AN ABSTRACT OF THESIS OF

Joy G. Waite for the degree of Master of Science in Microbiology presented on March 4, 2004.

Title: Antimicrobial Properties of a Wine Based Disinfectant.

Abstract approved: Redacted for privacy

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Wine has recently been shown to be a possible protective agent against microbial foodborne illness. The chemical environment of wine makes it difficult, if not impossible, for microorganisms to survive. The low pH, high concentration of organic acids, relatively high ethanol concentrations, and the potential for high levels of sulfur dioxide provide a series of hurdles for any microorganism that comes into contact with wine. Each and every wine contains a different amount of these known antimicrobial parameters and the combination of these components contribute to its' ability of to inactivate microorganisms. The objective of this research was to determine the role these parameters contribute to the observed antimicrobial effect.

Initial experiments focused on the addition of sulfur dioxide to increase antibacterial efficacy. Four bacterial strains of common household pathogens were used to measure antimicrobial activity in a suspension test, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and two strains of *Staphylococcus aureus*. A Chardonnay wine was produced at Oregon State University and different levels of sulfur dioxide and 1.5% sodium chloride were added. As expected, different bacterial species displayed varying levels of sensitivity towards the wine samples. *Klebsiella pneumoniae* and *P. aeruginosa* were significantly more sensitive to wine than *S.*

aureus. The former strains were inactivated by all treatments in less than five minutes. *Staphylococcus aureus* cells were capable of surviving in some of the wine treatments for nine minutes. Increased sulfur dioxide concentration and the presence of sodium chloride enhanced the ability of wine to inactivate these microorganisms.

Five commercial Chardonnay wines were analyzed for their ability to inactivate the most resistant strain *S. aureus* 649. Samples were enumerated after one and three minutes of exposure. Results were variable, however trends could be discerned. Sulfur dioxide concentration did not correlate with antimicrobial activity of the wines. Other wine components were measured: pH, titratable acidity, volatile acidity, and ethanol concentration. No single parameter correlated with efficacy from the suspension tests. It was determined that complex interactions between wine components and their availability for acting on the microorganism varied by wine system. It is possible that there are other wine components that contribute to efficacy that were overlooked (e.g. polyphenols).

The final stage of this study involved evaluating combinations of pH, titratable acidity, sulfur dioxide, and ethanol in a wine background. Suspension tests were performed against *Escherchia coli* O157:H7 and *Staphylococcus aureus* with samples being taken after twenty minutes of exposure. *Escherichia coli* O157:H7 was significantly more sensitive than *S. aureus* to the wine treatments. Analysis of overall trends showed that titratable acidity and ethanol concentration were not ideal predictors of efficacy. Molecular sulfur dioxide concentration was found to be a relatively good predictor of antimicrobial effects. Hydrogen ion concentration (pH) levels appear to be the most important parameter for predicting inactivation of *S.*

aureus. Varying pH levels are known to affect the antimicrobial effects of other components. Decreased pH shifts the equilibrium of organic acids to the more antimicrobial undissociated form. Decreasing pH also increases the concentration of molecular sulfur dioxide in solution. The antimicrobial effects of ethanol are known to be enhanced by lowering the pH of the solution. This study concluded that decreasing pH enhanced the antimicrobial effect of ethanol. Other combinations may also lead to some strong conclusions that may enhance the ability to predict disinfection ability of a given wine.

Overall results suggest that Chardonnay wine contains a combination of antimicrobial parameters that contribute to disinfecting ability. Of the tested parameters, pH appears to be the most influential; however the combination of all the components is more important than any one parameter.

ANTIMICROBIAL PROPERTIES OF A WINE BASED DISINFECTANT

By
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A THESIS

Submitted to
Oregon State University

In partial fulfillment of
The requirements for the
Degree of

Master of Science

Presented March 4, 2004
Commencement June 2004

Master of Science thesis of Joy G. Waite presented on March 4, 2004.

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Joy G. Waite, Author

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation of all the time, effort, support, expertise, and patience that Mark Daeschel has shown to me over the last several years. I would also like to thank my committee members: Yanyun Zhao, Daniel Rockey, and Mark Christensen for being flexible and patient throughout this process. I would like to express my sincere gratitude to Ned Morris, Laura Byrdy, Silvia Stan, Cindy Bower, and Rat Schilke for their time and friendship in the lab. Thank you to Alejo Giron, Mark Malowicki, and Garrett Berdan for making each day a little more fun. I would also like to express my sincere appreciation to the faculty, staff and students of the Department of Food Science and Technology for making me feel at home over the last several years. Thank you to Sujaya Rao and Devora Shamah for the educational and outreach opportunities over the last two years, but more importantly for their encouragement and friendship. Last but not least, thanks to my son Allan, my mother Sharon, my father Joe, and my sisters Laurie and Mindy for supporting and encouraging me and never saying I wasn't capable of great things.

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CHAPTER 1

Introduction

Incidence of Foodborne Illness

There are estimated to be 76 million cases of foodborne illness in the United States annually, of these cases 325,000 require hospitalization and 5,000 result in death (Tierney and others 2002). The overall number of outbreaks appears to be increasing, more than 10% of the population has a case of foodborne illness annually (Mullerat and others 1995). At least 250 different disease-causing microorganisms can be transmitted by food or drink (Doyle and others 2000).

Most people associate increased foodborne illness risk with situations beyond their control (e.g. restaurants) and not with eating at home (Tierney and others 2002). Data suggested in 1986 that more foodborne outbreaks were linked to private homes than all other sources combined (Hilton and Austin 2000).

Improper food handling in the home is thought to be the leading factor contributing to these illnesses (Zhao and others 1998, Tierney and others 2002). Epidemiological data indicates approximately 19-21% of foodborne illness occurs in the home, however this is likely a gross underestimate due to the unlikeliness of small outbreaks being reported or investigated (Zhao and others 1998, Tierney and others

2002). A study in the Netherlands predicted that 80% of *Salmonella* and *Campylobacter* infections were contracted in the home (Hilton and Austin 2000, Tierney and others 2002). In the United Kingdom, it was estimated that 95% of salmonellosis and 99.8% of *Campylobacter* infections are sporadic or restricted to immediate family members (Meredith 2001). It cannot be assumed that all of the sporadic cases are due to the household environment, however it was estimated that approximately 76% of *Salmonella* outbreaks recorded in the United Kingdom were associated with single households (Meredith 2001). A study in Italy found that 74% of *Salmonella* outbreaks were associated with home-cooked meals (Jones 1998). There are significant social and economical costs associated with outbreaks arising from the home (Bloomfield and Scott 1997). Increases in domestically associated foodborne illness outbreaks have been linked to lifestyle changes and poorer home hygiene (Jones 1998). While the risk to the community is greater if transmission of infection is in a public domain, the impact of infections originating in the home should not be ignored (Jones 1998).

Until recently, foods with low pH and high organic acid content were believed to be safe from food poisoning; however outbreaks linked to unpasteurized fruit juices have raised concerns (Just and Daeschel 2003). Acid-resistant organisms may survive in food products that were originally thought to be safe: apple cider, sausage, cheese, yogurt, orange juice, etc (Uljas and Ingham 1999, Cheng and others 2002). However, if the food source of the bacterial pathogen has been properly cooked and outbreaks

are still occurring, then it is likely that cross-contamination may be an extremely important factor (Meredith 2001).

Kitchen contamination

Consumers are being encouraged to eat more fresh foods for a number of reasons, however unprocessed or raw foods have the potential to introduce pathogens into the home potentially causing foodborne illness if not properly handled and stored (Roberts 1990, Jay and others 1999). Raw meats are considered to be a primary source of foodborne infection; undercooked meat is a known cause of foodborne illness. However, improper handling can lead to contamination of worksurfaces possibly leading to indirect incidences of foodborne illness (Roberts 1990). Other food items including eggs, raw milk, fish, shellfish, fruits and vegetables may contribute to the contamination of the kitchen (Roberts 1990). *Salmonella enteritidis* has been shown to remain on fingers and utensils following contact with contaminated egg products, even after washing (Bloomfield and Scott 1997, Hilton and Austin 2000, Meredith 2001). Roberts (1990) reported that between 1970 and 1982, 10% of food poisoning outbreaks in England and Wales were linked to cross-contamination and/or infected food handlers. Hands, surfaces, cooking utensils, and other kitchen equipment that have been inadequately cleaned and disinfected may cause cross-contamination (Roberts 1990, Bloomfield and Scott 1997). However, people that are in constant contact with raw foods may become asymptomatic carriers of pathogens (Roberts 1990). Cross-contamination risk is dependent on the frequency and

occurrence of pathogenic bacteria as well as the likelihood of transferring the contaminants (Bloomfield and Scott 1997).

Scott and others (1984) have performed numerous studies of the domestic environment and have determined the kitchen to be the most important area of the home for harboring and transferring infection. While it is difficult to estimate the number of foodborne illnesses arising in the home, it is even more difficult to estimate the percentage of household illnesses that are due to kitchen and toilet cross-contamination versus temperature abused food. There are numerous variables in the kitchen environment, either commercial or domestic, that can lead to contamination of various surfaces, items, and foods. Ideally, kitchens would have separate areas to handle raw and ready-to-eat foods and food preparers would regularly wash their hands (Roberts 1990). The three main routes by which microorganisms enter are foods are: raw food and ingredients, human contact, and the environment (Roberts 1990). An infected food handler appears to be a low risk for foodborne illness except in the case of *S. aureus* food poisoning (Roberts 1990). The domestic food handler may be capable of negating a large portion of the effort made by the food processors to keep products safe (Jay and others 1999).

Cross-contamination may be the result of direct or indirect contact. Direct cross contamination occurs if pathogens are transferred directly from a source (raw food, pets, and people) to the food (Jay and others 1999). Indirect cross contamination includes a source (*Salmonella* from raw chicken), a vehicle (hands, cloths, surfaces) for the transfer, and the food (Jay and others 1999). Jay and others (1999) investigated

domestic food-handling practices in Australia by videotaping 40 households for one week to seven months. During the video study, households displayed improper food-handling techniques, poor personal hygiene, inadequate cleaning, and insufficient food preparation area to separate raw and cooked foods. Moreover, food preparers left food at room temperature for long periods of time allowed the packaging of raw meat to sit on the work area during further preparation, failed to wash hands frequently or at all, and failed to clean work areas effectively. Forty-seven percent of food preparers did not wash their hands after handling raw meat, and of the 53% that did wash their hands, only slightly over half (56%) used soap. Almost half of the households surveyed did not use a detergent or cleaning agent on kitchen surfaces at any time during monitoring. From the video data, they estimated the total estimated Australian weekly incidence of poor food-handling events to be 8.2×10^6 that is about 8 million events in homes per week that may lead to foodborne illness.

A foremost concern is when cooked food comes into contact with microorganisms from the environment or from raw foods. This is important because the microbial load on the cooked food has been greatly reduced so there are few microorganisms to compete with pathogens. Cross-contamination in itself is not extremely dangerous, but coupled with temperature abuse; it is a major cause for alarm (Tebbutt 1984). Even extremely low inoculum levels of *E. coli* and *Salmonella* may reproduce to dangerous levels, and some pathogens, such as *E. coli* O157:H7 may cause illness by ingestion of only 10 cells (Scott and others 1982, Bloomfield and Scott 1997).

Tebbutt (1984) studied the specifics of how people use cloths for cleaning food surfaces. He found that 96% of 234 locations used reusable cloths to apply cleaning agents, and only 24% provided separate cloths for raw and cooked food areas (Tebbutt 1984). In these locations, the cleaning process may actually be contributing to the occurrence of contamination. Finch and others (1978) sampled various kitchen items and found approximately 47% to harbor *S. aureus* in small numbers; these could serve another vehicle for transferring pathogens. Draining boards and joints around the faucet were also areas with high contamination levels (Tierney and others 2002).

Scott and Bloomfield (1993) analyzed different procedures for using cloths to clean countertops in a college kitchen. They found that after cleaning with only detergent, cloths and surfaces were more heavily contaminated than before cleaning. These same cloths were used for several days to clean kitchen surfaces and were contributing to the contamination of kitchen surfaces. Some of the cloths were heavily contaminated after being in use in the kitchen for only three hours. They compared the detergent cleaning with self-disinfecting cloths and found these cloths to reduce general contamination levels on surfaces.

Josephson and others (1997) sampled various items in the kitchen for the incidence of microbial contamination. All kitchen surfaces exhibited some degree of contamination indicating that bacteria are ubiquitous; however it is possible that any surface could be linked to food contamination. Most bacteria found in the kitchen were nonpathogenic; however most homes did have some pathogenic species present (Scott and others 1982). Domestic kitchen contamination was less than for

commercial kitchens, however the contamination levels found were significant and could likely lead to illness (Bloomfield and Scott 1997). Small numbers of potential pathogens were isolated from 49% of all food contact surfaces (Scott and others 1982). *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus, Streptococcus faecalis,* and *Staphylococcus aureus* were some of the potentially pathogenic organisms found in the kitchen (Finch and others 1978, Scott and others 1982, Tebbutt 1984). Microbial loads of some surfaces were quite high, Josephson and others (1997) found fecal coliform levels to be 2.17×10^4 cfu/25 cm^2. They found that the sink and the sponge were consistently contaminated, likely due to the constant availability of moisture. The contamination of the sponge could contribute to contamination of other areas of the kitchen due to the household use of sponges to wash dishes as well as wiping off surfaces.

Hilton and Austin (2000) performed a similar study involving dishcloths used as a possible vehicle for spreading bacteria from surface to surface in the domestic environment. Dishcloths and sponges are commonly used to wipe food contact surfaces. Once these items are contaminated they may be used several times on several different sites, thus possibly spreading the bacteria around on the surface and transferring the bacteria from surface to another, perhaps clean surface. Sponges are known to harbor a significantly greater microbial population compared to dishcloths. However, dishcloths were found to transfer a large number of bacteria to the surface, whereas sponges did not allow as many bacteria to transfer to the surface from the sponge. Hilton and Austin (2000) theorized that the cavernous nature of the sponge

prevented transfer of the microorganisms to the food contact surface. However, rinsing the dishcloth significantly reduced the number of bacteria transferred to the surface. The mechanical action of rinsing dishcloths may help to reduce the potential cross-contamination in the kitchen.

The classic cross-contamination example is the use of a cutting board to cut a raw chicken and then using the same cutting board to cut raw vegetables for a salad, thus transferring potentially pathogenic species from a raw food to a ready-to-eat food (Ak and others 1994, Doyle and others 2000). According to one survey, upwards of 60% of domestic food preparers do not wash the cutting board after cutting raw meat and before cutting raw vegetables (Doyle and others 2000). Consequently, several studies have investigated the prevalence of pathogen contamination on cutting boards. Various types of cutting boards are used, however most commercial operations (90%) provide separate cutting boards for raw and cooked foods, however in the home this is not always the case (Tebbutt 1984). Tebbutt (1984) tested cutting boards used in the food industry and found that cutting boards that were cleaned 'as required' were more often contaminated with pathogens, such as *E. coli*, than boards that were cleaned after each use.

Ak and others (1994) investigated cutting boards as a potential source of cross contamination. Plastic cutting boards were theorized to be easier to clean and therefore less likely to be contributing to cross contamination when compared to wooden cutting boards. Cutting boards were contaminated with pure cultures of *E. coli* O157:H7, *Listeria monocytogenes,* or *Salmonella typhimurium*. Wooden cutting

boards were found to yield fewer bacteria than plastic cutting boards after contamination. Cutting boards were also inoculated and held overnight. The bacterial numbers were greatly reduced or undetectable on wooden cutting boards, whereas the microorganisms had doubled at least four times on the plastic boards.

Meredith (2001) investigated the extent to which proper food hygiene interventions would prevent the spread of bacteria in the kitchen while preparing a chicken casserole. There were 40 key steps in the chicken recipe and each was assessed for risk and determined critical control points. Most of the critical control points were for hand washing, the remaining points were cleaning the cutting board, knife, and all surfaces and utensils when finished. Hand washing protocols and cleaning protocols were chosen based on similar procedures followed by videotaped volunteer cooks. The chicken was contaminated with *E. coli* DH5a, which contains pLITE 27 plasmid, so that bioluminescence could be used to measure contamination. 35 sites around the kitchen were chosen for sampling including two sites that were never used during food preparation. The most frequently contaminated sites were those used for cleaning and personal hygiene (i.e. dishcloths, towels and sink). When critical control points were not controlled (food hygiene violations), contaminated site numbers increased. Only two of the 35 sites were not contaminated, and they were the sites that were not touched throughout preparation. Washed items were found to be contaminated, indicating the lack of efficacy of hot, soapy water in removing and/or killing microorganisms. Kitchen utensils that were cleaned and dried with a towel were found to be contaminated, so post-cleaning contamination is likely to occur in a

domestic environment. These towels are often commonly used to dry hands as well, and most people in hospitals (52-79.8%) do not comply with hand washing protocol.

Bloomfield and Scott (1997) assessed various locations in the home and categorized these into four groups: reservoirs, reservoir/disseminator, hand and food contact surfaces, and other surfaces. They also recommended disinfection procedures for these areas. Wet areas in both the kitchen and the bathroom were identified as microbial reservoirs being highly contaminated and should be continuously disinfected. They determined that raw food was likely the major contamination source in the kitchen; however wet cleaning cloths may also serve as permanent reservoirs for microbial populations. Reservoir/disseminators included items, such as wet cloths or cleaning utensils, were critical areas in need of improvement to prevent the transfer of pathogenic bacteria. These areas should be disinfected immediately before and during use. Hand and food contact surfaces (cutting board, sink taps, toilet handles) should be disinfected immediately prior to use. Risk of transfer from these areas is relatively small, however potentially pathogenic organisms are often found in high levels. Other surfaces, such as walls and floors, are less likely to be involved in bacterial contamination of food products; however it is suggested that these areas should be disinfected occasionally.

Large reductions in the microbial load, especially Gram-negative organisms, on countertops are due to air-drying (Tebbutt 1988). Drying of surfaces is known to have a bactericidal effect; however its effect is dependent on the organism, temperature, presence of organic material, and humidity (Bloomfield and Scott 1997).

Air-drying may not be significant. Studies have indicated that if fingers or utensils are placed in contact with a dry, contaminated surface, significant numbers of organisms are transferred. Some organisms can survive for drying periods longer than four hours. *Salmonella enteritidis* was shown to survive 24 hours on Formica surfaces after food preparation (Bloomfield and Scott 1997).

Two conditions must be met in order for there to be a foodborne infection risk: there must be pathogenic bacteria present and there must be a route for bacteria to transfer from a surface to the food (Hilton and Austin 2000). Infections arising from food prepared in the home may be reduced if adequate kitchen hygiene is practiced (Josephson and others 1997). The action of cleaning may help to reduce microbial numbers due to the mechanical removal of the bacteria, however studies have shown that bacteria can remain on surfaces after cleaning, especially the cutting board and sink (Josephson and others 1997). Without using disinfectants, bacteria including fecal coliforms and pathogens may propagate in the kitchen environment (Scott and others 1984, Josephson and others 1997). However, just the use of a disinfectant is not ample to significantly reduce microbial numbers. Scott and others (1984) investigated kitchen surfaces prior to disinfectant use, immediately after disinfectant use and again several hours after disinfectant use. Several hours after disinfectant use, a large percentage of the sites had reestablished high microbial levels, therefore it is important to disinfect prior to food preparation as well as after food preparation.

Another problem associated with cleaning and disinfecting these surfaces is that there are surface irregularities that may harbor pathogenic bacteria (Tebbutt 1988,

Frank and Chmielewski 1997). This is especially true for wooden and plastic cutting boards that are damaged by repeated use with sharp objects (Tebbutt 1988).

Jones (1998) ranked the top risks in the home to be: kitchen work surface/utensil contamination during food preparation, unhygienic cleaning materials and appliances, poor personal hygiene in kitchen and toilet, unhygienic or inadequate food storage, surface, floor, and hand contamination from pets, insects, handling/washing soiled clothes, exposure to respiratory allergens, and unhygienic waste disposal bins. Other areas of the kitchen may be of concern as well, including the refrigerator which encourages the formation of biofilms, and the pantry which contains food stored for longer periods of time that can enhance the risk of infestation (Doyle and others 2000).

