatment. However, about 37% drop in ascorbic acid (oxidized form of Vitamin C) content of tomatoes was detected, with an equivalent increase in its dehydroascorbic acid (reduced form of Vitamin C) content using the same treatment.

As commercial antimicrobial mixtures of components, the treatments were previously reported to cause slight bleaching in strawberries after treatment with 100 ppm POAA-based solution for 2 min followed by 5-sec treatment of 500 ppm Na thiosulfate 500 ppm (Lukasik et al., 2003). It has been shown that hydrogen peroxide is sometimes used generally used in much higher concentrations ranging from > 50,000 ppm to 350,000 ppm when used either as vapor or for immersion treatments. Lower concentrations of hydrogen peroxide (≤ 3 ppm) were also reported but these were only restricted to vapor treatment. Published information also indicated that hydrogen peroxide causes browning, bleaching and blistering of food materials.

5.3 Estimated Residues

Following application of the antimicrobial mixtures in food, the components are subject to lost due to drainage, further washing, trimming, blanching, and other food processing procedures. However, some

amounts of the components are bound to remain in food as residues even after food processing. The limited number of studies conducted to examine the residues of hydrogen peroxide, POAA, POOA, HEDP and octanoic acid in various foods treated with mixtures of components as antimicrobial treatments were again commissioned. Results of the studies are shown below:

1. Less than 1 ppm residues of POAA, POOA and hydrogen peroxide were detected in chicken carcasses treated with POAA-based spray solution (200 ppm, 15-sec contact time) at ambient temperature.
2. Samples of rinsate analyzed to monitor the residues of hydrogen peroxide and peroxyacids from beef carcass treated with POAA-based solution (spray treatment, 200 ppm, 10 min contact time) showed decrease in concentration of hydrogen peroxide residues from 1 ppm to less than 0.003 ppm and concentration of peroxyacid residues from 10 ppm to 0.05 ppm.
3. No detectable residues of hydrogen peroxide and total peroxyacids in treated trimmed beef tissue were detected (POAA-based immersion treatment, 200 ppm) at contact times: 1, 5, 10 and 20 min. For samples exposed to the same treatment using 200 ppm, contact time of 20 min, no residue of hydrogen peroxide was also detected, while residue of total peroxyacids were detected to dropped to as low as 6.2 ppm.
4. Residues of POAA and hydrogen peroxide in ground peas treated with a commercial POAA-based wash solution (200 ppm, 6 h contact time, 70-75ºF (21-24ºC)) were detected to drop to as low as 3.28 ppm and 3.71 ppm, respectively. Similarly, residues of POAA and hydrogen peroxide in ground tomatoes exposed to similar treatment were detected to drop to as low as 9.18 ppm and 2.49 ppm, respectively.

Estimated residues for both HEDP and octanoic acid were in the ppb levels. Both estimated residues of octanoic acid and HEDP, however, were actually corrected estimates based on several assumptions that are yet to be validated by additional experiments.

Estimation of HEDP residues that may remain in food was said to be conservatively assumed to be 10% higher than what maybe established using the prescribed analytical method. Justification for this corrective input to HEDP level was based on the possible variations that may occur in the use of the mixtures of components as a result of automatic dispensing equipment dosing configuration and/or variation in measurement of peroxyacid concentration.

Octanoic acid residues were determined using a bridging calculation based on HEDP concentration that was previously estimated multiplied by the predetermined ratio of octanoic acid to HEDP in the treatments to be used. What is apparent in the proposed calculations of residual HEDP and octanoic acid is that these are based on estimates and a lot of error maybe accommodated in the calculations.

6 References

Beuchat LR (1980) Comparison of anti-*Vibrio* activites of potassium sorbate, sodium benzoate and glycerol and sucrose esters of fatty acids. *Appl. Environ. Microbiol.,* **39**, 1178.

