# AN ABSTRACT OF THE THESIS OF

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Wine is known to have been enjoyed since ancient times. Although early civilizations where unaware of scientific mechanisms of the properties of wine, empirical methods allowed them knowledge of its intoxicating effects as well as its healthful properties. These properties lead the ancients to use wine for many purposes including its use in medicine. It has been proposed that civilizations in antiquity such as the Romans and Greeks may have used wine as a means to sanitize their drinking water and avoid sickness due to pathogenic organisms. Wine has been shown to have unique antimicrobial properties that may support these claims. The objective of this research was to determine the efficacy of wines at diluted levels to inhibit pathogens in drinking water and to and to analyze the sensory properties of hypothesized dilutions.

Initial experiments focused on the inhibitory effects of wine at varying dilutions. Three pathogenic strains were used to determine the efficacy of wine as a sanitizing aide; two strains of *Escherichia coli* and one strain of *Salmonella poona.*

Two Chardonnay wines produced at Oregon State University were used for the microbial study. One Chardonnay was produced with added sulfites while the other had no added sulfites. It was determined that wines at low concentrations in water have an inhibitory effect against the microorganism tested.

The second objective of this study was to investigate the sensory properties of wine diluted with water. Two sensory studies were carried out using consumer panels. We first analyzed the ability to detect wine in water at low concentrations. Secondly we sought to determine whether different dilutions of wine and water were considered by the consumer to be refreshing. The study determined that wine mixed into water is easily detected at low concentrations. Through preference testing it was further determined that a low concentration of wine mixed with water was statistically more refreshing than water alone.

# INACTIVATION OF BACTERIAL PATHOGENS IN DRINKING WATER

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Evelyn R. Hartlerode, Author

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# Inactivation of Bacterial Pathogens in Drinking Water

## Chapter One

## Introduction

### Wine in Roman Culture

Wine is known to have been enjoyed since ancient times. Although early civilizations where unaware of scientific mechanisms of the properties of wine empirical methods gave them knowledge of its intoxicating effects as well as its healthful properties (Graver, 1998). The unique properties of wine lead the ancients to use wine for many purposes including its use in medicine. It has been widely indicated that wine was also utilized as a means of sanitizing drinking water in antiquity (Singleton, 1997; Dolara and others 2005).

It is estimated that wine had been in Italy before the traditional date of the foundation of Rome in 753 B.C. (Mole, 1966). Much of what we know today about wine drinking practices in ancient Rome comes from the writings of the poet Martial (Leary, 1999). Romans, Greeks, and Egyptians praised wine through gods, literature, and song. Democritus, who professed to know all the different kinds of vines in Greece, was alone in thinking it possible for them to be counted, but all other writers have stated that there is a countless and infinite number of varieties (Pliny, 1986). The

truth of this will appear more clearly if we consider the multitude of different kinds of wine made both past and present (Pliny, 1986).

Although wine was prevalent though Greek and Roman history it was not always the predominant beverage (Eisinger, 1982). The acceptance of wine as a beverage varied throughout history. Pliny the Elder relates that under the rule of Romulus and King Numa, in the early days of the republic, wine-drinking was discouraged and was permitted only to men over thirty-five, and entirely forbidden to women. The wife of Egnatius Maetannus was clubbed to death by her husband for drinking wine from the vat, and Romulus acquitted him on the charge of murder (Pliny, 1986).

Heavy drinking became acceptable, first for men and later for women during the days of the Empire (Von Bassermann-Jordan, 1932). Ancient Greek and Roman texts extolled the vine's virtues, but cautioned moderation (Grivetti, 1997)
The ancients also knew that wine held a potential for joy and disaster (Grivetti, 1997). The ancients knew that wine can cause acute and chronic damage when consumed in excess (Feher and others 2005). The effects of wine was described in a continuum from *ebrius* through *ebriosus* (Graver, 1998; Humphries and International Medieval, 2002).