The general public needs to be aware of the risks of foodborne illness in the domestic environment and they should be encouraged to use a cleaning agent and disinfectant at least daily to minimize contamination issues (Bloomfield and Scott 1997, Tierney and others 2002). Health educators need to communicate to consumers that effective cleaning includes detergent, hot water, and a disinfecting agent (Meredith 2001). It is likely that disinfectants will have an increasing role to play in preventing foodborne illness as multi-drug resistant pathogens become more common and vaccination programs and antibiotics fail to control outbreaks (Jones 1998). An individual's domestic hygienic practices are based on their habits and their knowledge of potential risks (Jones 1998). Bacterial hazards are virtually impossible to visualize, therefore education about hygienic procedures in the home is a crucial step to control

disease. Opportunities for cross contamination in the domestic environment may be even greater than the commercial environment due to a number of factors: variety of foods, types of construction materials, layout and facilities, use of kitchen for non-food-related activities, presence of pets, cleaning processes, cleaning products, and lack of training of the food handler and/or cleaner (Worsfold and Griffith 1996, Jay and others 1999).

Disinfectants

Disinfectants are used in several different domestic situations: home hygiene, food hygiene, personal hygiene, and home healthcare (Bloomfield and Scott 1997). However, disinfectants available for use in the home are not as standardized as those available for commercial use (Worsfold and Griffith 1996). Very little information about the product's active ingredients or usage directions is available on the label and these products are usually more expensive than their commercial counterparts (Worsfold and Griffith 1996). Disinfectants and biocides are considered to be different from antibiotics because of their lack of selective toxicity (Denyer and Stewart 1998). Disinfectants may be bacteristatic and/or bactericidal. Their efficacy may be dependent on their concentration (Denyer and Stewart 1998).

Disinfectants may have a number of microbial target sites including: cell wall, cytoplasmic membrane, replication machinery, protein synthesis machinery, and enzyme function (Denyer and Stewart 1998, Ricke 2003). Biocidal compounds may damage microbial cells in a number of ways: disruption of the proton motive force,

inhibition of metabolic pathways, disruption of replication, compromised membrane integrity, lysis, and intracellular coagulation (Denyer and Stewart 1998). The cytoplasmic membrane is the most common target for biocides and disinfectants (Denyer and Stewart 1998). The cytoplasmic membrane offers a wide variety of potential targets including phospholipids, enzymes and structural proteins (Denyer and Stewart 1998).

Disinfection is the final step in a sanitation program that is designed to prevent foodborne illness (Taylor and others 1999). Conversely, it has been suggested that disinfectants are not required for food preparation surfaces because the cleaning process mechanically removes most of the contaminants from the work surface (Tebbutt 1984, Block 1991). However, it is also contended that detergent in hot water does not significantly reduce contamination levels on surfaces (Scott and others 1984). The proper use of a disinfectant may provide an extra margin of safety to protect against cross-contamination and reduce the risk of foodborne illness (Tebbutt 1984).

Targeted use of disinfectants is the most effective way to reduce contamination, this is particularly important after preparing foods that have a high risk for harboring pathogens, i.e. raw meat or seafood (Josephson and others 1997).

While the use of disinfectants is encouraged, casual or sporadic use does not decrease contamination levels (Josephson and others 1997). There is also no cumulative effect of repeated applications of disinfectants, contamination levels will return to base levels by six hours post-disinfection (Scott and others 1984). Whereas disinfection is commonly part of the cleanup procedure, studies have shown the

necessity of disinfecting prior to food preparation and between separate food preparation activities (Scott and others 1984). Tierney and others (2002) conducted a survey with responses indicating that only 20% used a cleaning agent at least daily, thus 80% are casually using cleaning agents. Assuming similar responses for disinfectant use, there would be a high majority of consumers using a disinfectant on a casual basis. Daily use of cleaning agents was observed by Tierney and others (2002) to keep total viable counts below 10^4 cfu/cm^2; however more casual use of cleaning agents displayed contamination levels up to 10^7 cfu/cm^2.

The efficacy of disinfectants greatly depends on environmental conditions. Contact time is crucial, longer contact times allow for greater reductions in microorganisms (Zhao and others 1998). Disinfection, like cleaning, can be done by applying and wiping the disinfectant over the entire surface. This process combines the killing properties of the disinfectant with physical removal of the microorganisms from the surface (Tebbutt 1988, Block 1991).

Concentrations of active ingredients are important to the efficacy of the disinfectant. Inadequately low concentrations will increase the risk of foodborne illness as well as the potential for microorganisms to develop resistance to the disinfectant (Heinzel 1998, Luppens and others 2002). Extremely high concentrations increase costs and environmental impact (Luppens and others 2002). It is important to have disinfectants that are easily applied, resist inactivation by organic matter, and are environmentally friendly (Gutierrez and others 1999).

Numerous compounds and processes have been used to decrease microbial contamination including: acids, bases, halogens, hydrogen peroxide, alcohols, mannose, ozone, and irradiation (Mullerat and others 1995).

There are four categories of commercially available disinfectants have active ingredients: hypochlorite, phenolic, quaternary ammonia compounds, or hydrogen peroxide. Hypochlorite based disinfectants are not commonly used for home food contact surfaces, due to their association with the toilet. However they are effective at reducing microbial loads on draining boards, sink surfaces, cutting boards, and countertops (Scott and others 1984). Daily application of hypochlorite disinfectants can produce substantial reductions in household contamination levels within 15 minutes of application; however the results are short-lived with reestablished contamination levels after 3 hours (Scott and others 1984). Phenolic disinfectants had the same effect as hypochlorite, being capable of reducing household contamination levels rapidly, but high contamination levels were reestablished after 3 hours (Scott and others 1984). However, phenolic based disinfectants performed poorly on countertops and cutting boards and residual disinfectant can cause food tainting (Scott and others 1984). Phenolic disinfectants act by disrupting the transmembrane pH gradient and membrane integrity (Denyer and Stewart 1998). Quaternary ammonia compounds that are available are innocuous at the concentrations used and they act by irreversibly binding to the cell membrane increasing membrane permeability (Gutierrez and others 1999). The major drawback of these compounds is that their efficacy is greatly reduced in the presence of organic matter (Gutierrez and others

1999). Hydrogen peroxide at relatively low concentrations (0.06%) is an effective disinfectant that is not affected by the presence of organic material (Aarnisalo and others 2000). Hydrogen peroxide acts by free radical oxidation of enzyme and protein thiol groups which inhibit cell metabolism (Denyer and Stewart 1998). However, hydrogen peroxide can be decomposed by metal impurities and reductive metabolites. Also hydrogen peroxide is naturally formed by some organisms, thus these organism have protective mechanisms such as catalase and peroxidases that provide resistance (Heinzel 1998). Other compounds have been explored for their potential use as disinfectants such as ethanol, tertiary alkylamines, and potassium persulfate however these are not major contributors to the commercial market (Aarnisalo and others 2000).

Several approaches are being explored to improve disinfectant action. Developing disinfectant formulations that enhance biocide accumulation or interaction with target site, determination of synergistic formulations, or possibly using compounds that encourage autocidal processes are among these (Denyer and Stewart 1998). The effectiveness of the disinfectant is an important parameter, however disinfectant use in preventing cross-contamination is also dependent on the application procedure, including both when and how it is applied (Bloomfield and Scott 1997). Disinfection for a specific purpose can be the most effective procedure for interrupting cross-contamination (Bloomfield and Scott 1997).

There are many factors that determine the efficacy of a disinfectant. Increasing exposure time of the disinfectant and increasing the concentration of the active

ingredients increased reduction of bacterial loads (Taylor and others 1999, Aarnisalo and others 2000). Microorganisms that are attached to surfaces are more resistant to disinfectants than unattached cells (Frank and Chmielewski 1997). Organic material protects bacteria from both desiccation and from inactivation by disinfectants (Gutierrez and others 1999). The presence of grease or dried food debris on surfaces may negatively impact disinfectant efficacy (Tebbutt 1988). Organic matter may provide a matrix that is difficult for the disinfectant to penetrate or may chemically inactivate the disinfectant (Mullerat and others 1995). Temperature may also play a crucial role in efficacy determination (Taylor and others 1999, Aarnisalo and others 2000).

Each of these methods have shown some efficacy as an antimicrobial agent or process, however all have disadvantages, e.g. food deterioration, stability issues, low target specificity, health risks for user, product safety concerns, or high cost (Mullerat and others 1995). Increasing environmental concern has encouraged the development of disinfectants that have lower infection risk and limit environmental impact (Bloomfield and Scott 1997).

Wine

Wine is characterized by high alcohol content, high organic acid content, and a low pH (Just and Daeschel 2003). The combination of these parameters contributes to the antibacterial environment of wine (Just and Daeschel 2003). Some studies involving survival of bacteria in wine have focused on survival of *Oenococcus oeni* for desirable malolactic fermentations. Temperature, ethanol, pH, and sulfur dioxide levels are believed to influence the malolactic fermentation (Vivas and others 1997).

Several studies have looked at wine as a protective tool against foodborne illness. Sheth and others (1988) studied the survival of *Salmonella typhimurium, Shigella sonnei*, and *Escherichia coli* in commercial beverages, all survived less than four hours in wine. The dramatic lethality of wine was contributed primarily to the low pH or possibly the combination of pH and 9.5% alcohol. Weisse and others (1995) analyzed beverages for safety of consumption to avoid infection from beverages and found *E. coli, S. typhimurium,* and *Shigella sonnei* to be completely inactivated after twenty minutes of exposure to red or white wine. Just and Daeschel (2003) determined that consumption of wine with a meal may protect individuals from foodborne illness by inactivating pathogens in a model stomach system. Bacteria survived longer in pinot noir wine compared to chardonnay (higher acidity) suggesting that acid concentrations play a significant role in microbial inactivation (Just and Daeschel 2003).

Schriever and others (2003) have demonstrated the ability of red wine to inhibit the growth of *Chlamydia pneumoniae*. Previous studies in their laboratory

(Mahady and Pendland 2001) have demonstrated the ability of red wine extract to inhibit *Helicobacter pylori* therefore possibly protecting consumers against peptic ulcers and gastric cancer. In both of these studies, resveratrol (a phenolic compound found in red wine) was also studied and found to have antimicrobial effects on the pathogens in question (Mahady and Pendland 2001, Schriever and others 2003)

Ariza and others (1992) tested 32 wines for mutagenic properties using the L-arabinose resistance test of *S. typhimurium*. One wine showed no mutagenic activity, while the other 31 wines showed a range of mutagenic activity to a maximum of 30-fold higher than the sensitivity limit. In general, red wines showed the highest level of mutagenicity. The production region and vintage did not correlate with mutagenicity, however individual production methods may be a primary factor involved with mutagenicity.

Titratable Acidity-Organic Acids

Organic acids are present in a number of foods, either present naturally or added as preservatives and contribute desirably to sensory attributes as well as to the overall retardation of microbial growth in the food (Marshall and others 2000, Zaika 2002, Ricke 2003). Inorganic acids may also have antimicrobial properties, however on an equimolar basis; organic acids are more inhibitory to microorganisms (Marshall and others 2000). Organic acids primarily are considered to be saturated straight-chain monocarboxylic acids and their derivatives (Ricke 2003). Acetic, citric, malic and tartaric acids are found in various fruits and vegetables at levels occasionally

exceeding 1% (Zaika 2002). Organic acids are also used in some decontamination procedures in the food industry (Zaika 2002). Citric, malic, and lactic acids at 2-5% are used in the meat industry to sanitize carcasses post-slaughter to decrease microbial load and prolong shelf-life (Zaika 2002). Organic acids (mainly tartaric, malic, and lactic acids) are present in wine due to their natural concentration in grapes as well as their increased (lactic) concentration during fermentation (Ricke 2003).

Just and Daeschel (2003) separated wines into volatile (ethanol) and non-volatile (acid) components and found the non-volatile fraction was more effective than the volatile fraction at killing *E. coli* O157:H7 and *S. typhimurium*. These results lead them to conclude that the acidic portion of wine is at least or more important than ethanol in decreasing bacterial survival when wine is consumed with a meal. Increased organic acid content may increase the efficacy of a wine-based disinfectant.

Organic acids are known to be bacteristatic and bactericidal depending on the health of the organism and the environment, however the antibacterial mechanism of the action is not fully understood (Ricke 2003). The antimicrobial activity of organic acids is dependent on several factors including: exposure time, temperature, type of acid, concentration of acid, dissociation level (pKa), and pH (Uljas and Ingham 1999, Marshall and others 2000). As the pH of the system decreases, the concentration of the more inhibitory undissociated form increases (Marshall and others 2000, Ricke 2003). Traditionally, it has been assumed that the undissociated form of the organic acid can cross the lipid bilayer, dissociate upon entry into the neutral cytoplasm and cause the internal pH to decrease (Uljas and Ingham 1999, Ricke 2003). The pH of

the bacterial cell needs to maintain neutrality in order to stabilize its functional macromolecules (e.g. enzymes, DNA) (Ricke 2003). In order to combat the decrease in pH, protons need to be actively pumped out of the cell consuming ATP, costing the cell a lot of energy (Ricke 2003). Organic acids may also interfere with the membrane structure and membrane proteins causing disruption of the electron transport chain thus preventing the cell from producing ATP (Ricke 2003).

The affect of organic acids on microorganisms has been described as causing a decrease in pH below the growth range and the inhibition of metabolic pathways (Russell 1992). Certain classes of organic acids and bases can cause disruption of the gradients across the cell membrane and cause an internal pH change (Russell 1992). However, it does not seem that high concentrations of organic acids can disrupt the proton motive force (Russell 1992). Acidic fermentation end products tend to accumulate intracellularly causing a detrimental drop in the cytoplasmic pH because of the cells inability to quickly expel protons to neutralize the internal pH (Russell 1992). The depressed intracellular pH causes the accumulation of anions leading to toxicity (Russell 1992).

Additional antibacterial mechanisms of organic acids include interference with nutrient transport, membrane damage causing leakage, disrupting outer membrane permeability, and interfering with macromolecular synthesis (Ricke 2003). Mechanisms are difficult to explore due to the complexity of the interactions between organic acids and cells (Ricke 2003). Organic acids target the transmembrane pH

gradient and disrupt membrane integrity causing leakage and disrupting transport, respiration, and energy coupling (Denyer and Stewart 1998).

It is difficult to assess the antimicrobial efficacy of individual organic acids due to their differing physical chemistry, as well as differences in bacterial cells (e.g. species, growth conditions, media composition, growth phase, etc) (Ricke 2003).

Hsiao and Siebert (1999) evaluated several organic acids to determine their minimum inhibitory concentration (MIC) against several microorganisms of concern in the food industry; the results are summarized as follows (Table 1.1):

Table 1.1. MIC values for selected organic acids.

Microorganism	**MIC Tartaric Acid (g/L)**	**MIC Lactic Acid (g/L)**	**MIC Malic Acid (g/L)**
B. cereus	5.90	3.48	13.6
B. subtilis	26.1	8.32	26.1
E. coli	50.0	3.72	50.0
L. fermentum	25.0	25.3	25.0
L. plantarum	26.0	30.7	26.0
Alicyclobacillus	7.07	5.39	4.58

From the table, it is not intuitive to determine which of these acids is the most effective. Lactic acid usually has the lowest MIC value for each organism. General antimicrobial rankings of organic acids used in the food industry are: tartaric acid is less effective than malic acid and citric acid, which are less effective than acetic acid and lactic acid (Zaika 2002). The efficacy appears to be related to the acid's pKa. Acids with higher pKa values are more effective than acids with lower pKa values (acetic pKa = 4.75, tartaric acid pKa = 2.98). This appears to be due to the

concentration of the undissociated form of the acid (Zaika 2002). Lactic acid has been shown to be an effective antimicrobial agent against *E. coli* O157:H7 in certain foods at pH 3.0 (Jordan, Glover and others 1999). Uljas and Ingham (1999) have shown that lactic acid has more of a bactericidal effect on *E. coli* O157:H7 than malic or citric acids. Malic acid concentrations in apple juice range from about 0.15% to 0.19% and are not significantly bactericidal (Uljas and Ingham 1999).

During malolactic fermentation, malic acid is converted to lactic acid by *Oenococcus oeni* (Vivas and others 1997). Enologists recommend malolactic fermentations in most red and some white wines, including chardonnay, to protect the wine from secondary fermentation after bottling (Vivas and others 1997). This organism has the amazing ability to not only survive the harsh conditions of wine but to grow and metabolize in a low pH, high ethanol, and sulfur dioxide environment (Carrete and others 2002).

Sulfur Dioxide

Sulfur dioxide has been used to control fermentation, regulate taste and extend the shelf life of many different food products, especially wine, for centuries (Dott and others 1976, Heinzel 1998, Jarvis and Lea 2000). Sulfur dioxide is added to wine to control oxidation reactions (by polyphenoloxidases), bind acetaldehyde, solubilize anthocyanins, and to restrict the growth or kill undesirable microorganisms, especially acetic acid bacteria (Dott and others 1976, Usseglio-Tomasset 1992, Carrete and others 2002). It is common for wine makers to add 50-100 parts per million of total

sulfur dioxide to the grape must (Carrete and others 2002). In the finished wine there may be sulfite levels of up to 50 parts per million in the free form and 300 parts per million of total sulfite (Dott and others 1976). Sulfur dioxide is usually at high levels in finished white wines due to their greater susceptibility to oxidation (Usseglio-Tomasset 1992). In our study, we are primarily concerned with the antimicrobial activity of sulfur dioxide.

In a wine, sulfur dioxide may be present as a result of the yeast activity during fermentation or as an intentional addition by the wine maker (Usseglio-Tomasset 1992). The formation of sulfur and sulfite by yeast is dependent on the yeast strain and the composition of the must (Eschenbruch and Bonish 1976). Wine-making yeast produce approximately 10-40 mg/L by enzymatic reduction of sulfate to sulfite which is actively secreted by the cell (Usseglio-Tomasset 1992, Heinzel 1998). Producing and excreting excessive levels of sulfite may be toxic to the yeast themselves, leading to a sluggish fermentation by inhibiting growth and influencing energy production (Dott and others 1976, Heinzel and Truper 1976). Although there are negative impacts to the yeast when sulfur dioxide is present, sulfur dioxide may encourage a desirable fermentation by suppressing the growth of other microorganisms leading to undesirable fermentations (Dott and others 1976).

Once added to wine, sulfur dioxide will come to equilibrium in its environment, being influenced by the pH as well as other chemical entities in the wine (Usseglio-Tomasset 1992). Therefore, sulfur dioxide will exist in equilibrium in its free form and bound forms (Usseglio-Tomasset 1992, Jarvis and Lea 2000). Binding

components arise in the juice from the berries as well as during the fermentation process (Jarvis and Lea 2000). Aldehyde and ketone groups react with sulfur dioxide, thus reducing its antioxidative and antimicrobial properties (Usseglio-Tomasset 1992). The three major sulfite binders produced during fermentation are acetaldehyde, pyruvate, and α-ketoglutarate (Dott and others 1976, Ussegio-Tomasset 1992, Jarvis and Lea 2000). Sulfur dioxide can also form complexes with anthocyanins (Usseglio-Tomasset 1992). The degree of binding is dependent on the concentration of the binding components as well as their reactivity (Jarvis and Lea 2000)

Bound sulfur dioxide is unable to inhibit microorganisms due to its complex with the binding components. Free sulfur dioxide exists in several forms dependent upon the pH of the solution (Hammond and Carr 1976). Of the various forms of sulfur dioxide, 'molecular SO_2' is the only form that can enter microbial cells and disrupt cell processes (Usseglio-Tomasset 1992, Jarvis and Lea 2000). A majority of the free sulfur dioxide in solution is in the form of bisulfite, acidifying the wine can cause an increase in the concentration of molecular SO_2 and thus increase the antimicrobial activity (Jarvis and Lea 2000, Block 1991). Concentration of molecular SO_2 can be determined by measuring the pH and free sulfur dioxide in the wine (Usseglio-Tomasset 1992). With decreasing pH, there is an increase in the percentage of free sulfur dioxide existing as molecular SO_2; at pH 4.00 only 0.64% of free sulfur dioxide is in the antimicrobial form, whereas at pH 3.00 6.06% is in the antimicrobial form (Usseglio-Tomasset 1992). Small concentrations of molecular SO_2 (0.5-1.0 parts per million) are effective at inhibiting microorganisms (Jarvis and Lea 2000). Molecular

SO_2 has been shown to be at least 1000 times more effective than bisulfite or sulfite ion against bacteria (Hammond and Carr 1976, Usseglio-Tomasset 1992). Molecular SO_2 has also been shown to be the most effective form against various yeasts and the mold *Aspergillus niger* (Hammond and Carr 1976, Usseglio-Tomasset 1992).

