Hydrogen Peroxide Treatment of Raisins, Walnuts, and Prunes Combined Progress Report

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Summary:

Vapor Phase Hydrogen Peroxide (VPHP) technology offers a promising method for the disinfection of surface-borne bacteria, yeasts, and molds on a number of agricultural commodities. The number of colony forming units per gram (cfu/g) on raisins was compared among untreated, VPHP-treated, and a sodium hypochlorite-ethanol wash. VPHP substantially reduced the cfu/g on both raisins coming into the plant (incoming) and raisins cleaned and oiled for packaging (outgoing). The cfu/g on incoming raisins were reduced to zero in 15 of 20 cases while outgoing raisins were reduced to zero in 17 of 20 cases. Preliminary data on walnut nut meats using three culture media showed a 95% reduction in the number of cfu/g of product. We expect similar results on prunes.

Introduction:

Liquid hydrogen peroxide has long been known as a useful sanitizing agent; its use on food was first reported for the preservation of milk (Schrodt, 1883). Hydrogen peroxide is approved and in wide spread use as an effective sterilizing agent for aseptic food container packaging material (Reuter, 1989). The employment of hydrogen peroxide for container sterilization has been much greater in Europe than in the United States. Hydrogen peroxide holds promise for some agricultural applications as an excellent surface sanitizing agent to replace some commonly used fungicides and fumigants (Block,1992).

The Food and Drug Administration (FDA) lists hydrogen peroxide as a "generally recognized as safe substance" (GRAS)(Code of Federal Regulations, Title 21, parts 180-189). Hydrogen peroxide is already produced in a food grade and can be safely handled by trained employees. The direct application of hydrogen peroxide to food requires approval on an item by item basis. A determination will need to be made if any residues remain. We will evaluate methods of determining residues.

Hydrogen peroxide is relatively environmentally friendly since its degradation yields only water and oxygen. It is composed of two atoms of hydrogen and two atoms of oxygen. During the

dissociation of the hydrogen peroxide molecule an intermediate molecule is formed called a free radical. The free radical is a strong oxidizer and is highly reactive (Schumb, Satterfield, and Wentworth, 1955). It attacks other molecular bonds, affecting the disruption of membranes for example, to cause the death of the targeted microbes. At room temperature the hydrogen peroxide molecule is highly reactive. However, the reactivity and microbial toxicity of this molecule can be enhanced with vaporization, elevated temperatures, radio waves, ultraviolet light, and metal ions.

A recently patented vapor phase hydrogen peroxide technology increases the potential applications of hydrogen peroxide (Moore and Perkinson, 1979). The American Sterilizer Company (AMSCO) has developed this technology and is producing equipment for the medical industry (Childers,1991). The USDA/ARS laboratory in Fresno, CA has a confidentiality agreement with AMSCO to research applications of this technology within agriculture. AMSCO has provided the laboratory with two different machines using the AMSCO VHP^{tm} technology.

The objectives of this research are to test VPHP technology as a method to reduce the number of microbe colony forming units on raisins, walnuts, and prunes. Currently, we are determining the optimum treatment parameters and efficacy of VPHP technology. Later, we will compare presently utilized practices including the use of propylene oxide on walnuts and the use of potassium sorbate on prunes to VPHP. Future work is also planned to develop methods to determine hydrogen peroxide residues, product storage life, and to evaluate quality.

Materials and Methods:

Standard microbiological methods were used to determine the number of microbes (AOAC, 1992). The personnel at the Dried Fruit Association laboratory in Fresno, California provided information and assistance.

Raisins

The raisin evaluations were made with product provided by four cooperating packing houses. Incoming raisins were obtained from the bins before the product was brought into the plant. Loose debris were removed from the dried fruit but pedicels, if present, remained attached. Outgoing raisins were obtained at the end of the packing line and held for approximately one week before treatment. VHP^{tm} treated raisins were held two days after treatment before plating on the agar media. Microorganisms on raisins were determined both by maceration of 50 gram samples (spread plating) and plating of whole intact raisins (direct plating).

Raisins for spread plating were homogenized 2 minutes in a blender (Waring) at high speed with phosphate buffer pH 7.2 (BBL,

Becton Dickinson) and subjected to a standard dilution series before plating. The agar medium for spread plating was dichloran rose bengal chloramphenicol agar base (DRBC, Oxoid). Inoculated spread plates were incubated for 5 days at 25 degrees centigrade before the colonies were counted. Each dilution was plated in triplicate and each dilution was counted when possible. The mean of the three plates is reported from appropriate dilutions within the range of 25-250 cfu/g per plate. Untreated controls were included to determine the natural level of infection.