Beuchat LR & Ryu JH (1997) Produce handling and processing practices: special issue. *Emerg. Infect. Dis.,* **3**(4), 459-65.

Clark JP (2003) New developments in sanitation help keep food plants clean. *Food Technol.,* **53**(10), 81, 87.

Conley AJ & Kabara JJ (1973) Antimicrobial action of esters of polyhydric alcohols. *Antimicrob. Agents Chemother.,* **4**, 501.

Cords BR & Dychdala GR (1993) Sanitizers: halogens, surface-active agents and peroxides. In: P M Davidson & A L Branen, eds. Antimicrobials in foods, second edition. New York, Marcel Dekker, Inc., pp. 469-537.

Doores S (1993) Organic acids. In: Davidson PM & Branen AL, eds. Antimicrobials in foods, second edition. New York, Marcel Dekker, Inc., pp. 95-136.

Dychdala GR & Koroma JM (1986) Bacterial activity of P3-oxonia active at different levels. Unpublished report from Henkel Corporation.

EC (2003) Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on the evaluation of antimicrobial treatments for poultry carcasses. European Commission, Health and Consumer Protection Directorate-General. http://europa.eu.int/comm/food/fs/sc/scv/out63_en.pdf. Date accessed: 15 March 2004.

Kabara JJ (1972) A new preservative for food. *J. Food Safety,* **4**(1), 13.

Lukasik J, Bradley ML, Scott TM, Dea M, Koo A, Hsu W-Y, Bartz JA & Farrah SR (2003) Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. *J. Food Prot.,* **66**(2), 188-193.

Masson RB (1990) Recherche de nouveax disinfectants pour les produits de 4eme gamme. In: *Proc Congress Produits de 4eme Gamme et de 5eme Gamme*, Brussels, CERIA, pp 101.

Orth R & Mrozek H (1989) Is the control of *Listeria, Campylobacter* and *Yersinia* a disinfectant problem? *Fleischwirtshaft* **69**(10), 1575-1576.

Parish ME, Beuchat LR, Suslow TV, Harris LJ, Garrett EH, Farber JN & Busta FF (2003) Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. In: Comprehensive reviews in food science and food safety, Vol. 2 (Supplement). Institute of Food Technologists/Food and Drug Administration Report on Task Order 3, pp 161-173.

Park CM & Beuchat LR (1999) Evaluation of sanitizers for killing *Escherichia coli O157:H7, Salmonella* and naturally occurring microorganisms on cantaloupes, honeydew melons and asparagus. *Dairy Food Environ. Sanit.*, **19**, 842-847.

Peters DL (1995) Control of enteric pathogenic bacteria on fresh produce. Unpublished M. S. thesis from Lincoln, Univ. of Nebraska Graduate College.

Sapers GM & Simons GF (1998) Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technol.,* **52**(2), 48-52.

Sapers GM, Miller RL & Mattrazzo AM (1999) Effectiveness of sanitizing agents in inactivating *Escherichia coli* in golden delicious apples. *J. Food Sci.,* **64**(4), 734-737.

Smulders FJM (1995) Preservation by microbial decontamination: the surface treatment of meats by organic acids. In: Gould GW, ed. New methods of food preservation. London, Blackie Academic & Professional.

Taormina PJ & Beuchat LR (1999) Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.,* **62**(4), 318-324.

Taylor JH, Rogers SJ & Holah JT (1999) A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against *Escherichia coli* O157:H7 *Psedomonas aeruginosa* at 10 and 20ºC. *J. App. Microbiol.*, **87**, 718-725. USDA (2002) The use of peroxyacids as an antimicrobial agent on poultry processing in the United States. United States Department of Agriculture, Foreign Agricultural Service, and Food Safety and Inspection Service, Office of International Affairs. December 2002.

Winniczuk PP (1994) Effects of sanitizing compounds on the microflora of orange fruit surfaces and orange juice. Unpublished M. S. thesis from Gainesville, Florida, University of Florida Graduate School.