## Wine as Medicine

The Roman acceptance of wine consumption was in part related to the knowledge of the benefits of wine as medicine. During the Roman period wine was considered the universal medicine (Lucia, 1963). Wine is frequently mentioned as a

constituent of medicine in ancient text (Lucas and Harris, 1999). New wine (mustum) in particular was regarded as healing and non-inebriating (Lucia, 1963). Pliny the Elder's regard for wine as medicine, goes as far as to say that 'wine acts as remedies in themselves, merely by supplying wine' (Pliny, 1986). Furthermore, the ability for wine to act as a disinfectant was known by the ancients (Feher et al., 2005).

The ideas of Hippocrates and his followers were fully embraced by the Roman medical professions (Grivetti, 1997). In Hippocrates' *Regimen in Acute Disease*, specific wines are prescribed for certain disorders (Grivetti, 1997). The ideas of Hippocrates, including wine consumption as a means to good health were fully embraced by the Roman medical profession (Grivetti, 1997).

Several ancient documents further suggest wine for specific ailments. In the Babylonian Nippur tablet (by 2200 years B.C.), considerably the oldest pharmacopeia, ointments mixed with wine were described to protect against cutaneous diseases (Feher et al., 2005). Similarly Galen a Greek physician who revered Hippocrates while serving as a physician to the gladiators, noted that dressings saturated with wine did not putrefy (Lucia, 1963). On the Egyptian Ebers Papyri (1500 B.C.) wine and preparations mixed with wine are recommended against constipation, dyspepsia, as well as the treatments for epilepsy and the prevention of jaundice (Feher et al., 2005). During the Roman Empire the military surgeon of Nero, Dioscordies (80 A.D.) recommended wine for wound disinfection, anesthesia, and to prevent suppuration (Feher et al., 2005). He compounded numerous preparations including several medicines dissolved or mixed with wine (Feher et al., 2005).

The medicated wine recipes of Galen, the famous second-century Greek physician who practiced in Rome, were preserved in the Byzantine and Arabic periods, and influenced European medicine for hundreds of years (Lucia, 1963).

The use of wine did not go without controversy. Ancient Greek physicians were divided into those who used wine therapeutically and those who did not (Lucia, 1963). Among the advocates of wine as medicine were such notable Roman physicians as Asclepiades of Bithynia, Hikesios of Smyrna, and Menecrates of Tralles (Lucia, 1963).

## Wine Mixed With Water

It is well documented from Roman and earlier sources that wine was usually diluted before consumption (Singleton, 1997; Quennell and Quennell, 1954). In addition, pottery remains from Central and Eastern Gaul that were used for wine, were decorated with the words, *da merum*' (served unmixed wine) and '*Misce mihi*' (mix me!), and another possibly reads '*Parce aquam*' (spare the water!) (Biddulph, 2008).

There are several proposed reasons for this practice. The practice of mixing wine with water may have been done for reasons of taste or avoiding intoxication. Alternatively, the ancients, knowing the medicinal effects of wine could have observed this practice as a means to sanitize their drinking water.

According to Singleton, the dilution was often of equal volumes of water to wine; sometimes more water was added, but seldom less than one-third water (1997). Detailed descriptions occur in Pliny's *Natural History* concerning the dilution of wine with water. As to the quantity of water he states that it depends upon the strength of

the wine; it is generally thought, however, that the best proportions are one '*cyathus*' of wine and two of water (Lucia, 1963). Pliny also recounts Homer's recorded mixing of Maronean wine with water in the proportion of 20 parts of water to one of wine (Pliny, 1986). Mucianus, a Roman statesman, ascertained that the custom was to mix with one pint of this wine eight pints of water (Pliny, 1986).