Lots of 100 treated and untreated individual raisins were direct plated on DRBC, held for 5 days at 25 degrees centigrade, and the percentage of raisins from which mold colonies grew were counted. Nearly all untreated control raisins had sufficient Aspergillus niger spores to be detected by direct plating (799 infected of 800 raisins).

Initial VHP^{tm} exposure times were two hours duration to determine treatment effectiveness. Raisins were enclosed in plastic mesh bags and treated with VHP^{tm} equipment for 60 minutes. The bags were removed from the equipment chamber, shaken to reorient the fruit, and returned to the chamber for an additional 60 minute exposure. In later experiments, exposure times were reduced to less than 5 minutes with results similar to the long duration treatments.

An ethanol and sodium hypochlorite treatment was included to compare the results of a known effective surface disinfectant with VHP^{tm} technology. Raisins were shaken in 70% ethanol for 2 minutes in sterile plastic bags, the ethanol was decanted, and sterile distilled water was added, and again shaken for 2 minutes. The sterile water was decanted, 5.25% sodium hypochlorite (Clorox^{tm} bleach) diluted to 0.525% was added, and shaken with the raisins. The bleach was decanted and the raisins were shaken with 3 additional sterile distilled water rinses.

Walnut Nut Meats

Preliminary VHP^{tm} work has been started on walnut nut meats. A comparison was made to determine the best media for conducting the evaluations of microbial populations. The media evaluated were: 1) dichloran rose bengal chloramphenicol agar base (DRBC, Oxoid); 2) aerobic plate count agar (APC, Oxoid); and 3) potato dextrose agar (PDA, Sigma). VHP^{tm} treated walnut nut meats (60 minutes) were compared with untreated controls.

A standard spread plate method was used to evaluate walnut halves and pieces for microbe presence (AOAC, 1992). The nut meats were shaken with phosphate buffer pH 7.2 (BBL, Becton Dickinson), serially diluted, 0.2 ml aliquot applied to the culture media, and spread with a glass dally stick.

APC media was incubated for 2 days at 35 degrees centigrade. APC media typically is utilized to evaluate the number of bacteria

and yeasts. The high incubation temperature suppresses most fungi. The DRBC and PDA media were incubated 5 days at 25 degrees centigrade. The DRBC media was utilized to evaluate the number of fungal colonies. Fungal colony spreading and bacteria are suppressed by DRBC media making it easier to obtain reliable cfu/g. The PDA agar is utilized as a good general media for the growth of microbes. Molds, yeasts, and bacteria can be frequent spreaders on PDA plates making it difficult to count colonies.

Future Prune Work

VPHP treatment is expected to reduce the colony forming units on prunes. A comparison to potassium sorbate will be made. The methods for evaluating treatment effectiveness may involve both techniques as given previously for raisins and walnuts. Pitted prunes will likely be ground in buffer while unpitted fruit will likely be shaken with a buffer to determine the microbial load. We are seeking industry cooperators for both walnuts and prunes at this time.

Raisin Results and Discussion:

The DRBC agar medium selected for both spread plating and direct plating suppresses the growth of bacteria and the spreading growth of fungal colonies. The numbers of colony forming units per gram of product is less when DRBC agar medium is used to evaluate untreated product because the bacteria are suppressed. Our experience, however, with other commodities and in using different media confirm the effectiveness of the VHP^{tm} treatment on yeasts, bacteria, and fungi.

All three raisin graphs showed washing raisins in ethanol-Clorox^{tm} was consistently less effective in reducing mold counts than VHP^{tm} treatment (Incoming, Outgoing, and Direct Plating).

The direct plating results show near 100 percent (799/800) infected fruit both incoming and outgoing. VHP^{tm} treatment reduced the percentage of infected raisins to 3% and 0%, respectively, for incoming and outgoing raisins. Ethanol-Clorox^{tm} treatment reduced the percentage of infected raisins to 23% and 32%, respectively, for incoming and outgoing raisins.

Data communicated from Dried Fruit Laboratory personnel show that perhaps only 10-15 percent of the naturally sun dried raisins will meet the low cfu/g needs of some buyers. Some highly selective buyers have also requested a "zero" tolerance for xerophytic yeasts. A "zero" tolerance is an extremely difficult target to achieve and probably does not warrant the effort to obtain it. Human pathogens would not likely occur on raisins.

A target was selected for having less than 250 cfu/g of product that would satisfy most buyers. Vapor phase hydrogen peroxide technology is capable of reaching this level of surface disinfection. The low water availability of dry raisins after

treatment provides natural protection from spoilage organisms. The VHP^{tm} process is dry, not adding additional moisture to the product.