Sulfur dioxide has been suggested to be taken up by yeast cells through passive diffusion and active transport (Hammond and Carr 1976). Microorganims can absorb sulfate ions and successively reduce them to sulfite and sulfide for the biosynthesis of sulfur-containing amino acids (Hammond and Carr 1976). The uptake mechanism of sulfur dioxide by bacteria is primarily through diffusion (Carrete and others 2002).

Sulfur dioxide acts on microorganisms by direct inhibition of growth; this is a more effective process in Gram-negative bacilli as compared with gram positive bacilli (Hammond and Carr 1976). Free sulfur dioxide diffuses into *O. oeni* and some is converted to sulfite (HSO_3^-) (Carrete and others 2002). Once in the cell, sulfur dioxide can disrupt enzyme functionality by interacting with disulfide bonds of cysteine-cysteine peptides (Hammond and Carr 1976, Block 1991). Sulfur dioxide may also breakdown thiamine, an essential vitamin for most microorganisms (Hammond and Carr 1976, Block 1991). Furthermore, sulfur dioxide can interrupt purine synthesis by cleaving folic acid (Hammond and Carr 1976). Nicotinamide adenine dinucleotide (NAD) also interacts with sulfur dioxide making it unavailable for other cellular reactions (Hammond and Carr 1976). Sulfur dioxide is known to be mutagenic to microorganisms: causing transformation of cytosine bases to uracil (bisulfite), interfering with double helix formation, inactivating RNA and interfering

with transcription (Hammond and Carr 1976, Block 1991). Sulfur dioxide can also peroxidize lipids, altering membrane functionality and metabolism (Hammond and Carr 1976, Block 1991). Moreover, sulfur dioxide is capable of binding enzymatic intermediates thus influencing reaction equilibrium (Block 1991). All of these interactions are detrimental to the cell and may lead to cell death (Carrete and others 2002).

Ethanol Effects

Alcohols have been used for a number of years as preservatives and disinfectants; however ethanol use as a food disinfectant has received little attention (Ingram and Buttke 1984, Huang and others 2001).

Alcohol toxicity is related to their hydrophobicity and chain length (Ingram and Buttke 1984). Long chain alcohols (greater than 10 carbons) are ineffective at inhibiting bacterial growth; however medium chain alcohols are better inhibitors than short chain alcohols (Ingram and Buttke 1984).

Microbial species may show variable resistance to alcohols. The short chain alcohols (less than six carbons) are the only alcohols that lead to cellular lysis of *E. coli* (Yamashita and others 2001). Alcohols (ethanol, propanol, and isopropanol) have been shown to be effective at inactivating *L. monocytogenes* when suspended in the presence or absence of organic material (Aarnisalo and others 2000). Seventy percent ethanol is commonly used as a laboratory surface disinfectant based on its ability to kill various bacteria with an exposure time of 30 seconds (Block 1991, Huang and

others 2001). Concentrations of 30-100% ethanol by volume have been shown to kill *P. aeruginosa* within 10 seconds (Block 1991). Lower concentrations (20%) were not as efficacious as higher concentrations, however complete kill of *P. aeruginosa* was observed after 30 minutes of exposure (Block 1991). *S. aureus* and *Streptococcus pyogenes*, both Gram-positive vegetative bacteria, were more resistant to inactivation by ethanol (Block 1991). Recent studies have demonstrated that stationary phase cells are more resistant to ethanol than growing cells (Yamashita and others 2001).

A significant amount of research has been performed to understand alcohol toxicity in microorganisms important to fermentation industries, especially *Saccharomyces cerevisiae*. High concentrations of ethanol inhibit the uptake of glucose and other nutrients possibly by altering the lipid bilayer and protein carriers (Leao and Van Uden 1984). Alcohols may possibly interfere with the membrane potential, the transmembrane proton gradient, and the protein carriers in the membrane (Leao and Van Uden 1984). Alkanols increase leakage of potassium and sodium ions through membranes (Leao and Van Uden 1984).

The presence of ethanol has been correlated with low survival rates of *Escherichia coli* O157:H7 in several foods (Jordan and others 1999b). Ethanol can also uncouple the lactose transporter in *E. coli* likely leading to increased proton influx (Leao and Van Uden 1984). Ethanol has been used for a substitute for salt in producing low-salt and salt free miso (Huang and others 2001). In food products, ethanol provides protection against spoilage but still allows enzymes to function

properly during fermentation (Huang and others 2001). Ethanol concentrations of greater than 15% immediately inactivate vegetative cells (Ingram and Buttke 1984).

Lower concentrations of ethanol can sensitize bacterial cells to lower concentrations of other bactericidal processes (e.g. radiation or acids) (Ingram and Buttke 1984). Bacterial pathogens have been shown to be sensitized to various components by adding ethanol to treatment combinations (Brewer and others 2002). Efficacy of ethanol as a bactericidal agent becomes more enhanced at higher temperatures (Yamashita and others 2001). Most bacteria respond to ethanol concentration in a dose-dependent manner, with very few organisms capable of surviving in concentrations greater than 10% by volume (Ingram and Buttke 1984). Environments with high alcohol concentrations are analogous to environments with extreme pH or extreme temperature (Ingram 1986).

Two mechanisms are hypothesized for the inhibition of fermentation by ethanol: cell membrane damage and end product inhibition of enzymes (Ingram 1986, Brewer and others 2002). Ethanol can also cause disruption in the cross-linking of peptidoglycan, decreasing the rigidity of the cell wall making the cell more susceptible to lysis (Ingram and Buttke 1984). Ethanol freely diffuses across the cell membrane (Ingram 1986, Denyer and Stewart 1998). Once ethanol is within the cell membrane, it can disrupt the organization of the phospholipids by replacing water as the hydrogen bonding molecule and increase the fluidity of the membrane by solubilizing hydrophobic groups (lipids) and denaturing proteins (Ingram and Buttke 1984, Casey and Ingledew 1986, Ingram 1986, Brewer and others 2002). Interference of ethanol in

the cell membrane may also cause increased passive proton influx into the cell, thus disrupting transportation of certain nutrients (e.g. maltose, ammonium, and some amino acids) (Casey and Ingledew 1986). The membrane increases in polarity thus compromising the barrier properties of the membrane resulting in intracellular ethanol concentration quickly reaching equilibrium with the environment (Ingram 1986). Once inside the cell, ethanol is capable of slowing DNA, RNA, and protein synthesis rates (Ingram and Buttke 1984). Low ethanol concentrations (10-20%) cause cells to leak compounds necessary for glucose metabolism (e.g. nucleotides and magnesium) (Ingram 1986, Yamashita and others 2001). Ethanol may also interfere with glycolysis by denaturing critical pathway enzymes (Casey and Ingledew 1986). At higher ethanol concentrations (20-40%), it is thought that ethanol causes some damage to the respiratory system which leads to hydrogen peroxide production, subsequently resulting in cell death (Yamashita and others 2001).

Ethanol is an important antimicrobial compound present in wine. Just and Daeschel (2003) demonstrated the importance of ethanol concentration by comparing differences in bactericidal efficacy of grape musts and wines. *Salmonella typhimurium* survival decreased from 16 days to 10 minutes in pinot noir must and wine, respectively (Just and Daeschel 2003). *Escherichia coli* O157:H7 survival decreased from 3-12 days in chardonnay juice to between 44 and 57 minutes in chardonnay wine (Just and Daeschel 2003). However, 10% ethanol alone has been shown to not be inhibitory on bacterial growth (Sheth and others 1988). Huang and others (2001) observed that bacteria are inhibited by ethanol and hypothesized the use

of alcoholic beverages as disinfectants against food decontamination. They predicted that higher proof liquors consumed with food may decrease the risk of foodborne illness by acting on ingested pathogens (Huang and others 2001).

pH Effects

The pH of wine is consistently between 3.0 and 4.0. Enzymatic activity is pH dependent; therefore changes in the internal pH of the microbial cell can drastically affect cellular metabolism and growth (Kobayashi and others 2000). *Escherichia coli* O157:H7 has been shown to survive in pH 3.0 conditions from 2-5 hours up to 3 days (Jordan, Oxford and others 1999).

Sheth and others (1988) concluded from their experiments with commercial beverages that the low pH of some beverages, singly or in combination, may cause non-repairable levels of damage to Gram-negative bacteria. pH alone may not be enough to kill bacterial cells. Low pH beverages, such as apple juice (pH 3.3-4.1), that were once thought to be free of pathogenic bacteria may harbor pathogens such as *E. coli* O157:H7 and *Salmonella* (Uljas and Ingham 1999). The pH alone does not ensure product safety, as some bacteria with a low infectious dose may survive, but not grow, in these products and may lead to outbreaks of foodborne illness. Uljas and Ingham (1999) found pH to be the most important determinant in reducing bacterial populations in apple cider.

The pH of a complex solutions such as wine, may affect other antimicrobial components of the solution. Changes in pH will change the level of titratable acidity

of the wine as well as shifting the equilibrium of the organic acids to the antimicrobial undissociated form of the acid. A lower pH will also shift the sulfur dioxide equilibrium to favor the antimicrobial molecular sulfur dioxide form (Usseglio-Tomasset 1992). Decreasing pH levels may also increase the antimicrobial efficacy of ethanol (Jordan and others 1999b).

Volatile Acidity

Historically, acetic acid has been used longer than any other preservative (Marshall and others 2000). Acetic acid inhibits bacteria, yeasts and molds and is the most effective against bacteria (Block 1991). Aarnisalo and others (2000) tested the efficacy of a disinfectant containing 0.02% v/v peracetic acid and 0.03% v/v acetic acid and found that it was capable of reducing *L. monocytogenes* by three log cycles in five minutes under soiled conditions.

When dissolved in water, acetic acid dissociates thus decreasing the pH of the solution (Marshall and others 2000). The increased proton concentration can disrupt membrane function, denature enzymes, and alter permeability (Marshall and others 2000). The undissociated form of acetic acid can cross the lipid bilayer and dissociate in the cytoplasm causing a decrease in cytoplasmic pH (Marshall and others 2000).

Commercially, acetic acid is formed in a four-step process (Marshall and others 2000). Starch is converted to sugar by amylases and then the sugars are converted to ethanol by anaerobic processes in yeast (Marshall and others 2000). The final two steps are performed by aerobic acetic acid-producing bacteria (Marshall and

others 2000). These bacteria convert ethanol to hydrated acetaldehyde and then convert it to acetic acid using aldehyde dehydrogenase (Marshall and others 2000). During wine fermentation, it is likely that most of the acetic acid is produced when the ethanol concentration is less than 5% and when there is still oxygen available to the microorganisms (Rosell and others 1968).

Acetic acid has been evaluated for its antimicrobial properties. Effectiveness increases as concentration increases, pH decreases, temperature increases, and microbial load decreases (Marshall and others 2000). Gram-positive bacteria tend to be more resistant to acetic acid than Gram-negative species (Marshall and others 2000). Also, facultative anaerobic bacteria are more resistant than aerobic bacteria (Marshall and others 2000).

Phenolic Compounds

Polyphenolic compounds are widely distributed in nature, and are present in large amounts in plants, especially in vegetables, fruits, tea and wine (Puupponen-Pimia and others 2001).

There are over 4000 different phenolic flavonoids which can be categorized into several different subgroups: flavonols, flavones, flavanones, and anthocyanins (Puupponen-Pimia and others 2001). Flavonols and flavones are the most common flavonoids in plants; however anthocyanins are present in high amounts in some fruits and berries (Puupponen-Pimia and others 2001). Many plant phenols are known to possess antimicrobial properties, possibly due to mutagenic activity (Puupponen-Pimia

and others 2001). Ariza and others (1992) tested various wines for their mutagenic activity using the Ames test and found most wines to be mutagenic. The mutagenicity of the red wines was primarily attributed to the flavonols, especially quercitin and rutin which are not present in white wines (Ariza and others 1992).

Phenolic compounds may inhibit the growth and metabolism of some bacteria (Vivas and others 1997). Phenolic acids and tannins may inhibit the growth of lactic acid bacteria during malolactic fermentation, whereas anthocyanins may encourage this process (Vivas and others 1997). Vivas and others (1997) studied the influence of phenolic compounds on *O. oeni* growth and metabolism. According to this study, gallic acid and free anthocyanins in phosphate buffer enhanced cell viability, while other phenolic acids showed no influence (Vivas and others 1997). Vanillic acid was inhibitory to *O. oeni* growth rate and peak population (Vivas and others 1997). Phenolic compounds affect microorganisms differently; generally Gram-negative organisms are more sensitive to phenolic compounds than Gram-positive organisms (Puupponen-Pimia and others 2001).

The mechanisms involved with the interactions between phenolic compounds and bacteria are likely to be complex and are dependent on the specific compounds present in the wine (Vivas and others 1997).

Salt

Wine does not traditionally contain a significant concentration of salt. A large percentage of the cost of a bottle of wine is due to the taxation of the product by the

Bureau of Alcohol, Tobacco, and Firearms (BATF). The wine-based disinfectant developed in this study is not intended to be consumed as an alcoholic beverage and thus must be "denatured" to no longer be considered an alcoholic beverage and thus tax exempt. Similar to cooking wine, wine can be denatured by the simple addition of sodium chloride to a minimum level 1.5%.

Sodium chloride is a salt that exhibits a very low order of toxicity. Salt concentrations as low as 0.5% are capable of inhibiting microbial growth (Block 1991). However, some organisms are highly resistant to salt concentrations, including *S. aureus* which can grow in 16% salt solution at 37 degrees C (Hui and others 1994).

Huang and others (2001) studied the combination effects of sodium chloride and ethanol against *E. coli* and *S. aureus*. The presence of salt in nutrient broth enhanced the lethality of ethanol (Huang and others 2001). This combination likely caused the inactivation of microbial enzymes and the inhibition of microbial growth (Huang and others 2001). Due to their studies, Huang and others (2001) hypothesized the use of ethanol and sodium chloride as possible food preservatives or intervention strategies in the home.

CHAPTER 2

Investigation of role of sulfur dioxide concentration on bacterial survival in Chardonnay and complexity of predicting efficacy from wine composition

Joy G. Waite and Mark A. Daeschel

ABSTRACT

Wine is an inhospitable environment for most microorganisms and its consumption may protect individuals against microbial foodborne illness. Both volatile and non-volatile fractions of Chardonnay and Pinot noir have been shown to exhibit lethal affects on *Salmonella typhimurium* and *Escherichia coli* O157:H7. Increased levels of volatile acidity or poor market price can leave wine producers with a surplus of wine that cannot be sold for profit. By denaturing the wine, the producer could sell the wine as a base material for use in a disinfectant formulation.

An objective of this research was to explore the effect of chardonnay wine with different levels of sulfur dioxide on reduction of bacterial pathogens: *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and two strains of *Staphylococcus aureus.* The second objective was to examine the efficacy of five commercial chardonnay wines against *S. aureus* and determine important parameters for disinfectant composition.

Suspension tests were performed to estimate the antimicrobial activity of wines. Overnight bacterial cultures were diluted and added to wine at approximately 10^7 cfu/ml. Samples were taken after one minute and every two minutes for a total exposure of nine minutes.

Adding sulfur dioxide to the base wine increased the log reduction for all strains. Pathogens varied in their sensitivity to wine; *K. pneumoniae* and *P. aeruginosa* were more sensitive than *S. aureus* strains.

Four commercial Chardonnay wines were analyzed for their effectiveness against *S. aureus* 649. Sulfur dioxide concentration did not predict disinfectant efficacy for the commercial products. Log reduction varied from 0.25 to 4.70 (complete kill) after one minute. While sulfur dioxide concentration has a positive effect on disinfecting ability, more complex interactions of wine components likely determine efficacy.

Wine has the potential to be used as a food-contact surface disinfectant. Consumers are likely to desire an alternative to the 'chemical' disinfectants on the market due to a desire for more natural, environmentally-friendly disinfectants.

INTRODUCTION

It has been estimated that between 19 and 21% of foodborne outbreaks originate in the home, however this may be a gross underestimate due to underreporting and lack of investigation of small outbreaks (Zhao and others 1998, Tierney and others 2002). These outbreaks may be attributed to a number of causes, including temperature abuse, undercooking, and cross-contamination. Roberts (1990) reported that between 1970 and 1982 in England and Wales, 10% of foodborne illness outbreaks were linked to cross-contamination and/or infected food handlers. While there are numerous pathways in which cross-contamination can occur, the risk can be minimized by the food preparer if they are knowledgeable about the risk. Meredith (2001) determined critical control points in the preparation of a chicken casserole and found that washing hands and cleaning work surfaces significantly reduced

contamination from raw products to surfaces in the kitchen. Jones (1998) applied HACCP procedures to the domestic environment and ranked kitchen work surfaces as a high risk of cross contamination.

Disinfectants, if used correctly, can be a good tool to minimize the risk of cross contamination in the domestic environment. Targeted use of disinfectants, especially immediately prior to using the work surface, is highly effective at reducing the bacterial load on the work surface (Scott and others 1984, Josephson and others 1997). Numerous products are available on the market, however little information about the product's active ingredients or usage directions are available on the label (Worsfold and Griffith 1996). Some of the commercially available products possess undesirable attributes, including deterioration, stability issues, low target specificity, health risks to the user, product safety concerns, or high cost (Mullerat and others 1995). These concerns along with increased consumer awareness of environmental impact are encouraging the development of more environmentally friendly disinfectants (Bloomfield and Scott 1997).

Wine contains a number of components that are known to have antimicrobial properties. The low pH of wine combined with relatively high ethanol concentrations, organic acids, sulfur dioxide and polyphenols provide a composition that should be capable of killing pathogenic microorganisms. Several studies have investigated wine for its ability to protect the consumer against microbial foodborne illness (Sheth and others 1988, Weisse and others 1995, Just and Daeschel 2003). There are certain situations that could make wine desirable as a disinfectant. Wines containing

excessive volatile acidity can no longer be sold as wine, but could be denatured and formulated into a disinfectant. Poor market conditions resulting in surplus bulk wine may lead wine producers to consider making a disinfectant product rather than selling it to ethanol distilleries.

The easiest antimicrobial component for a wine producer to adjust is the level of sulfur dioxide present in the wine. Sulfur dioxide is present in wine either as a natural product of yeast fermentation or chemically added to grapes, juice, must, or finished wine to protect it from oxidation and microbial spoilage (Usseglio-Tomasset 1992).

This study was conducted to analyze the efficacy of wine as a disinfectant with increasing the level of sulfur dioxide against several common pathogens found in the home. The second portion of this study was to analyze several commercial Chardonnay wines for their effectiveness against *S. aureus*.

MATERIALS AND METHODS:

Culture information:

The strains used for these experiments were *Klebsiella pneumoniae* (601), *Pseudomonas aeruginosa* (603), *Staphylococcus aureus* (609), and *Staphylococcus aureus* (649).

All strains were cultured in tryptic soy broth at 30 degrees C and overnight cultures were used for all experiments.

Klebsiella pneumoniae was enumerated on tryptic soy agar for all experiments and plates were counted after incubation overnight at 30 degrees C. *Pseudomonas aeruginosa* was enumerated on plate count agar for all experiments and plates were counted after incubation overnight at 30 degrees C. *Staphylococcus aureus* strains were enumerated on brain-heart infusion agar for all experiments and enumerated after 24-48 hours incubation.

Treatments for sulfur dioxide trials:

A low sulfite chardonnay that had been produced at Oregon State University was used as the base wine. A 1% stock solution of potassium metabisulfite was made and added to the base wine to increase the sulfur dioxide level of the base wine to 100 parts per million and 200 parts per million. Sodium chloride was also added to these treatments at a level of 1.5%.

A commercial hydrogen peroxide based disinfectant served as a comparative control for these experiments.