In the books of poetry left by Martial, he documents the mixing of fine wines with snow ice. This is done to dilute the wine and tone down the "fiery" quality of the wine(Leary, 1999). One obvious reason for this custom, which was likely more than simple fashion, is that the weather where grapes thrive is hot, at least in the summer, and thirst may not be quenched without drunkenness with undiluted wine (Singleton, 1997). On the other hand, ancient wines were often sweet. Of course, a sweet wine is not particularly thirst-quenching. The only way that the earliest vintners could have produced sweet wine was to use very ripe grapes and partially dried grapes. Adding honey to a dry wine would work, but was likely too expensive and would cause refermentation. Various reasons could be proposed for diluting alcoholic, sweet wines ranging from "taste" to more economical transport of the more concentrated beverage (Singleton, 1997). Fine wine of the ancients especially those for medicinal purposes, were concentrated and often required dilution to be palatable (Lucia, 1963). The ancients also found it expedient to concentrate their wines. Later when ready for use, the thick, often desiccated liquids were diluted with selected waters which were reputed to have special medicinal qualities (Lucia, 1963).

The person who drank undiluted wine was considered unsophisticated at best (Singleton, 1997). Pliny advised water to counteract inebriation (Lucia, 1963; Pliny

1986). The Roman writer Ammianus Marcellinus alludes to the baseness of the Gauls for not drinking wine mixed with water (Biddulph, 2008).

Perhaps contributing to the success of the Roman legions was that they carried wine and mixed it with local water. Wine seems to have been an expected ration of the legionnaires (Singleton, 1997). Successful sieges were sometimes noted as partly the result of the plentiful wine that was supplied to the troops, since contamination of the water would be particularly likely under siege conditions (Singleton, 1997). If an army is to "travel on its stomach" while foraging for most of its food and water, the health value of wine in the water was likely recognized by the leaders, as well as appreciated by the soldiers (Singleton, 1997). Roman soldiers always added small amounts of wine vinegar to their portable water supply, making a drink called 'oxos' which, according to the Gospel, was offered with a sponge by a soldier to Jesus Christ on the cross (Dolara et al., 2005).

One compelling reason for mixing wine with water is that much of the drinking water would make a person ill, but mixed with wine the water would be sanitized (Singleton, 1997). It's been suggested that ancient Mediterranean civilizations routinely mixed wine with water to benefit from the antimicrobial effects (Dolara et al., 2005). Wine could have been used to make contaminated wine safe or palatable (Singleton, 1997).

## Wine Production

The cultivation of the grape vine was established well before the founding of Rome and ancient Egypt. The earliest convincing indications for *Vitis vinifera* cultivation come from the Chalcolithic period (ca. 3700-3200 B.C. non-calibrated radiocarbon time) and Early Bronze Age (ca. 3200-1900 B.C.) (Zohary, 1997). The variety *Vitis vinifera* was used in ancient times for the production of many varieties of wine. Hundreds of varieties of wines are described by Pliny the Elder in his *Natural History* (Pliny, 1986).

Wine production in the ancient world was thought of in the same regards as we do today. Wine in ancient writings usually denotes the fermented juice of fresh grapes; which was the principal wine of the ancient Egyptians, though they had also other kinds, namely, palm wine, date wine, according to Pliny (Lucas and Harris, 1999). Egyptian and Roman paintings and artwork depict the process of the grapes being picked, tredded, and pressed (Stanley, 1999). The preparation of wine included separating the juice from the stalks and skins and allowed to ferment (Lucas and Harris, 1999). Pliny, states that the first pressing produces the finest wines; after this, water is added to the skin and juice; these are then pressed again to produce a lower quality wine (Stanley, 1999). Fermentation was carried out primarily by yeast naturally present on the grapes, and to a lesser extent from enzymes present in the juice (Lucas and Harris, 1999).

Wine production in ancient Rome was a relatively large production. Columella describes production on a commercial scale with vats that could hold in

excess of 8,000 liters (Eisinger, 1982). Production required the use of specialized equipment including presses and vats (Younger, 1966). It appears that the Romans used wine-presses to a far greater extent than the Greeks. This does not mean that treading was generally discarded (Younger, 1966). The Romans were also aware of the importance of cleanliness and fumigation in wine-making (Younger, 1966). Vats and vessels were washed out before the vintage with either sea water or fresh water (Younger, 1966).