The spread plate data in the incoming raisin mold count graph show that no samples were below the 250 cfu/g target. After treatment, 17 of the 20 samples evaluated were reduced below the target number.

The spread plate data in the outgoing raisin mold count graph show that 8 of the 20 samples passing through the packing plant could meet the target of less than 250 cfu/g. The frequency data showed 19 of 20 VHP^{tm} treated samples met the goal.

Walnut Nut Meat Results and Discussion:

Substantial numbers of yeasts, bacteria, and fungi were found on walnut nut meat surfaces. The graph reporting the number of cfu/g for walnut nut meat spread plate data showed 17,100-28,600 depending upon the agar media. The VHP^{tm} treated walnuts were reduced to 480-1370 cfu/g. This is a 95% reduction in the number of microbes.

The PDA agar media showed the highest number of microbes (28,600 cfu/g). The VHP^{tm} treatment reduced the counts on PDA to 1370 cfu/g. PDA is a general purpose media on which yeasts, bacteria, and fungi will grow. All three groups of these microbes were found to be present in large numbers. The lack of selectivity of this media and the spreading nature of some of the fungi and yeasts which were found make this a difficult agar medium for counting colony forming units.

DRBC suppresses bacteria multiplication and the spreading growth of fungal colonies. The DRBC spread plate data showed the least number of colonies (17,100 cfu/g). The VHP^{tm} treatment reduced the counts on DRBC to 480 cfu/g. The bacterial growth was suppressed which accounts for the reduced number. This agar medium would be the best choice if the number of fungal colonies is selected as the best indicator of treatment effectiveness and if fungi are determined to be an important portion of the microbe population on walnut nut meats.

The high temperature (35 degrees centigrade) at which the APC agar medium is incubated favors the growth of bacteria and yeasts. Most fungal growth is suppressed at this temperature. The APC plate count data showed 28,100 cfu/g, nearly all bacteria and yeasts. The VHP^{tm} treatment reduced the counts on APC to 1070 cfu/g. An advantage of the APC agar medium is its brief incubation requirement, only two days for microbe growth versus five days for DRBC and PDA. We have not compared VHP^{tm} treatment to propylene oxide for microbe control yet.

Conclusion:

Vapor phase hydrogen peroxide technology is an effective treatment to reduce the number of surface borne microbes on raisins and walnut nut meats. We are seeking industry cooperators for our research efforts on walnuts and prunes.

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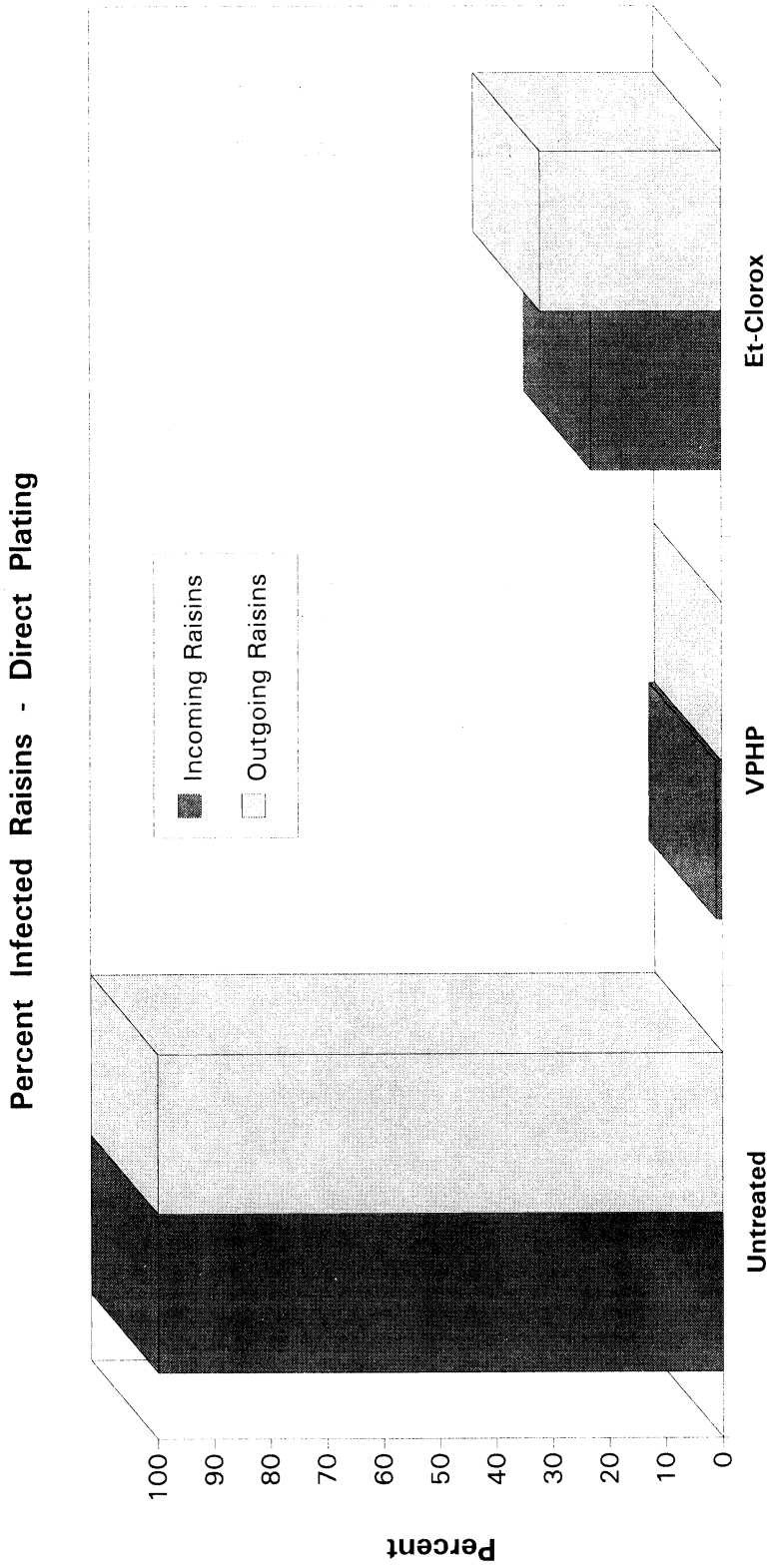