Suspension test for sulfur dioxide trials:

One mL of overnight culture was transferred to a 99-ml dilution blank (Butterfields) to serve as the inoculum. Sixty-seven hundredths mL of inoculum was added to 6 mL of treatment in a small screwcap glass test tube. Test tube was shaken thoroughly and samples were enumerated after 1, 3, 5, 7, and 9 minutes of exposure

time. All enumeration was done using a spiral plater (Autoplate 4000, Spiral Biotech). Experiments were done in duplicate.

Treatments for wine trials:

Five different chardonnay wines were received from California wine brokers: a 2000 vintage from the Mendocino appellation (029), a 2000 vintage from the Monterey appellation (321), a 2000 vintage from the Lake appellation (339), and two 2001 vintages from the Clarksburg appellation (503 and 794). The base Oregon wine (940) was also included as a treatment.

Wine analyses:

Wines were analyzed for free and total sulfur dioxide, pH, titratable acidity, volatile acidity, and alcohol content. Free and total sulfite levels were determined by the pararosaniline method (Grant 1947, Morris 2003) as described (Assocation of Anayltical Chemists 1990, Morris 2003), using standards at 25, 50, 75, 100, 150, 200, 250, and 300 ppm sulfur dioxide. Molecular sulfur dioxide levels were calculated using graph from data in Usseglio-Tomasset (1992). The pH was measured using a pH meter (ORION ionanalyzer/501). Titratable acidity was determined by procedure of Zoecklein and others (1990) determining the endpoint of the titration at pH 8.2 using the pH meter previously mentioned. Volatile acidity and alcohol content were determined by procedure of Zoecklein and others (1990).

Suspension Test for wine trials:

An overnight culture of *S. aureus* (649) was diluted 10-fold to serve as the inoculum. Nine and nine tenths ml of wine was transferred to sterile 17x100 mm plastic culture tubes with dual position closures. One tenth mL of inoculum was transferred to the wine. Culture tube was vortexed and samples were enumerated after 1 and 3 minutes of exposure time. All enumeration was done using a spiral plater (Autoplate 4000). Experiments were done in duplicate.

RESULTS AND DISCUSSION:

Sulfur dioxide trials:

Wine was shown to be capable of killing four strains of bacteria in a suspension test. Studies by Sheth and others (1988), Weisse and others (1995), and Just and Daeschel (2003) showed similar effects against different bacterial species, *Salmonella, Shigella,* and *E. coli.*

Sulfur dioxide is known to inhibit the growth of microorganisms; the addition of sulfur dioxide to the wine used in this study enhanced the ability of the wine to kill bacteria in a suspension test (Table 2.1). Increased sulfur dioxide concentration dramatically improved the effect of the wine on *S. aureus* 649. The four different strains used in this study showed differences in their sensitivity to wine. *K. pneumoniae* 601 seemed to be the most sensitive, followed by *P. aeruginosa*, and the two *S. aureus* strains (609 and 649). None of the wine treatments achieved complete kill for either *S. aureus* strain. There appear to be differences in the two *S. aureus*

strains, strain 609 did not shown any real difference in sensitivity between the 100 ppm and the 200 ppm sulfur dioxide treatments, whereas strain 649 tended to have a higher sensitivity to the increased sulfur dioxide concentration.

Salt was included in the wine treatments with additional sulfur dioxide to mimic the level that would be added to the commercial wine disinfectant product. It was assumed that the salt would have a limited affect on the destruction of the microorganisms, but that may not be the case. An additional treatment including the base wine with salt would have made the possible affect more clear.

While the hydrogen peroxide disinfectant was very effective in this study, some additional factors need to be examined. Neutralization is an issue that is important when analyzing disinfectant abilities. Wine is a complex solution containing a number of known antimicrobial compounds. It would be extremely difficult to develop a neutralizer that would halt the effect of the wine on the microorganisms, however it seems likely that most of the antimicrobial compounds in wine are rapidly bound or neutralized when in contact with organic material, otherwise there would have been no microbial colonies on any of the plates. When designing the experiment, it was thought that neutralization would unnecessarily increase the variability of the experiments and therefore no neutralization step was performed. However, in previous studies, a hydrogen peroxide disinfectant was found to perform well under conditions with high organic material present (Aarnisalo and others 2000).

Table 2.1. Average log reduction of bacterial pathogens during suspension test in chardonnay wine with different levels of sulfur dioxide and a commercial hydrogen peroxide disinfectant.

Treatment	Exposure Time (min) Log Reduction				
Klebsiella pneumoniae 601[1]	1		3		5
A) Hydrogen Peroxide Disinfectant	7.14		7.14		7.14
B) Oregon Chardonnay	6.25		7.14		7.14
C) Oregon Chardonnay + 100 ppm Total SO_2 + 1.5% NaCl	6.71		7.14		7.14
D) Oregon Chardonnay + 200 ppm Total SO_2 + 1.5% NaCl	7.14		7.14		7.14
Pseudomonas aeruginosa 603[1]	1		3		5
A) Hydrogen Peroxide Disinfectant	7.49		7.49		7.49
B) Oregon Chardonnay	4.31[3]		7.49		7.25
C) Oregon Chardonnay + 100 ppm Total SO_2 + 1.5% NaCl	7.49		7.49		7.49
D) Oregon Chardonnay + 200 ppm Total SO_2 + 1.5% NaCl	7.49		7.49		7.49
Staphylococcus aureus 609[1]	1	3	5	7	9
A) Hydrogen Peroxide Disinfectant	7.42	7.09	7.42	7.42	7.42
B) Oregon Chardonnay	1.37	2.90[3]	4.04[3]	5.87	6.19
C) Oregon Chardonnay + 100 ppm Total SO_2 + 1.5% NaCl	2.96	6.16	6.45	6.94	6.71
D) Oregon Chardonnay + 200 ppm Total SO_2 + 1.5% NaCl	3.02	6.24	6.30	6.94	6.28
Staphylococcus aureus 649[2]	1	3	5	7	9
A) Hydrogen Peroxide Disinfectant	6.81	6.81	6.81	6.81	6.81
B) Oregon Chardonnay	1.94[4]	2.24	2.78[4]	3.18[4]	3.81
C) Oregon Chardonnay + 100 ppm Total SO_2 + 1.5% NaCl	3.23	4.31	5.87	6.16	6.81
D) Oregon Chardonnay + 200 ppm Total SO_2 + 1.5% NaCl	3.80	5.18	6.32	6.81	6.32

[1] Average of three replicates
[2] Average of two replicates
[3] One replicate was estimated at greater than 10^5 CFU/ml
[4] One replicate was enumerated using only one plate

This suggests that applying the sample to culture media may have not been significant to neutralize the disinfectant and may influence the results in this study. For all of the hydrogen peroxide treatments and platings, only one enumeration had any colonies present (*P. aeruginosa* 603 at 3 minutes).

Wine trials:

Five commercial Chardonnay wines from California and the base wine from Table 2.1 were used in suspension tests against *S. aureus* 649 (Table 2.2). Samples were taken after one and three minutes of exposure time.

Table 2.2. Log reduction of *Staphylococcus aureus* in Chardonnay after one and three minutes of exposure time in suspension tests.

Wine	029	321	339	503	794	940
1 minute	1.51, 1.44	2.27, 4.70	4.70, 4.70	0.25, 0.59	3.70, 4.70	1.62, 1.61
3 minutes	1.40, 1.21	4.70, 3.70	2.80, 4.70	0.31, 0.61	4.70, 1.01	1.46, [1]

[1] Enumeration was incomplete due to difficulty with wet plates

From the data, wines numbered 321, 339, and 794 appear to be the most effective against this strain. Samples 029 and 940 were less effective, and sample 503 appears to be the least effective disinfectant. Comparison of relative disinfecting ability and sulfur dioxide levels (Table 2.3) there appears to be no obvious correlation. Samples 339, 503, and 794 had the highest total sulfur dioxide concentrations, ranging from 155.73 ppm to 165.37 ppm. Total sulfur dioxide levels are not a good indication of antimicrobial activity, because a significant portion may be bound and therefore

inactive. All samples except 339 and 940 had relatively high levels of free sulfur dioxide, but the important component is the level of molecular sulfur dioxide in the system. Molecular sulfur dioxide concentration in samples ranged from 0.357 ppm (sample 339) to 0.804 ppm (sample 321).

Table 2.3. Sulfur dioxide concentration in Chardonnay wines.

Wine	pH	Total SO_2 (ppm)	Free SO_2 (ppm)	% of Free SO_2 in Molecular Form	Molecular SO_2 (ppm)
029	3.64	72.22	37.22	1.45	0.539
321	3.51	73.46	41.44	1.94	0.804
339	3.23	155.73	9.77	3.65	0.357
503	3.57	165.37	25.36	1.70	0.430
794	3.56	159.95	24.74	1.73	0.429
940	3.11	2.8	0.98	4.78	0.047

Each individual wine produced is different and contains different concentrations of hundreds of compounds. The pH, titratable acidity, ethanol, and volatile acidity concentrations were analyzed (Table 2.4). No single parameter appeared to correlate with the effectiveness of the wine against *S. aureus* 649. A combination of these components is likely to be important in determining the efficacy of each as a disinfectant. Wine samples 503 and 794 show extremely similar values for parameters tested, however there seemed to be drastic differences between results from the suspension tests. There are other possible wine components with antimicrobial properties, including polyphenolics, that could influence the disinfecting ability.

Table 2.4. Parameters of Chardonnay Wines.

Sample	Molecular SO_2 (ppm)	Free SO_2 (ppm)	Total SO_2 (ppm)	pH	Titratable Acidity (g/L tartaric acid)	Ethanol (%)	Volatile Acidity (g/L acetic acid)
029	0.539	37.22	72.22	3.64	6.44	14.35	0.480
321	0.804	41.44	73.46	3.51	7.33	13.50	0.762
339	0.357	9.77	155.73	3.23	6.39	12.85	0.648
503	0.430	25.36	165.37	3.57	8.88	13.05	0.552
794	0.429	24.74	159.95	3.56	8.33	12.85	0.546
940	0.047	0.98	2.80	3.11	9.32	14.10	0.264

There is high variability among log reduction values after one and three minutes. This strain had some difficulty growing under laboratory conditions so the inoculum level for these experiments was quite low (5.0×10^4 CFU/ml). Lower inoculum levels can lead to increased variability of the response (Johnston and others 2000). Another important factor to consider is the variability of resistance of the cells in the population (Johnston and others 2000). Johnston and others (2000) studied the effect of biomass on reproducibility of the suspension test and concluded that the presence of dead bacterial cells could negatively impact the disinfecting ability of a solution. The low inoculum level used in this experiment was based on growth of the culture overnight at 30 degrees C, the culture itself had only reached a density of approximately 10^7 cfu/ml prior to the experiment. It could be possible, though unlikely, that the culture was beginning to die and therefore dead cells present in the inoculum could have influenced the results. Probably the most likely cause of the variability was due to inadequate mixing of the sample prior to enumeration, samples should be more thoroughly mixed in order to minimize these possible effects.

CONCLUSION:

While there are some drawbacks to using suspension tests, in general these are seen as a good tool to estimate the efficacy of potential disinfectants. In this study, wine was found to be a good disinfectant candidate based on suspension test results against *K. pneumoniae, P. aeruginosa*, and two *S. aureus* strains. Increasing the concentration of sulfur dioxide and adding salt increased the ability of the wine to reduce microbial populations. When analyzing commercial Chardonnay wines, it became apparent that sulfur dioxide concentration, be it total, free or molecular, did not correlate with disinfection ability. Other wine parameters: pH, titratable acidity, ethanol, and volatile acidity, also did not simply correlate with disinfecting ability. Combinations of these parameters are likely important in determining effectiveness. Other wine components not measured in this study may be important as well (e.g. polyphenols).

Further studies should investigate combinations of different wine variables in attempt to predict more efficacious combinations. Surface tests should also be explored to more accurately assess wine as a disinfectant.

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CHAPTER 3

Determination of efficacious levels of pH, titratable acidity, ethanol, and sulfur dioxide in Chardonnay against *Escherichia coli* O157:H7 and *Staphylococcus aureus*

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ABSTRACT:

Wine is a complex solution containing several components that are known to have antimicrobial properties. Low pH (3.0-4.0), high organic acid content (titratable acidity greater than 6.0 g/L tartaric acid), relatively high ethanol (10-15%), and potentially high sulfur dioxide concentration (0-300 ppm) are some of the components that may contribute to the efficacy of wine as a disinfectant.

The objective of this research was to determine effective combinations of these four parameters on reducing bacterial populations of *Escherichia coli* O157:H7 and *Staphylococcus aureus*. A factorial design was used to test the four variables (pH, titratable acidity, sulfur dioxide, and ethanol) in combinations of low, medium, and high levels.

Suspension tests were performed to compare the efficacy of the 81 treatments against the two bacterial pathogens. Overnight cultures were added directly to the wine treatments to achieve a concentration of approximately 10^7 cfu/ml. Samples were plated after 20 minutes of exposure time. Suspension tests were performed in duplicate for each of the pathogens.

Increasing the concentration of any one component increased the lethality of the wine; however some combinations were more effective than others. The harshest treatment achieved complete reduction of both bacterial pathogens; however less harsh treatments achieved the same effect. Overall, *E. coli* O157:H7 was more sensitive to the wine treatments than *S. aureus*.

Wine destined to be used as a disinfectant could be enhanced by increasing any of the parameters tested in this study (acidity, organic acid content, ethanol, and sulfur dioxide). Other components, such as volatile acidity or phenolics, in the wine likely contribute to the overall effectiveness as well but likely play a smaller role.

INTRODUCTION:

The domestic environment may be contributing to upwards of 19% of foodborne outbreaks (Zhao and others 1998, Tierney and others 2002). Some percentage of these outbreaks is likely due to poor household hygiene. Targeted disinfectant use may minimize the infections risk in the home (Scott and others 1984, Josephson and others 1997).

Wine is a complex solution containing a number of antimicrobial parameters. Several studies have demonstrated the possible ability for wine consumption to protect individuals from foodborne illness (Sheth and others 1988, Weisse and others 1995, Just and Daeschel 2003). These antimicrobial properties may be effective against household pathogens and application on household surfaces therefore a wine-based disinfectant may be a useful tool to minimize cross contamination in the domestic environment.

High concentrations of ethanol are used in laboratories to decontaminate surfaces with short exposure times (Block 1991, Huang and others 2001). Extensive research on the ethanol toxicity to yeast and lactic acid bacteria have been studied due

to their importance in beer and wine production (Leao and Van Uden 1984, Ingram 1986, Brewer and others 2002).

Sulfur dioxide is present in wine comes from two different sources. Sulfur dioxide is a natural byproduct of yeast which may contribute levels between 10 and 40 mg/L (Usseglio-Tomasset 1992, Heinzel 1998). Winemakers may also add sulfur dioxide, in various forms, primarily to control oxidation and prevent microbial spoilage throughout the winemaking process (Dott and others 1976, Usseglio-Tomasset 1992, Carrete and others 2002).

Wine is a very acidic environment, primarily due to the presence of tartaric and lactic acids. Organic acids are known to possess antimicrobial properties, but their effectiveness is dependent on the type of acid, the concentration of the acid, dissociation level and pH (Uljas and Ingham 1999, Marshall and others 2000). Wine consistently has a low pH in the range of 3.0-4.0. The pH level cause considerable variation to the effectiveness of antimicrobial compounds. Alone, pH can cause a loss of enzyme function; however low pH alone does not ensure sterilization (Kobayashi and others 2000, Uljas and Ingham 1999). Decreasing pH enhances the activity of ethanol against microorganisms, shifts the equilibrium of organic acids towards the undissociated form, and increases the titratable acidity.

Several of the known antimicrobial parameters are able to be adjusted in order to produce an optimal disinfectant. This study was designed to look at four wine parameters: pH, titratable acidity, sulfur dioxide concentration, and ethanol

concentration in various combinations to evaluate antimicrobial activity against *S. aureus* 710 and *E. coli* O157:H7 716.

MATERIALS & METHODS

Culture information:

The strains used for these experiments were *Staphylococcus aureus* (710) and *Escherichia coli* O157:H7 (716).

The strains were cultured in brain-heart infusion broth at 37 degrees C and overnight cultures (stationary phase cells) were used for all experiments. All experiments were performed in a class II biological safety cabinet. Strains were enumerated on brain-heart infusion agar (Difco) for all experiments. *Escherichia coli* plates were counted after incubation overnight at 37 degrees C. *Staphylococcus aureus* plates were counted after 48 hours of incubation at 37 degrees C.

Treatments:

The base wine for these experiments was Badger Mountain (no sulfites added) Organic Chardonnay 2002 Columbia Valley. To this wine, combinations of pH, titratable acidity, sulfur dioxide, and ethanol were added in a factorial design with three levels of each treatment (Table 3.1). The pH was adjusted using 6N hydrochloric acid to either pH 3.25 or pH 3.00 as measured by pH meter (ORION ionanalyzer/501). Titratable acidity was adjusted by adding 37%(w/v) tartaric acid to increase the titratable acidity by 2 grams per liter or 4 grams per liter. Sulfur dioxide levels were adjusted by adding potassium metabisulfite to increase the total sulfur

dioxide concentration by 50 ppm or 150 ppm. Ethanol levels were adjusted by adding 95% ethanol to increase the alcohol concentration by 1.5% or 3.0%.

Table 3.1. Factorial design of wine treatments.

ADGJ	**AEGJ**	**AFGJ**	**BDGJ**	**BEGJ**	**BFGJ**	**CDGJ**	**CEGJ**	**CFGJ**
ADGK	**AEGK**	**AFGK**	**BDGK**	**BEGK**	**BFGK**	**CDGK**	**CEGK**	**CFGK**
ADGL	**AEGL**	**AFGL**	**BDGL**	**BEGL**	**BFGL**	**CDGL**	**CEGL**	**CFGL**
ADHJ	**AEHJ**	**AFHJ**	**BDHJ**	**BEHJ**	**BFHJ**	**CDHJ**	**CEHJ**	**CFHJ**
ADHK	**AEHK**	**AFHK**	**BDHK**	**BEHK**	**BFHK**	**CDHK**	**CEHK**	**CFHK**
ADHL	**AEHL**	**AFHL**	**BDHL**	**BEHL**	**BFHL**	**CDHL**	**CEHL**	**CFHL**
ADIJ	**AEIJ**	**AFIJ**	**BDIJ**	**BEIJ**	**BFIJ**	**CDIJ**	**CEIJ**	**CFIJ**
ADIK	**AEIK**	**AFIK**	**BDIK**	**BEIK**	**BFIK**	**CDIK**	**CEIK**	**CFIK**
ADIL	**AEIL**	**AFIL**	**BDIL**	**BEIL**	**BFIL**	**CDIL**	**CEIL**	**CFIL**

pH - ABC	Titratable Acidity - DEF	SO_2 - GHI	Ethanol - JKL
A = Base	D = Base	G = Base	J = Base
B = 3.25	E = +2.0 g/L	H =+50 ppm	K = +1.5%
C = 3.00	F = +4.0 g/L	I =+150 ppm	L = +3.0%

Suspension test:

Sixteen hour (stationary phase) cultures were used as the inoculum. Nine and nine-tenths ml of wine was transferred to sterile 17x100 mm plastic culture tubes with dual position closures. One-tenth mL of overnight culture was transferred to the wine. Test tube was vortexed after inoculum was added and again prior to plating. Samples were plated after 20 minutes of exposure time. All plating was done using a spiral plater (Autoplate 4000, Spiral Biotech). Experiments were performed in duplicate. True replicates were not performed for these experiments; technical replicates were completed, so as to estimate variability among inocula.

Wine analyses:

Wines were analyzed for free and total sulfur dioxide, pH, titratable acidity, and alcohol content. Free and total sulfite levels were determined by the pararosaniline method (Grant 1947, Morris 2003) as described (Association of Analytical Chemists 1990), using standards of 5, 10, 15, 20, 50, 100, 150, and 200 ppm sulfur dioxide. Molecular sulfur dioxide levels were calculated using free sulfur dioxide levels and pH (Usseglio-Tomasset 1992). The pH was measured using a pH meter (ORION ionanalyzer/501). Titratable acidity was determined by the procedure described by Zoecklein and others (1990). Alcohol content was measured using an ebuilliometer which is based on boiling point depression.