Early records from Mesopotamia, Egypt, Greece, and Rome indicate that sweet wines were made and highly esteemed (Singleton, 1997). Ancient Greco-Romans distinguished against wine quality; they differentiated between levels of quality, vintage and appellation (Stanley, 1999). Greeks preferred resin-based additives for the purpose of preservation. The Romans on the other hand commonly used a syrup that was called sapa, defrutum, or caroenum. These syrups were prepared by simmering must in a leaden vessel over a slow fire until it was reduced by one third (Eisinger, 1982). Boiled down must and raisins also appear to have been used as additives, at least by Roman times (Singleton, 1997). Cato recommends the addition of lye-ashes boiled with boiled-down must to the wine skin, or else a pound and a half of salt. He further recommends occasionally adding pounded marble; he also mentions sulfur (Pliny, 1986).

Although sulfur was used in Roman life it remains unclear when sulfur dioxide was first used in preserving wine and if ancient Romans deliberately used sulfur in wine production (Singleton, 1997). Sulfur was known to the ancients Romans for many hundreds of years, but to them its use was medical or magical (Younger, 1966).

Sulfur would have been available from the venting of volcanic sulfur dioxide through mines. Roasting of pyrite ores for metal recovery was recognized as a source of this potent gas (Singleton, 1997). Furthermore, sulfur was exported from Sicily and from, or through, Puteoli; and sulfur 'matches' were sold in Rome (Younger, 1966).

Sulfur was used in Roman agriculture, mining and home use (Singleton, 1997; Pliny, 1986). The Romans burned sulfur as a means of fumigation (Hammond and Carr, 1976). Mines and dwellings as well as ships have been reported to be fumigated with sulfur dioxide in ancient Rome (Singleton, 1997).

Documented use of sulfur in early Roman wine production is mostly limited to viticulture practices (Younger, 1966). Pliny the Younger expressed that farmers who were anxious for the safety of their vines were advised to make bonfires so that the smoke should protect the vineyards, and expedients still practiced by modern growers against the disastrous frosts of early summer (Younger, 1966; Pliny 1986). Pliny adds that 'Some growers are content with submitting vines for three days on end to smoke from this concoction boiled to the windward of them' and this again might be thought to anticipate the modern sulfuring of vineyards (Younger, 1966; Pliny 1986).

There is some suggestion of the use of sulfur in wine production in Pliny's *Natural History* (Pliny, 1986) . Pliny the Elder relates that Cato recommends that a plaster for mending cracks in wine jars should be made from resin, wax, sulfur, and pulverized gypsum. However, there is nothing in these recipes from which we can infer that the sulfur was added because of its sterilizing quality (Pliny, 1986; Younger, 1966).

## Antimicrobial Properties of Wine

Research has shown that several of the compounds associated with wine have antimicrobial properties (Carneiro and others 2008; Cushnie and Lamb, 2005; Ganan and others 2009; Fernandes and others 2007; Boban and others 2010; Waite and Daeschel, 2007; Over and others 2009; Just and Daeschel, 2003). Wine contains organic acids, low pH, ethanol, sulfur dioxide, phenolic compounds, all which are known to have bactericidal or bacteriostatic effects (Waite and Daeschel, 2007).

While many studies have shown that the certain individual properties of wine have an antimicrobial effect and that wine can contribute to the protection of individuals from food borne pathogens, others have demonstrated that there is a synergistic relationship between wine components that make it antimicrobial (Boban et al., 2010; Møretrø and Daeschel, 2004; Waite and Daeschel, 2007).

## Organic Acids

Wine is an acidic environment, primarily due to the presence of organic acids. Organic acids are naturally present in fruits and vegetables, including grapes and are commonly used as food additives and preservatives as a means to limit microbial contamination (Ricke, 2003).