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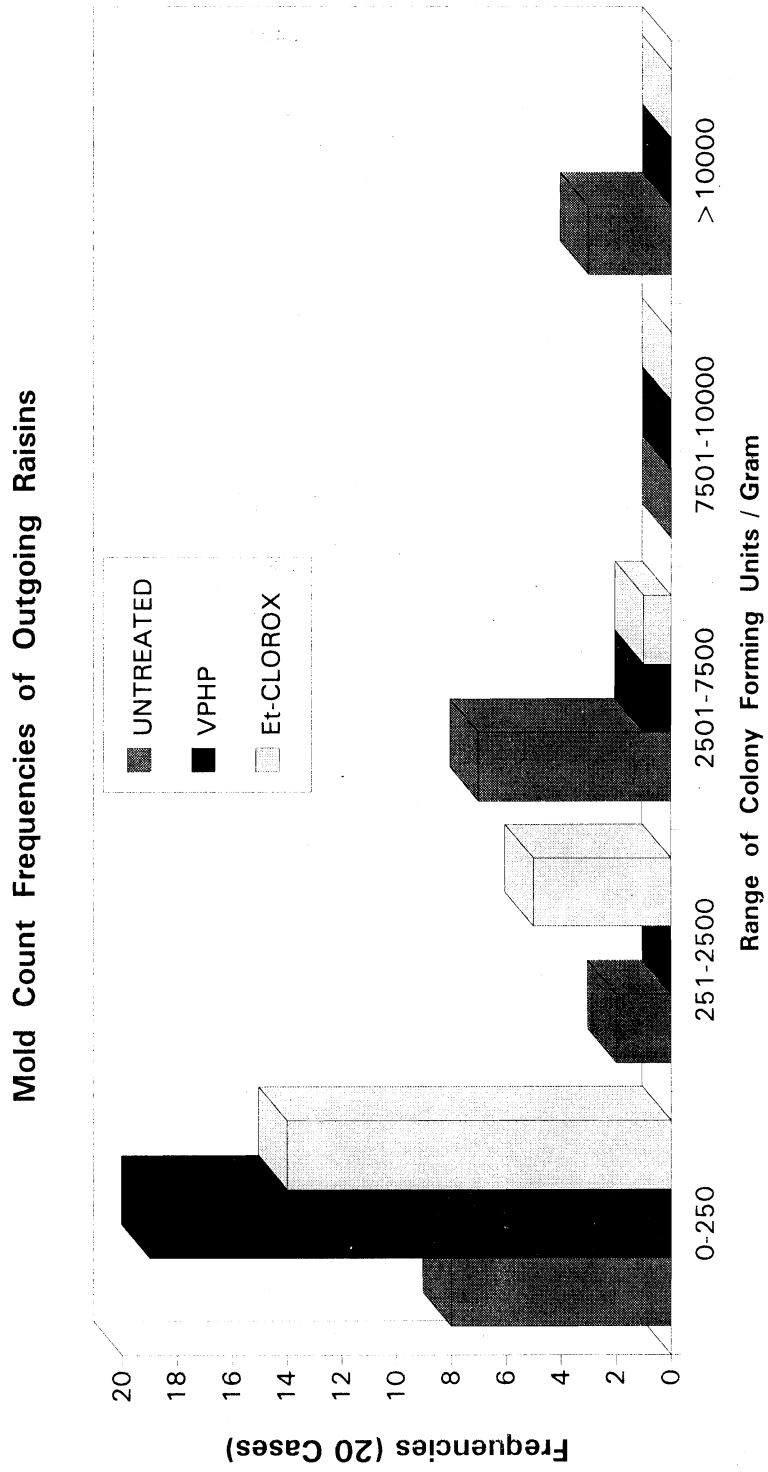