RESULTS & DISCUSSION

Wine treatments were adjusted and average parameter values are shown in Table 3.2. Several of the individual adjustments affected the levels of other parameters in the wine. Decreasing the pH caused the titratable acidity to increase and vice versa. Decreasing the pH and/or increasing the titratable acidity caused an increase in the concentration of molecular sulfur dioxide concentration. Increasing the concentration of sulfur dioxide or ethanol did not appear to affect the other parameters.

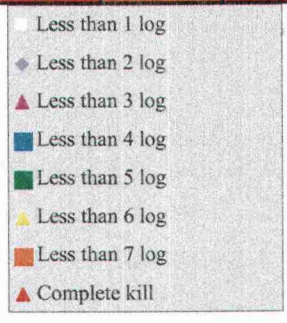
Table 3.2. Average measured values and standard deviation for wine treatments.

Sample	pH		Titratable Acidity (g/L)			
	Average	Standard Deviation	Average	Standard Deviation		
AD	3.72	0.02	6.42	0.35		
AE	3.51	0.05	8.73	0.34		
AF	3.27	0.08	10.55	0.08		
BD	3.21	0.02	7.65	0.30		
BE	3.03	0.02	9.69	0.40		
BF	2.91	0.01	11.09	0.94		
CD	2.96	0.02	8.37	0.41		
CE	2.84	0.01	10.04	0.45		
CF	2.74	0.01	12.10	0.61		
	Total Sulfur Dioxide (ppm)		Free Sulfur Dioxide (ppm)		%Molecular Sulfur Dioxide	Molecular Sulfur Dioxide (ppm)
	Average	Standard Deviation	Average	Standard Deviation	Average	Average
ADG	27.9	13.1	3.9	5.2	1.21	0.05
AEG	27.9	13.1	3.9	5.2	1.94	0.08
AFG	27.9	13.1	3.9	5.2	3.33	0.13
BDG	27.9	13.1	3.9	5.2	3.82	0.15
BEG	27.9	13.1	3.9	5.2	5.73	0.22
BFG	27.9	13.1	3.9	5.2	7.51	0.29
CDG	27.9	13.1	3.9	5.2	6.71	0.26
CEG	27.9	13.1	3.9	5.2	8.79	0.34
CFG	27.9	13.1	3.9	5.2	11.02	0.43
ADH	78.6	17.1	24.5	6.5	1.21	0.30
AEH	78.6	17.1	24.5	6.5	1.94	0.48
AFH	78.6	17.1	24.5	6.5	3.33	0.82
BDH	78.6	17.1	24.5	6.5	3.82	0.94
BEH	78.6	17.1	24.5	6.5	5.73	1.40
BFH	78.6	17.1	24.5	6.5	7.51	1.84
CDH	78.6	17.1	24.5	6.5	6.71	1.64
CEH	78.6	17.10	24.5	6.5	8.79	2.15
CFH	78.6	17.10	24.5	6.5	11.02	2.70
ADI	191.1	28.20	115.0	19.0	1.21	1.39
AEI	191.1	28.20	115.0	19.0	1.94	2.23
AFI	191.1	28.20	115.0	19.0	3.33	3.84
BDI	191.1	28.20	115.0	19.0	3.82	4.39
BEI	191.1	28.20	115.0	19.0	5.73	6.59
BFI	191.1	28.20	115.0	19.0	7.51	8.64
CDI	191.1	28.20	115.0	19.0	6.71	7.71
CEI	191.1	28.2	115.0	19.0	8.79	10.11
CFI	191.1	28.2	115.0	19.0	11.02	12.67
	Ethanol (%)					
	Average	Standard Deviation				
J	12.08	0.33				
K	13.28	0.23				
L	14.66	0.54				

Overall suspension test results (log reduction after 20 minutes of exposure time) for *E. coli* O157:H7 and *S. aureus* are shown in Figures 3.1-3.4. Due to the large amount of data, treatments have been grouped by log reduction values. Log reduction values of *E. coli* O157:H7 after exposure to different wine treatments for 20 minutes ranged from 2.15 to 7.39. *Staphlococcus aureus* was more resistant to treatments with log reductions ranging from 0.82 to 7.31.

Table 3.3. *Escherchia coli* O157:H7 treatments color coded for average log reduction after 20 minutes of exposure time.

ADGJ	AEGJ	AFGJ	BDGJ	BEGJ	BFGJ	CDGJ	CEGJ	CFGJ
ADGK	AEGK	AFGK	BDGK	BEGK	BFGK	CDGK	CEGK	CFGK
ADGL	AEGL	AFGL	BDGL	BEGL	BFGL	CDGL	CEGL	CFGL
ADHJ	AEHJ	AFHJ	BDHJ	BEHJ	BFHJ	CDHJ	CEHJ	CFHJ
ADHK	AEHK	AFHK	BDHK	BEHK	BFHK	CDHK	CEHK	CFHK
ADHL	AEHL	AFHL	BDHL	BEHL	BFHL	CDHL	CEHL	CFHL
ADIJ	AEIJ	AFIJ	BDIJ	BEIJ	BFIJ	CDIJ	CEIJ	CFIJ
ADIK	AEIK	AFIK	BDIK	BEIK	BFIK	CDIK	CEIK	CFIK
ADIL	AEIL	AFIL	BDIL	BEIL	BFIL	CDIL	CEIL	CFIL



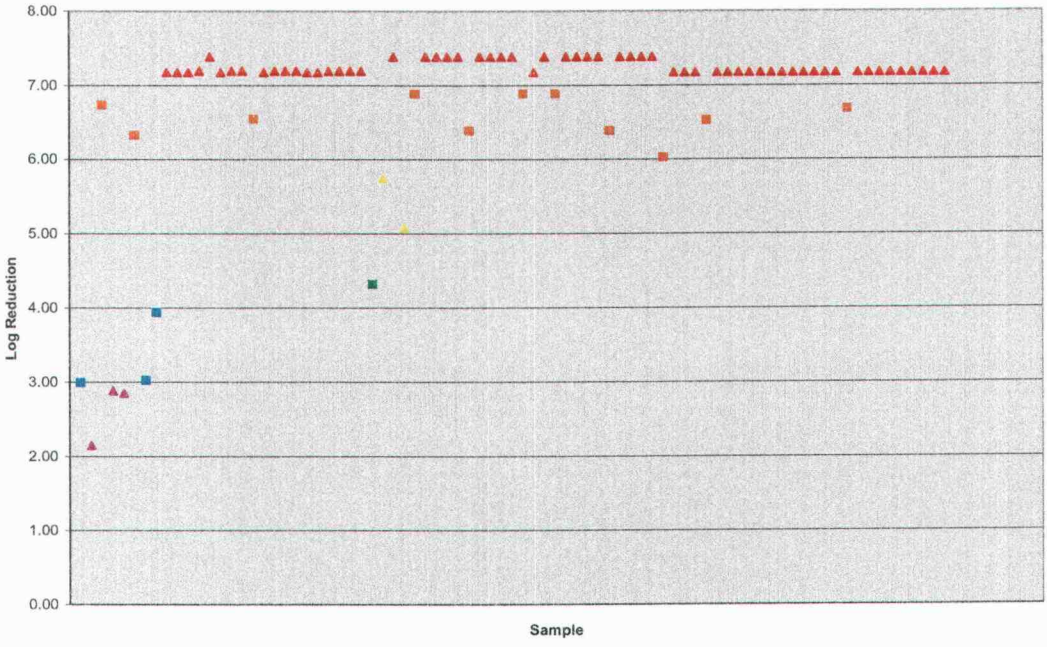


Figure 3.1. Log reduction of *Escherichia coli* O157:H7 after 20 minutes exposure to different wine treatments.

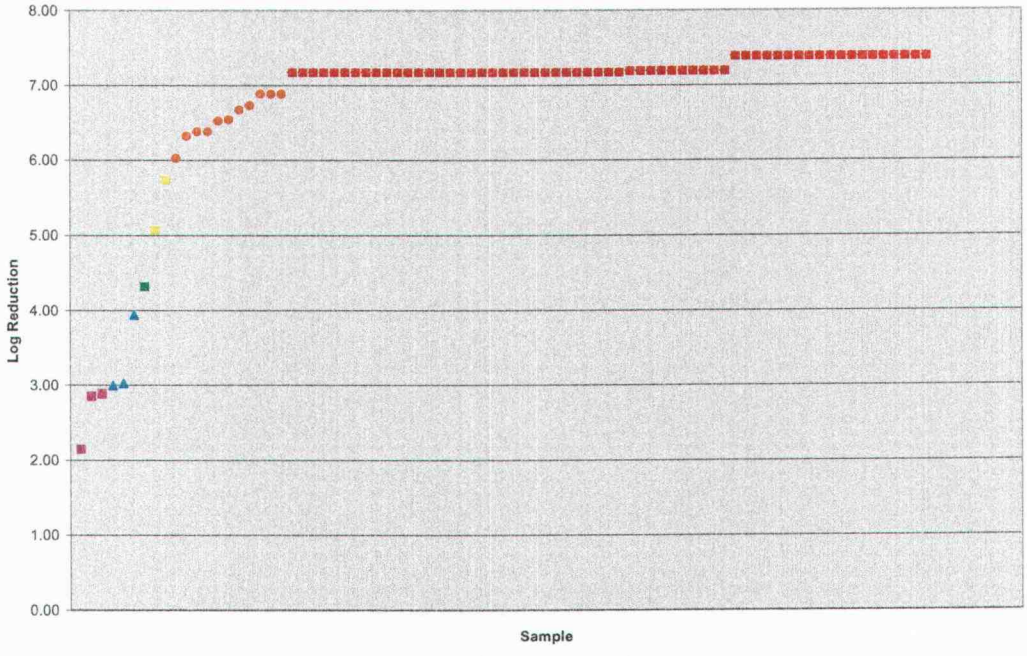


Figure 3.2. Log reduction of *Escherichia coli* O157:H7 after 20 minutes exposure to different wine treatments - sorted by treatment effectiveness.

Table 3.4. *Staphylococcus aureus* treatments color coded for average log reduction after 20 minutes of exposure time.

ADGJ	AEGJ	AFGJ	BDGJ	BEGJ	BFGJ	CDGJ	CEGJ	CFGJ
ADGK	AEGK	AFGK[1]	BDGK	BEGK	BFGK	CDGK	CEGK	CFGK
ADGL	AEGL	AFGL	BDGL	BEGL	BFGL	CDGL	CEGL	CFGL
ADHJ	AEHJ	AFHJ	BDHJ	BEHJ	BFHJ	CDHJ	CEHJ	CFHJ
ADHK	AEHK	AFHK	BDHK	BEHK	BFHK	CDHK	CEHK	CFHK
ADHL	AEHL	AFHL	BDHL	BEHL	BFHL	CDHL	CEHL	CFHL
ADIJ	AEIJ	AFIJ	BDIJ	BEIJ	BFIJ	CDIJ	CEIJ	CFIJ
ADIK	AEIK	AFIK	BDIK	BEIK	BFIK	CDIK	CEIK	CFIK
ADIL	AEIL	AFIL	BDIL	BEIL	BFIL	CDIL	CEIL	CFIL

[1] Treatment not repeated

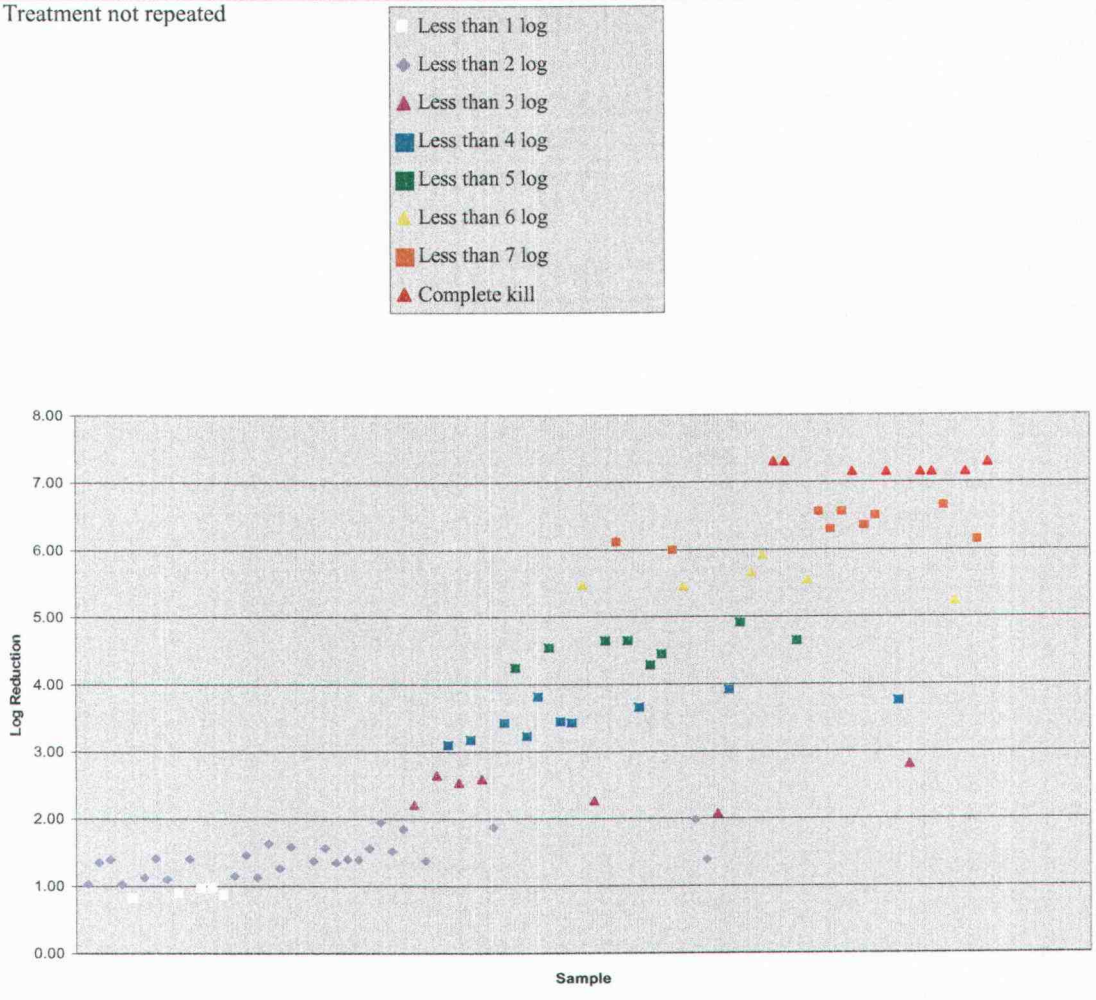


Figure 3.3. Log reduction of *Staphylococcus aureus* after 20 minutes exposure to different wine treatments.

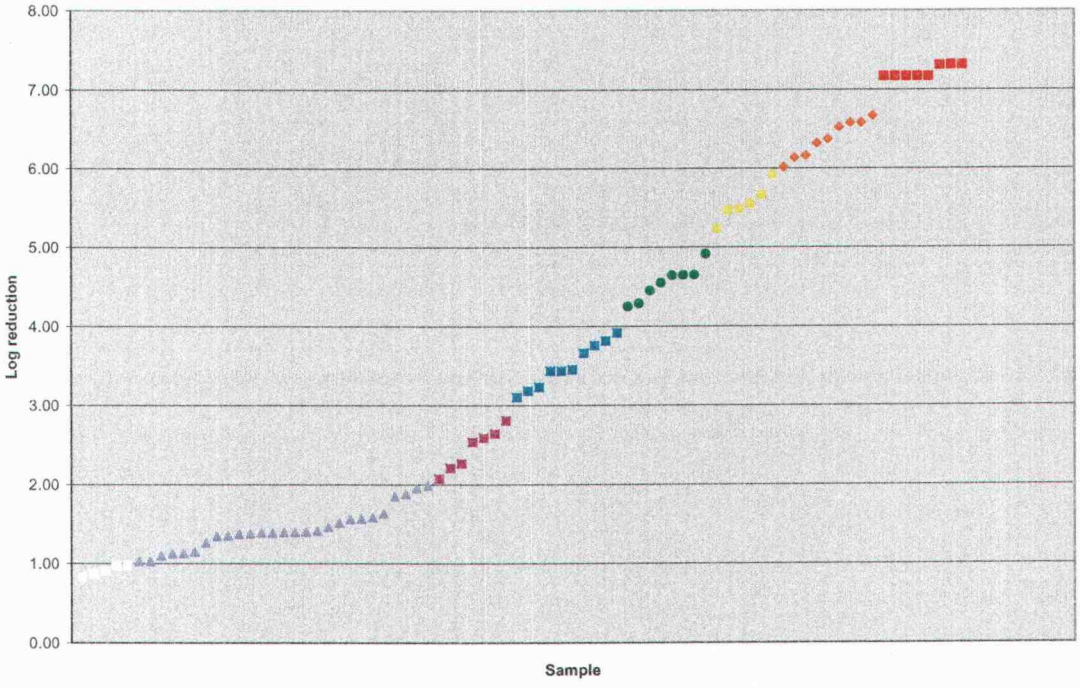


Figure 3.4. Log reduction of *Staphylococcus aureus* after 20 minutes exposure to different wine treatments – sorted by treatment effectiveness.

Figures 3.1-3.4 have been color coded to match log reduction values on Tables 3.3 and 3.4 to visually display the treatment combinations and there effectiveness against *E. coli* O157:H7 (Figures 3.1 and 3.2) and *S. aureus* (Figure 3.3 and 3.4). The base wine (ADGJ) was not always the least effective treatment; however it was consistently producing little over a 1 log reduction in *S. aureus*. The difference in log reduction among the least effective treatments is unlikely to be significant. The 'harshest' treatment (CFIL) consistently inactivated all cells of both *S. aureus* and *E. coli* O157:H7.

Figures 3.1 and 3.2 have the x-axis arranged to display the order of samples shown in Table 3.1. In Figure 3.2, the data from Figure 3.3 has been sorted by

effectiveness (log reduction) in order to more easily visualize the data; this is the same for Figure 3.4 from Figure 3.3. By looking at the overall data from these experiments, it is more apparent to see that there are differences in the resistance of *E. coli* O157:H7 and *S. aureus*. *Escherichia coli* O157:H7 was much more sensitive to the wine combinations that *S. aureus*. *Escherichia coli* O157:H7 is capable of causing life threatening illness with a low infectious dose and is known to be able to persist in acidic environments (Just and Daeschel 2003). Taylor and others (1999) have shown that although *E. coli* O157:H7 is known to persist in acidic conditions; it is usually not considered a resistant organism in suspension tests. From these results, the focus of determining an effective combination of parameters is focused on the results from *S. aureus*.

Figures 3.5-3.8 display the same data as Figure 3.4, with different variables being highlighted, to aid in determining simple relationships between single variables and effectiveness. Figure 3.5 has differing ethanol concentration highlighted. The three levels of ethanol (12.1, 13.3, and 14.7%) are scattered throughout the graph. There appears to be no simple correlation between increased ethanol concentration and increased bactericidal activity. Higher levels of ethanol did not predict disinfectant efficacy.

Figure 3.6 focuses on log reduction with respect to titratable acidity. There were nine different levels of titratable acidity in the experimental set, ranging from 6.42 g/L tartaric acid to 12.10 g/L tartaric acid. These levels were categorized into

three groups to aid visualization. Titratable acidity levels did not simply correlate with increased bactericidal actions.