The antimicrobial mechanisms are not fully understood, however organic acids exhibit bacteriostatic and bactericidal properties (Ricke, 2003). Although the mechanisms are not fully understood it is well known that the ability for organic acids to inhibit microbial growth is primarily due to direct pH reduction (Beuchat. L, 1998).

The lower the external pH, the more undissociated weak acid will be available. (Bearson and others 1997). The undissociated form of these weak acids is able to diffuse across the cell membrane and dissociate inside the cell, lowering the pH in the process (Beuchat. L, 1998; Bearson et al., 1997). Organic acids also disrupt substrate transport by increasing the cell membrane permeability (Beuchat. L, 1998). Membrane uncoupling capabilities of organic acids have also been linked to the antimicrobial ability (Ricke, 2003).

Of the organic acids found in wine, malic and tartaric acids are the two most prevalent. Both malic and tartaric acids are known to have antimicrobial effect, especially at the low pH found in wine (Hsiao and Siebert, 1999; Ricke, 2003). Kim and others (2009) reported that malic, tannic, and tartaric acids were antimicrobial against *Escherichia coli* O157:H7 (2009). While tannic acids had the greatest antimicrobial action against *E.coli* 0157:H7 the three organic acids together were found to be most effective. The mixture of these acids inactivated all inoculated cells of *E.coil* within two hours ( 7.5 log reduction) (Kim et al., 2009). In a study by Daglia and others (2007) to determine the effectiveness of red and white wines against oral streptococci, they concluded that amongst other organic acids malic and tartaric were responsible for the antimicrobial activity at concentrations commonly found in wine. Furthermore, Over and others (Over et al., 2009) found citric, malic, and tartaric acids exhibited strong antibacterial activity at 75.0mM against *E.coli* OH7:157, *Listeria monocytogenes*, and *Salmonella typhimurium*.

Several other organic acids found in wine include volatile fatty acids. These weak acids are produced as a result of fermentation. Fatty acids are prevalent in wine

at varying carbon chain length (Yunoki and others 2004). Unsaturated fatty acids inhibited cellular fatty acid synthesis (Zheng and others 2005). Unsaturated fatty acids are bactericidal against Gram positive but not Gram negative organisms, and saturated fatty acids are not active against Gram positive or Gram negative bacteria (Zheng et al., 2005).

## pH

Wine is an acidic environment with a pH range of about 3.0 to 4.0. The pH of most wines makes it a hostile environment for pathogenic microorganism. Waite and Daeschel (2007) in their study of the antimicrobial properties of wine components, concluded that pH was the most effective factor in the log reduction on *Stapholoccocus aureus and E.coli* O157:H7. Low pH has two primary mechanisms for inhibition; disrupting the functioning of its enzymes as well as the transport of nutrients into the cell (Jay and others 2006).

The pH is detrimental to the microbial cell by increasing the effectiveness of antimicrobial components in wine. The bacterial cell has a residual negative charge and is relatively impermeable to $H^+$ and $OH^-$ ions however at acid pH values, compounds such as organic acids disassociate and can enter the negatively charged cell (Jay et al., 2006; Uljas and Ingham, 1999). In an acidic environment such as wine the cell must keep H+ from entering or expel H+ ions as rapidly as they enter (Jay et al., 2006). Low pH also adversely affects the cell due to disruption of enzymatic activity and cellular metabolism (Bearson et al., 1997; Kobayashi and others 2000).

Furthermore, the effectiveness of ethanol is increased in a low pH environment (Boban et al., 2010). At low pH ethanol has a greater effect on the cytoplasmic pH (Jordan and others 1999).

Although low pH has been shown to have an antimicrobial effect, enteric pathogens have developed mechanisms to tolerate acidic environments. Gorden and Small (1993) found that *E.coli* isolates were able to survive an environment of pH 2.5 for 2 hours while *Salmonella* species tested were unable to survive at pH 3. Similarly Benjamin and Datta reported the survival of enterohemorrhagic *E.coli* after five hours at pH 2.5 and 3.0 (1995). Due to acid tolerance, pH of wine cannot assure sterilization.