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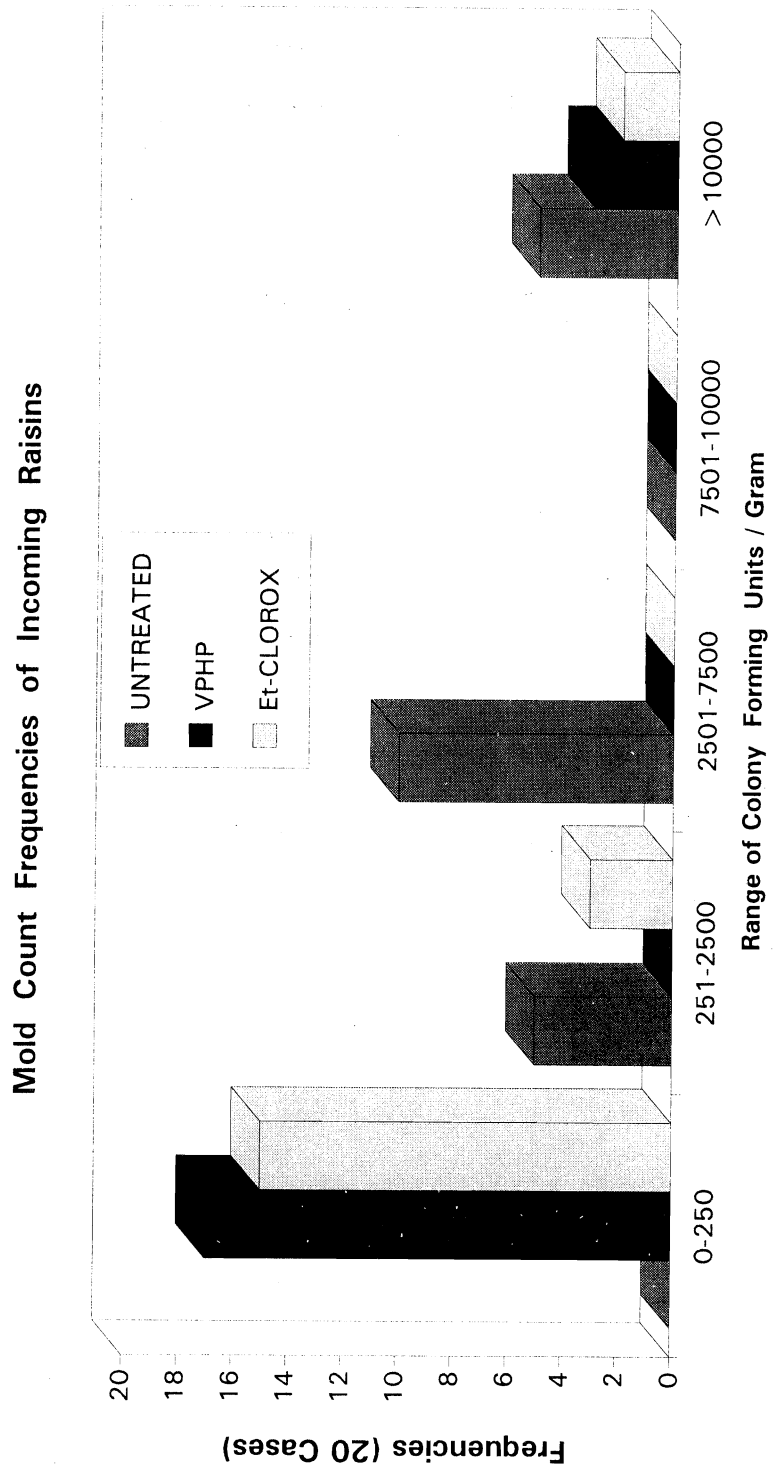

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