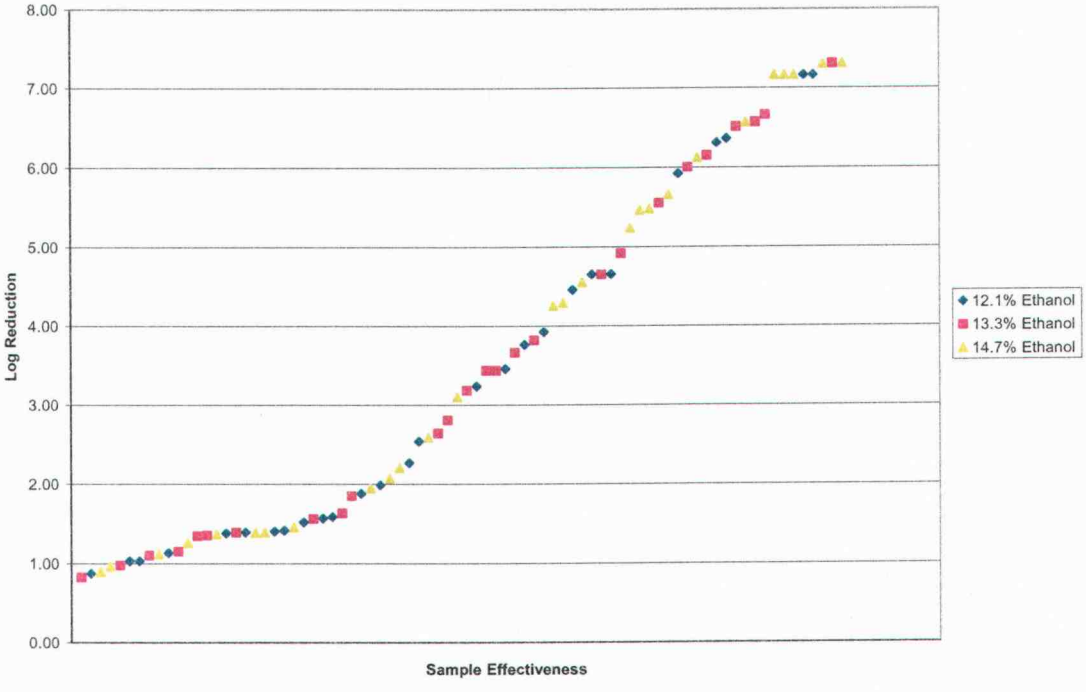


Figure 3.5. Relationship between effectiveness and ethanol concentration in suspension tests against *Staphylococcus aureus.*

Figure 3.7 focuses on the effect of molecular sulfur dioxide concentrations on *S. aureus*. Molecular sulfur dioxide levels are based on the amount of free sulfur dioxide present in the solution and the pH; calculated molecular sulfur dioxide levels ranged from 0.05 mg/L to 12.67 mg/L. These were categorized into three groups: less than 1 ppm, between 1 and 4 ppm, and greater than 4 ppm molecular sulfur dioxide. From this graph (Figure 3.7), it appears that treatments with high molecular sulfur dioxide concentrations (greater than 4 mg/L molecular sulfur dioxide) tend to be more

effective than samples with low molecular sulfur dioxide levels (less than 1 mg/L molecular sulfur dioxide) at reducing *S. aureus* in a suspension test. Molecular sulfur dioxide levels are dependent on pH. The pH of the solution shifts the equilibrium in favor of the more antimicrobial form of sulfur dioxide.

From Figure 3.8, it is apparent that pH plays an important role in the bactericidal effect of wine. pH values of the treatments ranged from 2.74 to 3.72 and were grouped into three ranges. Those treatments with very low pH values (less than 2.95) tend to be the most effective treatments. Mid pH range treatments (between 2.95 and 3.25) tended to be moderately effective, and high pH treatments tended to be the least effective at reducing cell numbers of *S. aureus*. Low pH solutions have antimicrobial characteristics; however several antimicrobial compounds may have enhanced activity when supplemented with a low pH. Low pH shifts the equilibrium of organic acids to have a higher concentration of the more efficacious undissociated form (Marshall and others 2000, Ricke 2003). The pH also affects the antimicrobial properties of sulfur dioxide. As the pH is lowered, the concentration of the antimicrobial molecular form increases (Usseglio-Tomassett 1992, Jarvis and Lea 2000).

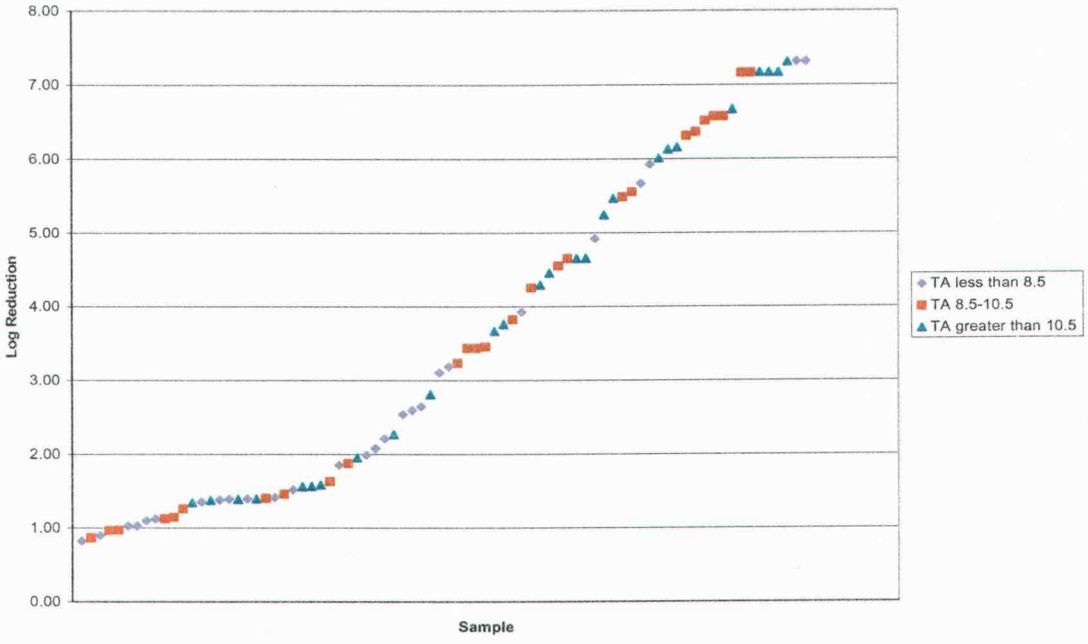


Figure 3.6. Relationship between effectiveness and titratable acidity in suspension tests against *Staphylococcus aureus.*

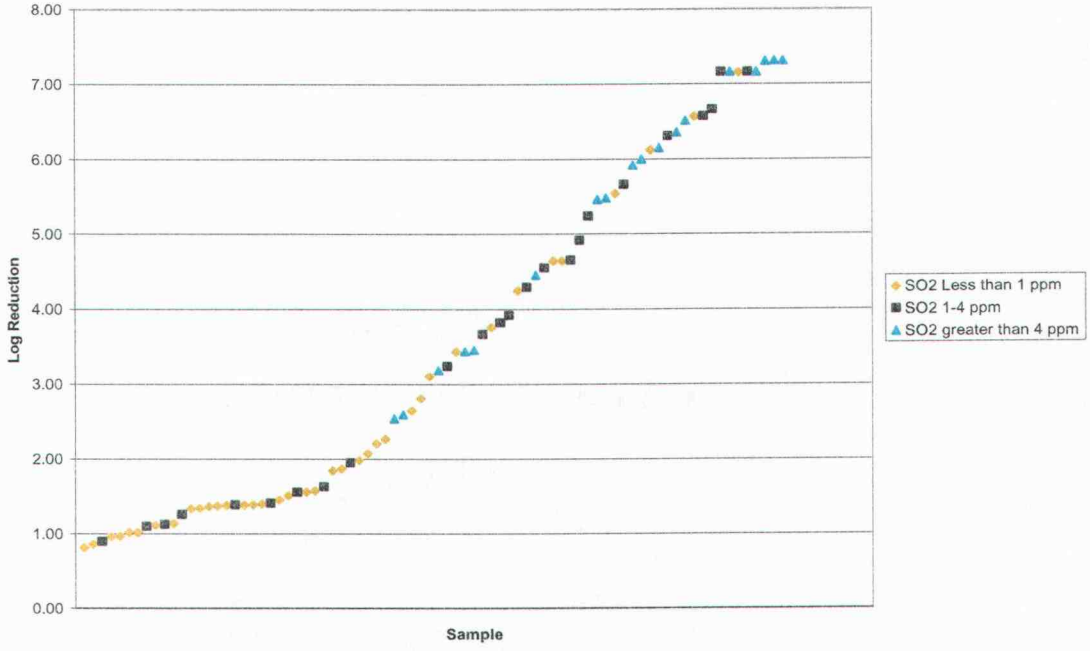


Figure 3.7. Relationship between effectiveness and molecular sulfur dioxide concentration in suspension tests against *Staphylococcus aureus.*

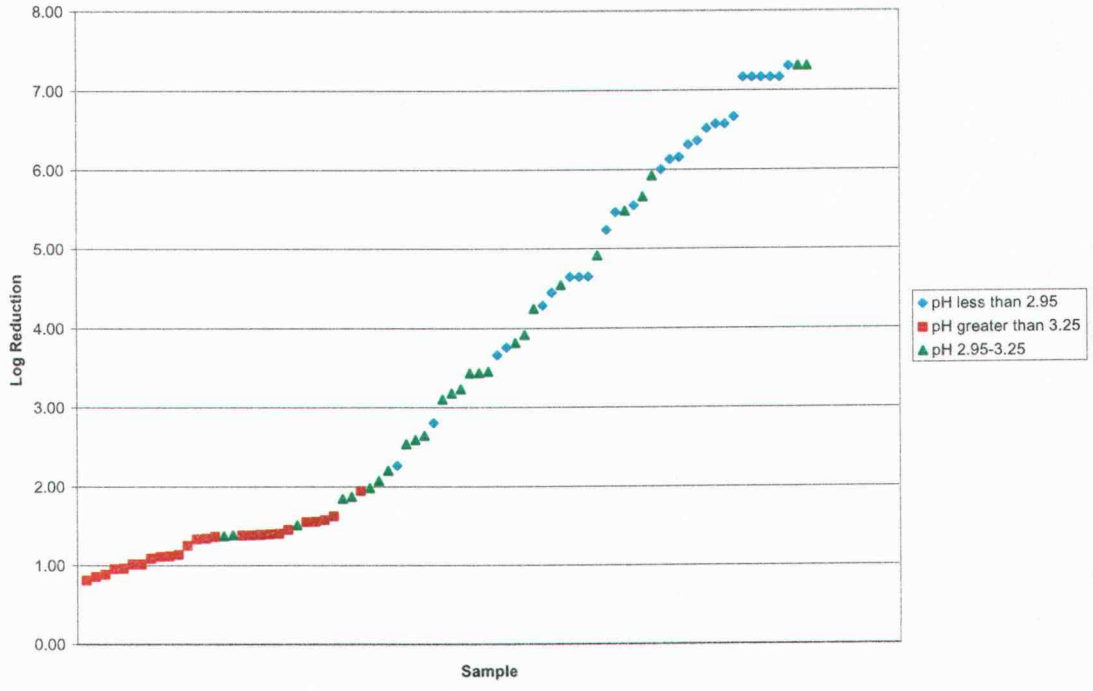


Figure 3.8. Relationship between effectiveness and pH in suspension tests against *Staphylococcus aureus.*

Ethanol has been shown to have enhanced ability at destroying microorganisms when adjusted to pH 3 (Jordan and others 1999). The results given in Figure 3.9 explore this relationship between ethanol and pH. The combination of low pH and increased ethanol concentrations showed some complementation. Decreasing the pH of the wine increases the effectiveness of higher concentrations of ethanol against *S. aureus*. These results agree with the finding of Jordan and others (1999). Other combination effects are likely, however difficult to separate in this study.

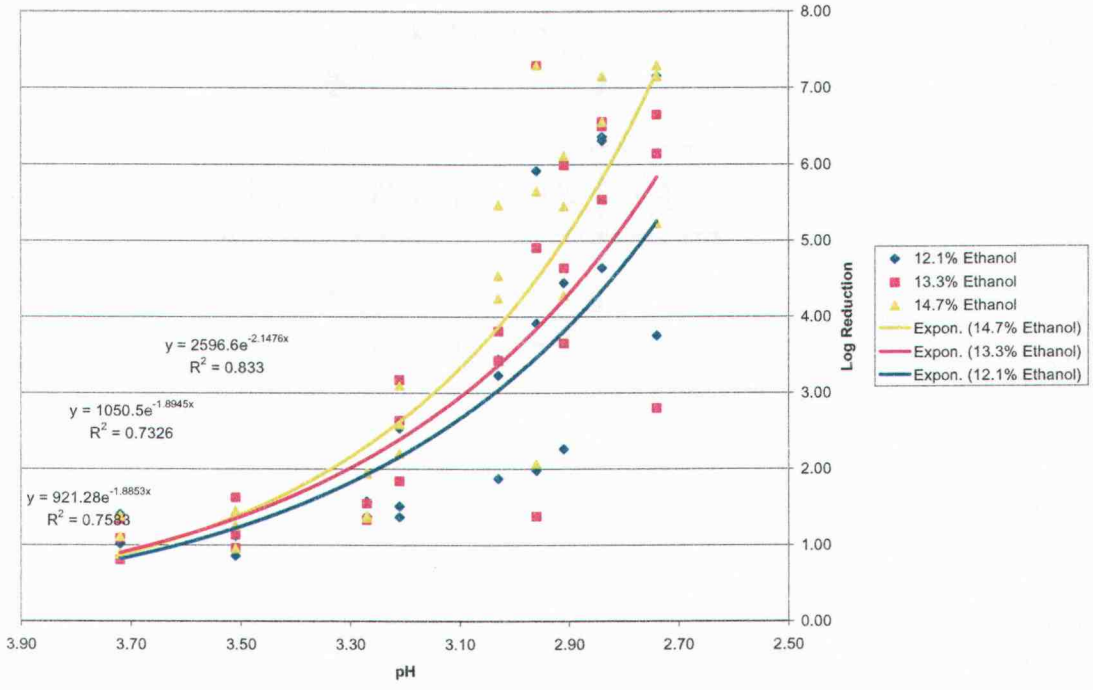


Figure 3.9. Relationship between pH and ethanol concentration in suspension tests against *Staphylococcus aureus*.

Suspension test exposure time may vary from study to study. For this study, the exposure time for these experiments was kept at twenty minutes. This is a rather lengthy exposure time, however due to the resistant nature of these microorganisms it was deemed appropriate to allow enough time so some inactivation occurred but not all populations were killed.

Suspension tests are the first test in determining possible disinfectants. These tests have some inherent problems including reproducibility (Taylor and others 1999). Suspension tests may or may not be indicative of a disinfectant's performance on surface tests (Aarnisalo and others 2002). While disinfectants are designed to be used after a cleaning step, often they are applied under 'dirty' conditions, therefore it is

desirable for effectiveness to be determined in the presence of organic matter (Gutierrez and others 1999). These are steps that should be approached in the future.

CONCLUSIONS:

This study investigated the antimicrobial effects of different levels of wine components in different combinations at varying concentrations. Titratable acidity does not appear to correlate with disinfecting ability in a suspension test. Molecular sulfur dioxide and pH appear to be the best single predictors of efficacy of a wine based disinfectant, however the combination of these parameters is important. The effectiveness of ethanol concentrations appears to be enhanced by lower pH, as previously shown. A wine based disinfectant is likely to be enhanced by adding some combination of sulfur dioxide, acid and/or ethanol. The proposed wine based disinfectant would also contain salt at a level of 1.5%; this may also contribute to the antimicrobial effect of the solution. Further studies need to be done to determine the effectiveness of wine as a surface disinfectant in environments representative of commercial and domestic food preparation.

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CHAPTER 4

CONCLUSIONS

Wine is a complex solution containing several different components that are known to individually have antimicrobial activity. From these studies, it can be concluded that sulfur dioxide concentration plays a role in enhancing the antimicrobial activity of Chardonnay wine. The addition of sulfur dioxide increased the ability of a base Chardonnay wine to reduce the number of several different pathogens. *Staphylococcus aureus* strains were found to be more resistant to wine compositions than *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Several commercial wines were analyzed for their effectiveness at reducing cells numbers in an effort to correlate sulfur dioxide levels with disinfecting ability. However, due to the complex interactions of wine components it was found that sulfur dioxide levels did not directly correlate with antimicrobial efficacy. Further wine analysis was completed and measurements of pH, titratable acidity, volatile acidity, and ethanol concentrations were taken. None of the measured components simply correlated with efficacy, and two of the wines with significantly different antimicrobial effects had similar measured values for all of the components. It is likely that there are interactions between the wine components that may influence efficacy, however it is also possible that there may be other constituents of wine that contribute to the antimicrobial properties (e.g. polyphenols).

Combination adjustments of a base Chardonnay wine in suspension tests against *Escherichia coli* O157:H7 and *Staphylococcus aureus* displayed some important results. *Escherichia coli* O157:H7 was found to be much more sensitive to the wine suspension tests that *S. aureus*. Titratable acidity and ethanol appeared to show little correlation with effectiveness. Molecular sulfur dioxide levels and pH were important parameters in predicting efficacy. Molecular sulfur dioxide levels are dependent on free sulfur dioxide concentration and pH. Low pH conditions are known to enhance the antimicrobial activity of the other components that were tested. Examining combinations of decreasing pH and ethanol content, displayed some interesting results. Decreasing pH levels enhanced antimicrobial activity among samples with increasing ethanol concentration. More complicated interactions are likely to exist among these samples.

Further exploration of a wine based disinfectant may be necessary to determine more encompassing conclusions. Disinfectant testing usually begins with suspension tests similar to those completed in this study. From here, challenge tests in the presence of organic material and surface tests are the next logical steps. Additional studies could include analyzing wines for antimicrobial activity contributed by polyphenol compounds present in white wines and/or possible mechanism of inactivation studies. Significant industry interest was encountered throughout the course of these studies and may provide further opportunities for advanced research.

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APPENDICES

Appendix I. Raw data from suspension tests for Chapter 2.

601 Klebsiella pneumoniae

9-Jul		Survivors					
Treatment	Inoculum		1	3	5	7	9
Disinfectant		2.05*10^7	0.00	0.00	0.00	0.00	0.00
Wine		2.05*10^7	0.00	0.00	0.00	0.00	0.00
Wine + 100 ppm + salt		2.05*10^7	0.00	0.00	0.00	0.00	0.00
Wine + 200 ppm + salt		2.05*10^7	0.00	0.00	0.00	0.00	0.00

18-Jul		Survivors			
Treatment	Inoculum		1	3	5
Disinfectant		4.36*10^7	20.00	0.00	0.00
Wine		4.36*10^7	330.00	0.00	0.00
Wine + 100 ppm + salt		4.36*10^7	0.00	0.00	0.00
Wine + 200 ppm + salt		4.36*10^7	0.00	0.00	0.00

26-Aug		Survivors			
Treatment	Inoculum		1	3	5
Disinfectant		3.2*10^6	0.00	0.00	0.00
Wine		3.2*10^6	0.00	0.00	0.00
Wine + 100 ppm + salt		3.2*10^6	0.00	0.00	0.00
Wine + 200 ppm + salt		3.2*10^6	0.00	0.00	0.00

603 Pseudomonas aeruginosa

9-Jul		Survivors					
Treatment	Inoculum		1	3	5	7	9
Disinfectant		2.31*10^7	0.00	0.00	0.00	0.00	0.00
Wine		2.31*10^7	0.00	0.00	0.00	0.00	0.00
Wine + 100 ppm + salt		2.31*10^7	0.00	0.00	0.00	0.00	0.00
Wine + 200 ppm + salt		2.31*10^7	0.00	0.00	0.00	0.00	0.00

18-Jul		Survivors			
Treatment	Inoculum		1	3	5
Disinfectant		2.69*10^7	0.00	0.00	0.00
Wine		2.69*10^7	>10^5	0.00	0.00
Wine + 100 ppm + salt		2.69*10^7	0.00	0.00	0.00
Wine + 200 ppm + salt		2.69*10^7	0.00	0.00	0.00

26-Aug		Survivors			
Treatment	Inoculum		1	3	5
Disinfectant		4.68*10^7	0.00	0.00	0.00
Wine		4.68*10^7	1.57*10^3	0.00	5.00
Wine + 100 ppm + salt		4.68*10^7	0.00	0.00	0.00
Wine + 200 ppm + salt		4.68*10^7	0.00	0.00	0.00

609 Staphylococcus aureus

9-Jul		Survivors				
Treatment	Inoculum	1	3	5	7	9
Disinfectant	5.72*10^7	0.00	10.00	0.00	0.00	0.00
Wine	5.72*10^7	4.08*10^5	396.00	10.00	0.00	10.00
Wine + 100 ppm + salt	5.72*10^7	1.02*10^4	10.00	20.00	0.00	10.00
Wine + 200 ppm + salt	5.72*10^7	1675.00	10.00	10.00	10.00	10.00

18-Jul		Survivors				
Treatment	Inoculum	1	3	5	7	9
Disinfectant	5.11*10^7	0.00	0.00	0.00	0.00	0.00
Wine	5.11*10^7	6.11*10^5	>10^5	>10^5	390.00	35.00
Wine + 100 ppm + salt	5.11*10^7	>10^5	220.00	15.00	10.00	0.00
Wine + 200 ppm + salt	5.11*10^7	6.37*10^4	5.00	80.00	0.00	95.00

26-Aug		Survivors				
Treatment	Inoculum	1	3	5	7	9
Disinfectant	6.23*10^6	0.00	0.00	0.00	0.00	0.00
Wine	6.23*10^6	2.02*10^6	3.2*10^5	4.97*10^3	40.00	5.00
Wine + 100 ppm + salt	6.23*10^6	8.28*10^3	0.00	0.00	0.00	5.00
Wine + 200 ppm + salt	6.23*10^6	5.27*10^4	25.00	0.00	0.00	0.00

649 Staphylococcus aureus

18-Jul		Survivors				
Treatment	Inoculum	1	3	5	7	9
Disinfectant	2.75*10^6	0.00	0.00	0.00	0.00	0.00
Wine	2.75*10^6	2.52*10^5	9.71*10^4	2.59*10^4	1.31*10^3	170.00
Wine + 100 ppm + salt	2.75*10^6	4.4*10^3	10.00	0.00	0.00	0.00
Wine + 200 ppm + salt	2.75*10^6	1140.00	0.00	0.00	0.00	0.00

30-Aug		Survivors				
Treatment	Inoculum	1	3	5	7	9
Disinfectant	1.56*10^7	0.00	0.00	0.00	0.00	0.00
Wine	1.56*10^7	3.85*10^3	5.55*10^3	2.45*10^3	1.41*10^4	5.5*10^3
Wine + 100 ppm + salt	1.56*10^7	2.92*10^3	6.02*10^3	70.00	20.00	0.00
Wine + 200 ppm + salt	1.56*10^7	8.22*10^2	1.07*10^3	10.00	0.00	10.00

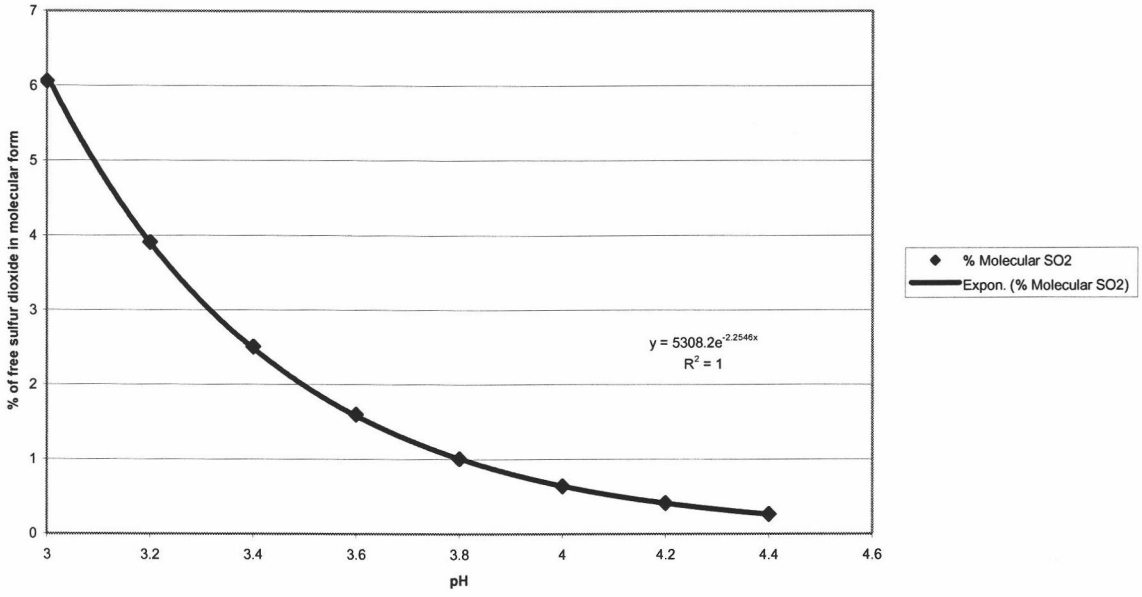
gray cells = counts from only one plate not an average of two plates

Appendix II. Raw data for commercial wine suspension tests.

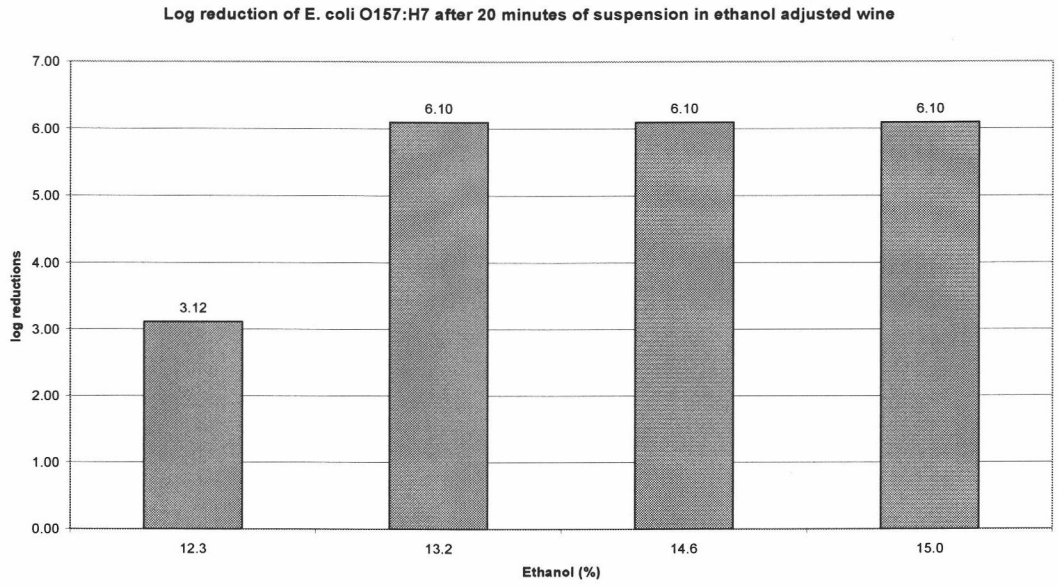
Wine Suspension Tests		Replicate 1		Replicate 2	
	24-Sep Inoculum	1	3	1	3
2000 Chardonnay Mendocino - 029	50000	1560	2010	1830	3110
2000 Chardonnay Monterey - 321	5.00*10^4	0	0	270	10
2000 Chardonnay Lake - 339	5.00*10^4	0	80	0	0
2001 Chardonnay Clarksburg PC01-001 - 503	5.00*10^4	27900	24600	12800	12400
2001 Chardonnay Clarksburg PC01-002 - 794	5.00*10^4	10	0	0	4900
Base Oregon Chardonnay - 940	5.00*10^4	1200	1730	1220	

gray cells = counts from only one plate not an average of two plates

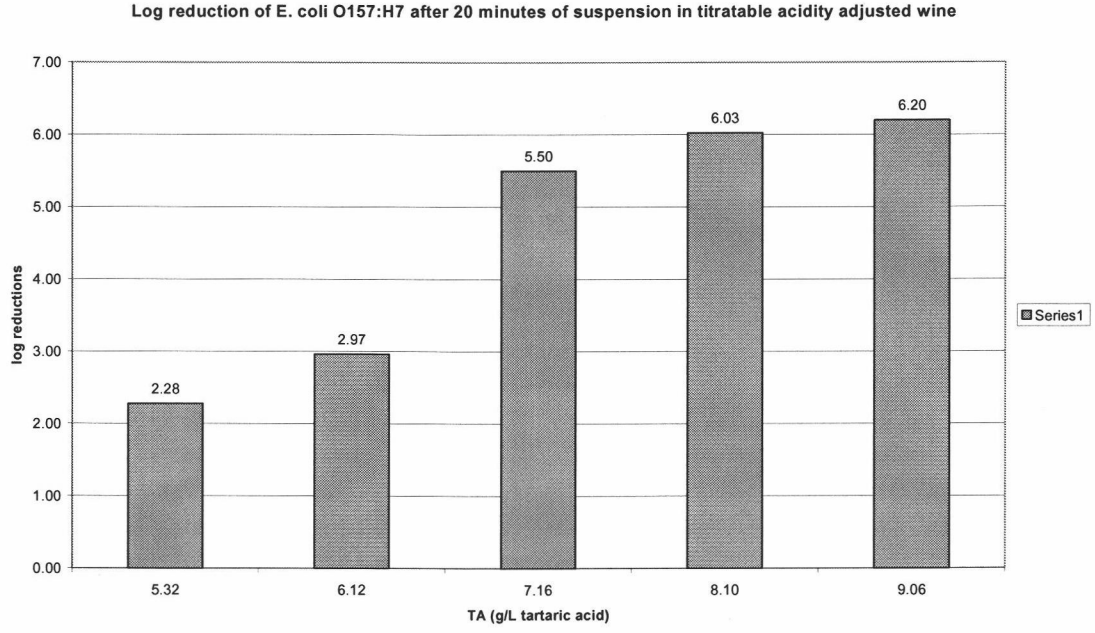
Appendix III. Determination of percentage of molecular sulfur dioxide present in wine as a function of pH and free sulfur dioxide concentration.



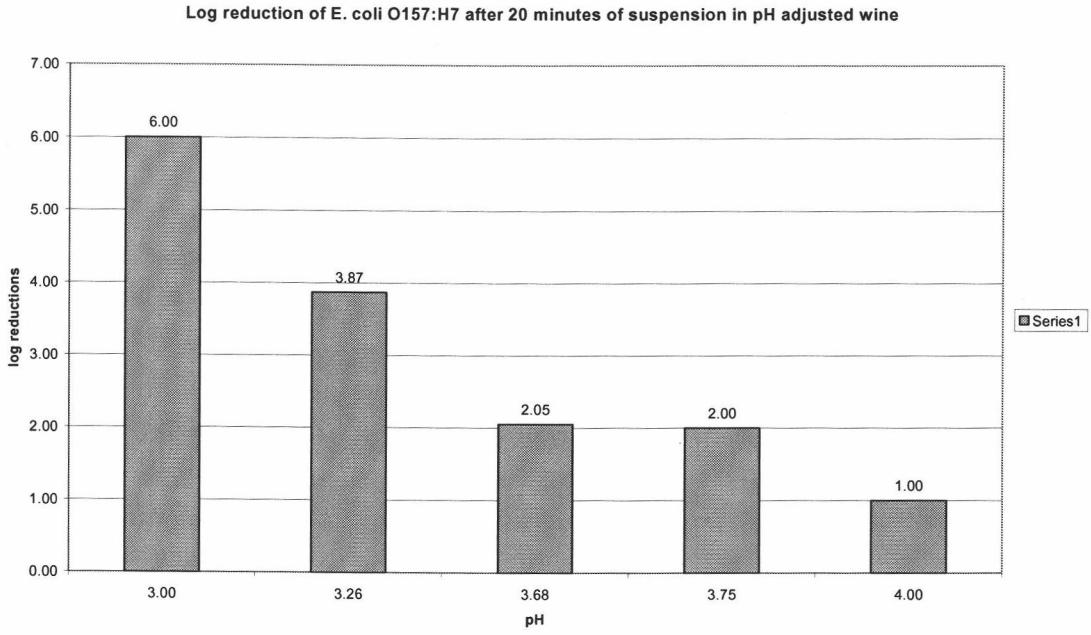
Appendix IV. Log reduction of *Escherichia coli* O157:H7 after 20 minutes of suspension in Badger Mountain 2001 Chardonnay with increasing ethanol.



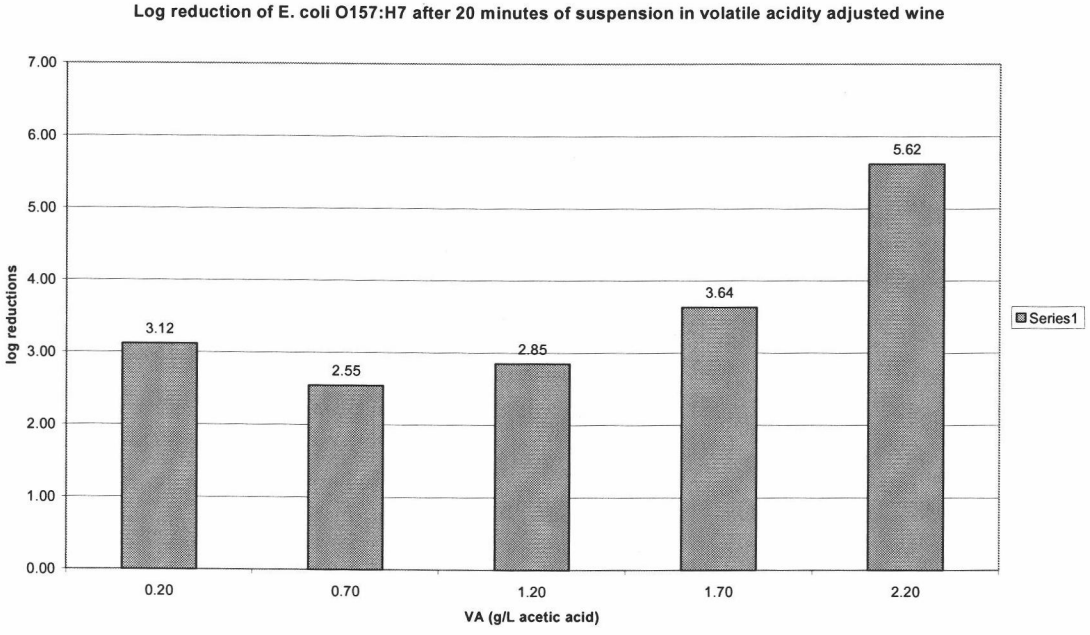
Appendix V. Log reduction of *Escherichia coli* O157:H7 after 20 minutes of suspension in titratable acidity adjusted wine.



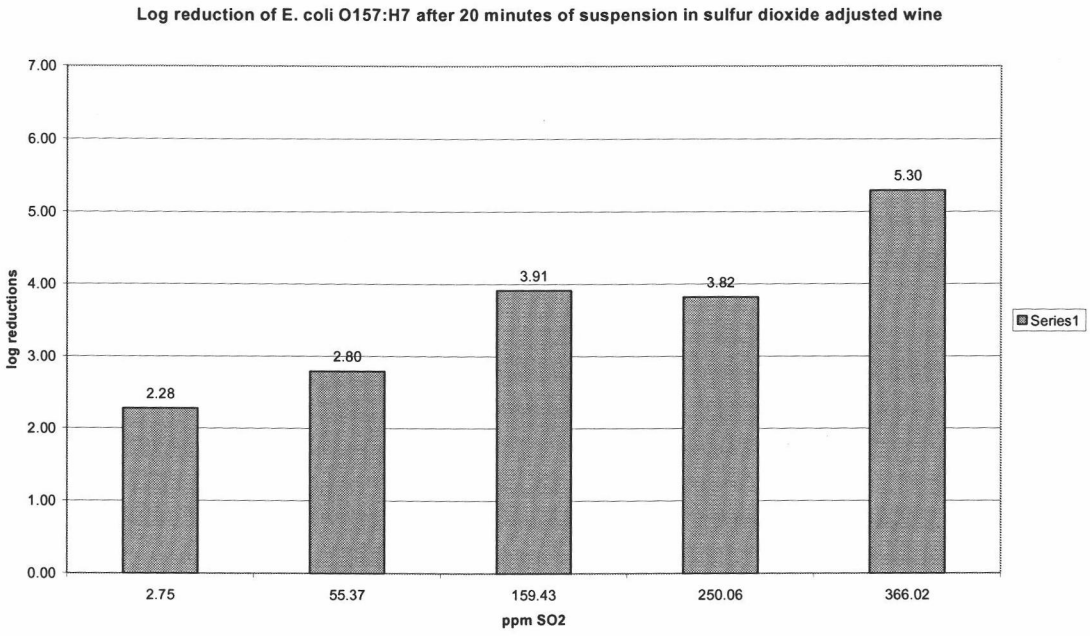
Appendix VI. Log reduction of *Escherichia coli* O157:H7 after 20 minutes of suspension in pH adjusted wine.



Appendix VII. Log reduction of *Escherichia coli* O157:H7 after 20 minutes of suspension in volatile acidity adjusted wine.



Appendix VIII. Log reduction of *Escherichia coli* O157:H7 after 20 minutes of suspension in sulfur dioxide adjusted wine.



Appendix X. Measured values for wine samples.

Sample	pH	Titratable Acidity	Total Sulfur Dioxide	Free Sulfur Dioxide	Molecular Sulfur Dioxide	Ethanol
ADGJ	3.72	6.42	27.90	3.90	0.05	12.08
ADGK	3.72	6.42	27.90	3.90	0.05	13.28
ADGL	3.72	6.42	27.90	3.90	0.05	14.66
ADHJ	3.72	6.42	78.60	24.50	0.30	12.08
ADHK	3.72	6.42	78.60	24.50	0.30	13.28
ADHL	3.72	6.42	78.60	24.50	0.30	14.66
ADIJ	3.72	6.42	191.10	115.00	1.39	12.08
ADIK	3.72	6.42	191.10	115.00	1.39	13.28
ADIL	3.72	6.42	191.10	115.00	1.39	14.66
AEGJ	3.51	8.73	27.90	3.90	0.08	12.08
AEGK	3.51	8.73	27.90	3.90	0.08	13.28
AEGL	3.51	8.73	27.90	3.90	0.08	14.66
AEHJ	3.51	8.73	78.60	24.50	0.48	12.08
AEHK	3.51	8.73	78.60	24.50	0.48	13.28
AEHL	3.51	8.73	78.60	24.50	0.48	14.66
AEIJ	3.51	8.73	191.10	115.00	2.23	12.08
AEIK	3.51	8.73	191.10	115.00	2.23	13.28
AEIL	3.51	8.73	191.10	115.00	2.23	14.66
AFGJ	3.27	10.55	27.90	3.90	0.13	12.08
AFGK	3.27	10.55	27.90	3.90	0.13	13.28
AFGL	3.27	10.55	27.90	3.90	0.13	14.66
AFHJ	3.27	10.55	78.60	24.50	0.82	12.08
AFHK	3.27	10.55	78.60	24.50	0.82	13.28
AFHL	3.27	10.55	78.60	24.50	0.82	14.66
AFIJ	3.27	10.55	191.10	115.00	3.84	12.08
AFIK	3.27	10.55	191.10	115.00	3.84	13.28
AFIL	3.27	10.55	191.10	115.00	3.84	14.66

BDGJ	3.21	7.65	27.90	3.90	0.15	12.08
BDGK	3.21	7.65	27.90	3.90	0.15	13.28
BDGL	3.21	7.65	27.90	3.90	0.15	14.66
BDHJ	3.21	7.65	78.60	24.50	0.94	12.08
BDHK	3.21	7.65	78.60	24.50	0.94	13.28
BDHL	3.21	7.65	78.60	24.50	0.94	14.66
BDIJ	3.21	7.65	191.10	115.00	4.39	12.08
BDIK	3.21	7.65	191.10	115.00	4.39	13.28
BDIL	3.21	7.65	191.10	115.00	4.39	14.66
BEGJ	3.03	9.69	27.90	3.90	0.22	12.08
BEGK	3.03	9.69	27.90	3.90	0.22	13.28
BEGL	3.03	9.69	27.90	3.90	0.22	14.66
BEHJ	3.03	9.69	78.60	24.50	1.40	12.08
BEHK	3.03	9.69	78.60	24.50	1.40	13.28
BEHL	3.03	9.69	78.60	24.50	1.40	14.66
BEIJ	3.03	9.69	191.10	115.00	6.59	12.08
BEIK	3.03	9.69	191.10	115.00	6.59	13.28
BEIL	3.03	9.69	191.10	115.00	6.59	14.66
BFGJ	2.91	11.09	27.90	3.90	0.29	12.08
BFGK	2.91	11.09	27.90	3.90	0.29	13.28
BFGL	2.91	11.09	27.90	3.90	0.29	14.66
BFHJ	2.91	11.09	78.60	24.50	1.84	12.08
BFHK	2.91	11.09	78.60	24.50	1.84	13.28
BFHL	2.91	11.09	78.60	24.50	1.84	14.66
BFIJ	2.91	11.09	191.10	115.00	8.64	12.08
BFIK	2.91	11.09	191.10	115.00	8.64	13.28
BFIL	2.91	11.09	191.10	115.00	8.64	14.66
CDGJ	2.96	8.37	27.90	3.90	0.26	12.08
CDGK	2.96	8.37	27.90	3.90	0.26	13.28
CDGL	2.96	8.37	27.90	3.90	0.26	14.66
CDHJ	2.96	8.37	78.60	24.50	1.64	12.08
CDHK	2.96	8.37	78.60	24.50	1.64	13.28
CDHL	2.96	8.37	78.60	24.50	1.64	14.66
CDIJ	2.96	8.37	191.10	115.00	7.71	12.08
CDIK	2.96	8.37	191.10	115.00	7.71	13.28
CDIL	2.96	8.37	191.10	115.00	7.71	14.66
CEGJ	2.84	10.04	27.90	3.90	0.34	12.08
CEGK	2.84	10.04	27.90	3.90	0.34	13.28
CEGL	2.84	10.04	27.90	3.90	0.34	14.66
CEHJ	2.84	10.04	78.60	24.50	2.15	12.08
CEHK	2.84	10.04	78.60	24.50	2.15	13.28
CEHL	2.84	10.04	78.60	24.50	2.15	14.66
CEIJ	2.84	10.04	191.10	115.00	10.11	12.08
CEIK	2.84	10.04	191.10	115.00	10.11	13.28
CEIL	2.84	10.04	191.10	115.00	10.11	14.66
CFGJ	2.74	12.10	27.90	3.90	0.43	12.08
CFGK	2.74	12.10	27.90	3.90	0.43	13.28
CFGL	2.74	12.10	27.90	3.90	0.43	14.66
CFHJ	2.74	12.10	78.60	24.50	2.70	12.08
CFHK	2.74	12.10	78.60	24.50	2.70	13.28
CFHL	2.74	12.10	78.60	24.50	2.70	14.66
CFIJ	2.74	12.10	191.10	115.00	12.67	12.08
CFIK	2.74	12.10	191.10	115.00	12.67	13.28
CFIL	2.74	12.10	191.10	115.00	12.67	14.66

Appendix XI. Raw data for *Escherichia coli* O157:H7 suspension tests.

Sample	Inoculum	Rep 1	Rep 2				
ADGJ	2.46*10^7	2.64*10^4	2.27*10^4	BDGJ	2.46*10^7	860	1570
ADGK	1.59*10^7	1.48*10^5	8.49*10^4	BDGK	2.46*10^7	50	40
ADGL	2.46*10^7	0	20	BDGL	2.46*10^7	0	0
ADHJ	2.46*10^7	2.97*10^4	3.40*10^4	BDHJ	2.46*10^7	210	200
ADHK	1.59*10^7	7.54*10^4	6.50*10^3	BDHK	2.46*10^7	10	0
ADHL	1.52*10^7	50	0	BDHL	2.46*10^7	0	0
ADIJ	2.46*10^7	2.68*10^4	1.95*10^4	BDIJ	2.46*10^7	0	0
ADIK	1.59*10^7	1.48*10^3	2.20*10^3	BDIK	2.46*10^7	0	0
ADIL	1.52*10^7	0	0	BDIL	2.46*10^7	0	0
AEGJ	1.52*10^7	0	0	BEGJ	2.46*10^7	10	10
AEGK	1.52*10^7	0	0	BEGK	2.46*10^7	0	0
AEGL	1.59*10^7	0	0	BEGL	2.46*10^7	0	0
AEHJ	2.46*10^7	0	0	BEHJ	2.46*10^7	0	0
AEHK	1.52*10^7	0	0	BEHK	2.46*10^7	0	0
AEHL	1.59*10^7	0	0	BEHL	2.46*10^7	0	10
AEIJ	1.59*10^7	0	0	BEIJ	1.52*10^7	0	0
AEIK	1.59*10^7	20	0	BEIK	2.46*10^7	0	0
AEIL	1.52*10^7	0	0	BEIL	2.46*10^7	10	0
AFGJ	1.59*10^7	0	0	BFGJ	2.46*10^7	0	0
AFGK	1.59*10^7	0	0	BFGK	2.46*10^7	0	0
AFGL	1.59*10^7	0	0	BFGL	2.46*10^7	0	0
AFHJ	1.52*10^7	0	0	BFHJ	2.46*10^7	0	0
AFHK	1.52*10^7	0	0	BFHK	2.46*10^7	10	10
AFHL	1.59*10^7	0	0	BFHL	2.46*10^7	0	0
AFIJ	1.59*10^7	0	0	BFIJ	2.46*10^7	0	0
AFIK	1.59*10^7	0	0	BFIK	2.46*10^7	0	0
AFIL	1.59*10^7	0	0	BFIL	2.46*10^7	0	0
CDGJ	1.52*10^7	10	20				
CDGK	1.52*10^7	0	0				
CDGL	1.52*10^7	0	0				
CDHJ	1.52*10^7	0	0				
CDHK	1.52*10^7	20	0				
CDHL	1.52*10^7	0	0				
CDIJ	1.52*10^7	0	0				
CDIK	1.52*10^7	0	0				
CDIL	1.52*10^7	0	0				
CEGJ	1.52*10^7	0	0				
CEGK	1.52*10^7	0	0				
CEGL	1.52*10^7	0	0				
CEHJ	1.52*10^7	0	0				
CEHK	1.52*10^7	0	0				
CEHL	1.52*10^7	0	0				
CEIJ	1.52*10^7	0	0				
CEIK	1.52*10^7	0	0				
CEIL	1.52*10^7	10	0				
CFGJ	1.52*10^7	0	0				
CFGK	1.52*10^7	0	0				
CFGL	1.52*10^7	0	0				
CFHJ	1.52*10^7	0	0				
CFHK	1.52*10^7	0	0				
CFHL	1.52*10^7	0	0				
CFIJ	1.52*10^7	0	0				
CFIK	1.52*10^7	0	0				
CFIL	1.52*10^7	0	0				

Appendix XII. Raw data for *Staphylococcus aureus* suspension tests.

Sample	Inoculum	Survivors Rep 1	Rep 2	Sample	Inoculum	Rep 1	Rep 2
ADGJ	1.27*10^7	1.20*10^6	1.18*10^6	BDGJ	1.73*10^7	4.74*10^5	5.81*10^5
ADGK	1.73*10^7	9.19*10^5	6.44*10^5	BDGK	1.73*10^7	2.03*10^5	2.93*10^5
ADGL	1.73*10^7	4.70*10^5	1.04*10^6	BDGL	1.73*10^7	1.37*10^5	8.30*10^4
ADHJ	1.73*10^7	1.23*10^6	2.15*10^6	BDHJ	1.73*10^7	6.41*10^5	8.17*10^5
ADHK	1.36*10^7	2.09*10^6	2.00*10^6	BDHK	2.02*10^7	5.19*10^4	4.00*10^4
ADHL	1.36*10^7	9.79*10^5	1.07*10^6	BDHL	2.02*10^7	1.91*10^4	1.30*10^4
ADIJ	1.73*10^7	8.79*10^5	5.08*10^5	BDIJ	2.02*10^7	6.72*10^4	5.03*10^4
ADIK	1.36*10^7	6.37*10^5	1.83*10^6	BDIK	2.02*10^7	1.49*10^4	1.16*10^4
ADIL	1.36*10^7	2.57*10^6	1.15*10^6	BDIL	2.06*10^7	4.58*10^4	6.00*10^4
AEGJ	1.73*10^7	6.04*10^5	7.70*10^5	BEGJ	1.73*10^7	2.18*10^5	2.40*10^5
AEGK	1.36*10^7	1.13*10^6	1.85*10^6	BEGK	1.35*10^7	3.55*10^3	6.83*10^3
AEGL	1.36*10^7	1.65*10^6	1.29*10^6	BEGL	1.35*10^7	5.80*10^2	9.84*10^2
AEHJ	1.36*10^7	1.77*10^6	1.90*10^6	BEHJ	1.35*10^7	7.86*10^3	7.74*10^3
AEHK	1.35*10^7	7.97*10^5	1.15*10^6	BEHK	2.02*10^7	3.15*10^3	2.94*10^3
AEHL	1.35*10^7	3.74*10^5	5.84*10^5	BEHL	2.02*10^7	4.90*10^2	6.60*10^2
AEIJ	1.36*10^7	1.18*10^6	8.64*10^5	BEIJ	2.02*10^7	1.01*10^4	4.91*10^3
AEIK	1.35*10^7	3.01*10^5	3.27*10^5	BEIK	2.06*10^7	8.91*10^3	6.30*10^3
AEIL	1.27*10^7	1.02*10^6	4.70*10^5	BEIL	2.02*10^7	1.10*10^2	40
AFGJ	1.73*10^7	4.35*10^5	4.67*10^5	BFGJ	1.73*10^7	9.35*10^4	9.28*10^4
AFGK				BFGK	1.35*10^7	6.17*10^2	1.48*10^2
AFGL	1.36*10^7	5.21*10^5	6.35*10^5	BFGL	1.35*10^7	0	1.02*10^2
AFHJ	1.35*10^7	3.61*10^5	3.74*10^5	BFHJ	1.35*10^7	4.24*10^2	2.12*10^2
AFHK	1.27*10^7	6.44*10^5	5.13*10^5	BFHK	2.06*10^7	2.10*10^3	9.42*10^3
AFHL	1.27*10^7	4.69*10^5	5.48*10^5	BFHL	2.06*10^7	7.80*10^2	1.42*10^3
AFIJ	1.36*10^7	4.65*10^5	6.57*10^5	BFIJ	2.02*10^7	1.01*10^3	5.00*10^2
AFIK	1.27*10^7	4.00*10^5	3.06*10^5	BFIK	2.02*10^7	20	20
AFIL	1.27*10^7	1.65*10^5	1.21*10^5	BFIL	2.06*10^7	50	100
CDGJ	2.05*10^7	1.97*10^5	2.27*10^5				
CDGK	2.06*10^7	1.08*10^6	6.52*10^5				
CDGL	2.06*10^7	1.82*10^5	1.63*10^5				
CDHJ	2.05*10^7	3.19*10^3	1.88*10^3				
CDHK	2.05*10^7	369	166				
CDHL	1.46*10^7	100	10				
CDIJ	2.05*10^7	30	20				
CDIK	2.05*10^7	0	0				
CDIL	2.05*10^7	0	0				
CEGJ	2.05*10^7	1.21*10^3	175				
CEGK	2.05*10^7	184	18				
CEGL	2.05*10^7	0	30				
CEHJ	2.05*10^7	10	10				
CEHK	2.05*10^7	0	30				
CEHL	1.46*10^7	0	0				
CEIJ	1.46*10^7	0	40				
CEIK	1.46*10^7	0	20				
CEIL	1.46*10^7	0	0				
CFGJ	1.46*10^7	3.36*10^3	1.90*10^3				
CFGK	2.06*10^7	3.20*10^4	3.17*10^4				
CFGL	1.46*10^7	0	0				
CFHJ	1.46*10^7	0	0				
CFHK	1.46*10^7	10	0				
CFHL	2.06*10^7	140	100				
CFIJ	1.46*10^7	0	0				
CFIK	2.02*10^7	20	10				
CFIL	2.02*10^7	0	0				

Appendix XIII. Cross contamination routes in the home.
Jones MV. 1998. Application of HACCP to identify hygiene risks in the home. Int Biodeterior Biodegrad 41:191-199.

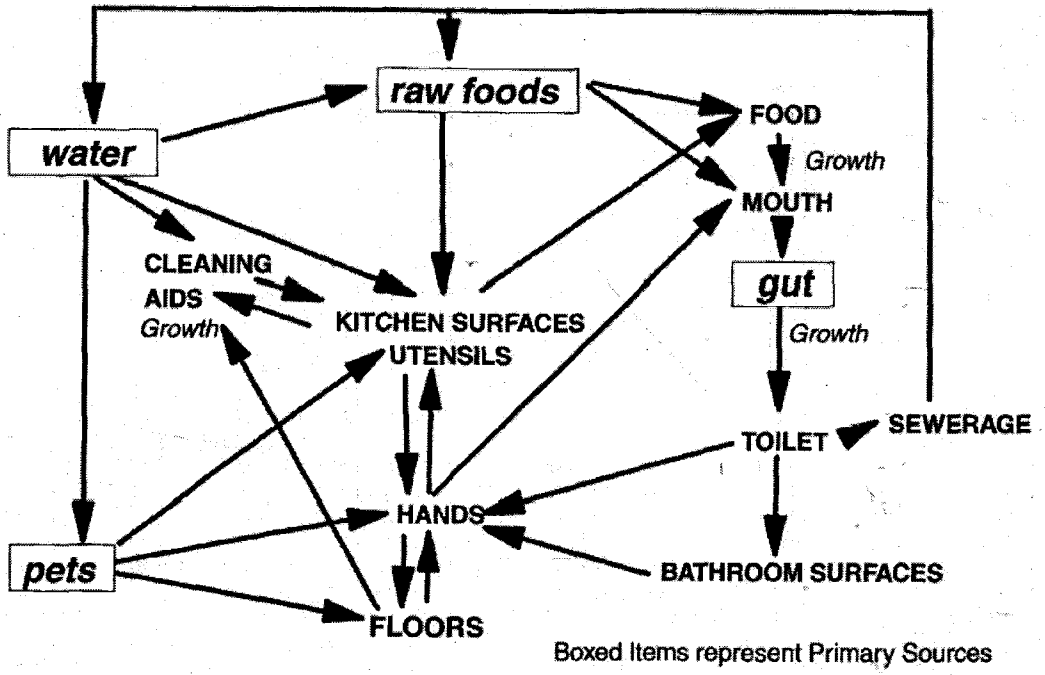


Fig. 2. Cross contamination routes for Enteric Pathogens.

Appendix XIV. Hygiene risk in domestic kitchen
Jones MV. 1998. Application of HACCP to identify hygiene risks in the home. Int Biodeterior Biodegrad 41:191-199.

Appendix: Hygiene risk analysis by activity and room function[1]

Kitchen

Activities
Preparing Food(cleaning, chopping, cutting, mincing, etc.)

Raw meat
High risk of contamination of sink, tap handles, work surfaces, utensils, equipment and hands with pathogens.
Scraps fed to pets may introduce gut pathogens.
Vegetables/fruit
Moderate risk of salads/fruit eaten raw becoming cross contaminated from kitchen surfaces, hands of cook, kitchen sink or from water.
Bread, pastries, cake
Very low risk for products to be cooked.
Fish
Cross contamination risk, low but moderate risk of direct food poisoning from seafoods.
Desserts/ice cream
Low risk if raw materials good bacteriological quality.
Some ingredients have moderate risk (raw eggs, dairy products).
Cooking
Very low risk except where inadequate internal temperatures are reached e.g. cooking from frozen.

Serving/finishing cooked foods

Low risk if food eaten immediately. **High risk if food left warm and contaminated from addition of raw materials or cross contaminated from hands or kitchen surfaces.**
Storage
High risk if time/temperature limits for food cooked or unpreserved fresh food abused.
Moderate to high risk of cross-contamination of cooked food from raw in 'fridge.
Low to moderate risk with long term storage of dry goods if sufficient moisture to allow moulds to grow.
Low to moderate risk of contamination by vermin/insects.

Eating

Main meals for adults/older children-low risk

Babies-**Moderate to high risk if baby feeding utensils, crockery, bottles etc. not hygienically clean or cross contaminated from adults' hands, kitchens surfaces etc.**

Snacking

Moderate risk if prepared by children/teenagers with less regard to hygiene than normal.

Cleaning-difficulties in cleaning adequately

Washing up

Very low risk from machine dish wash. Moderate risk from hand dish wash if heavily soiled, contaminated items difficult to clean. Kitchen sink bowl often contaminated.

Equipment

Moderate risk from poorly designed equipment which is difficult to clean and dry (mincers, slicers, food processors) or which have damaged/cracked surfaces.

Surfaces

Moderate to high risk if surfaces rough, damages, porous etc. Food contact surfaces which remain damp after cleaning are potentially high risk. Kitchen sink is a high risk surface which becomes rapidly recontaminated after cleaning.

Drains

Low risk unless flow rates low or blockages occur.

High risk if back flooding into sink or onto kitchen floor.

Cleaning-unhygienic cleaning

Cleaning aids/cloths/mops etc.

Very high risk of kitchen surface contamination from reusable cloths, mops, brushes etc.

Cleaning materials

Water-Moderate if water not of potable quality.

Chemicals

Low risk unless detergents/disinfectants etc diluted and stored by householder.

Waste disposal

Moderate risk from hand contact with waste bins. Risk of attracting flies, insects, vermin especially if ambient temperatures high and odours develop.

Laundry

Machine wash

Normal and soiled wash low risk especially if water temperature >50°C.

Hand wash

Normal wash low risk. Moderate risk of contaminating kitchen surfaces, hands, other clothes if hand washing soiled fabrics in kitchen.

Nappies (Diapers)

Moderate risk if nappies soaked in bucket in kitchen.

Disposal of nappy soak water liable to create bacterial aerosols.

Drying

Low risk activity but adds to humidity/dampness problems.

Ironing

No risk, can kill microbes on fabrics.

Storage

Low risk. Damp storage will assist survival of skin pathogens and moulds.

Pets

Handling/presence in kitchen

Moderate to high risk of cross contamination from pets to food/food preparation surfaces directly or via hands.

Specific diseases via direct contact (pet-hand-mouth etc)

Fouling/cleaning

High risk if floors/surfaces not adequately disinfected.

Young children at high risk.

Feeding

Low to moderate risk of cross contamination between feeding bowls and kitchen worksurfaces, cutlery, sink etc. Low to moderate risk of certain animal feeds attracting insects/vermin into kitchen or food store area.

General activities and leisure

Low risk

[1]Excludes direct or aerosol droplet person-to-person routes of transmission.

Appendix XV. Observed unhygienic practices in the domestic environment.
Jay LS, Comar D, Govenlock LD. 1999. A video study of Australian domestic food-handling practices. J Food Protect 62(11):1285-1296.

TABLE 2. *Common unhygienic practices observed from video data*

Description of unhygienic practices
Infrequent washing of hands and poor hand washing technique.
Handling garbage or garbage bin and returning to food preparation without washing hands.
Smoking during food preparation.
Serving food, blowing the nose, and returning to serving food without washing hands.
Using bare hands to wipe the nose during food preparation with no hand washing.
Food handlers touching hair, mouth, and face during food preparation.
Not using separate hand towels and dish towels.
Infrequent cleaning of work surface without cleaning the area before and after use.
Preparing food on a bench surface without cleaning the area before and after use.
Not removing contaminated packaging from food preparation area during food preparation.
Not keeping the preparation area tidy, e.g., removing vegetable peelings, used utensils, cleaning down the bench surface after the various stages in the meal preparation.
Interruptions in food preparation that result in cross-contaminating episodes, such as smoking a cigarette, returning to the gardening, or feeding the pet and then returning to the food preparation without washing hands; this also resulted in leaving food for excessive time in the temperature danger zone.
Placing unhygienic objects on the food preparation area, such as shoes, hairbrush, or toys.
Pet handling during food preparation.
Pets on kitchen benches.
Pets on kitchen benches licking plates or food containers.

TABLE 3. *Less common unhygienic practices observed from video data.*

Description of unhygienic practices
Using the same dishwater for the entire day.
Using the same dish towel for dishes and hands for 1 week.
Dish towel dropped to the kitchen floor and remaining on the floor for a number of hours before being retrieved and used.
Dirty dishes remaining in water in the sink for more than 12 h.