## Ethanol

Alcohol is present in wine as a product of fermentation. The high concentrations of alcohol in wine (10-15%) creates an osmotically stressful environment for microbial cells (Ganan et al., 2009; Ingram and Buttke, 1984). Alcohols are considered good disinfectants because they have a bactericidal effect against vegetative cell, and are relatively inexpensive (Larson and Morton, 2001).

The effects of alcohol on bacteria is a function of chain length and hydrophobicity (Larson and Morton, 2001; Ingram and Buttke, 1984). Chain lengths of ten or more carbons are much more toxic to cells than short chain length alcohols (Ingram and Buttke, 1984). Long chain ($C_6$ to $C_{20}$) alcohols are effective primarily against Gram-positive bacteria, along with some yeasts and molds and show little or

no activity against Gram-negatives (Kubo and others 1995). A study by Kubo and others (1995) showed that the maximum activity against the five Gram-positive bacteria tested was between $C_{12}$ and $C_{16}$ chain lengths(1995). The bactericidal action of the aliphatic alcohols intensifies with increasing molecular weight with the exception of tertiary alcohols (Larson and Morton, 2001).

Ethanol is known to damage the cytoplasmic membrane, causing an increase in permeability of the membrane (Waite and Daeschel, 2007; Ingram and Buttke, 1984). High concentrations of ethanol solubilize lipids and denature proteins in the cell membrane. The increase in permeability leads to enhanced efficacy of organic acids (Harding and Maidment 1996; Barker and Park 2001; Just and Daeschel 2003). Ethanol inhibits the cells ability to regulate internal pH and maintain a strong electrochemical gradient across the plasma membrane (Meyrial and others 1997). It is believed that an increase in leakage of ions and metabolites may be responsible for the decreased rate of growth in the presences of alcohols (Ingram and Buttke, 1984).

Alcohols also have a lytic effect on microbial cells. Lysis of microorganisms occurs with many antiseptics when used at concentrations approximately twice the minimum concentrations producing bacteriostasis. Cells become swollen and lyse due to the large osmotic pressure difference across the plasma membrane (Ingram and Buttke, 1984).

The mechanism of inhibition is also related to alcohols ability to denature proteins (Larson and Morton, 2001; Meyrial et al., 1997). In the absence of water, proteins are not denatured as readily as when water is present, explaining why

absolute ethanol is less bactericidal than mixtures of alcohol and water (Larson and Morton, 2001).

Extensive research on the toxicity of ethanol to pathogenic bacteria has been conducted. Most microorganisms are inhibited by (v/v) above 17% (Ingram and Buttke, 1984). Waite and Daeschel showed that there was an increase in inactivation of *S. aureus* and *E. coli* as ethanol concentrations increased in wine. In their study ethanol concentration of 14.66% was significantly more effective than concentrations of 13.28% and 12.08% (Waite and Daeschel, 2007). In contrast Sugita-Konishi and colleagues reported that a 14% ethanol concentration had no effect on *Salmonella enteritidis* or *E.coli O157:H7* and was therefore not a factor in the antimicrobial composition of red and white wine. Furthermore, Just and Daeschel (2003) reported that bacteria survived better in ethanol than in the volatile fractions of wine. These studies suggest that while ethanol is antimicrobial it does not alone inhibit growth at the concentrations commonly found in wine.

## Phenolics

In addition to organic acids, ethanol, and low pH, phenolic compounds contribute to the antimicrobial effects of wine. Fresh fruits and vegetables contain a variety of antimicrobial phenolic compounds (Wen et al., 2003). Phenolics occur in plant tissue as simple substituted phenols, glycosides and amides, or complex, polymerized molecules with high molecular weights (Wen et al., 2003). Phenolic

derivatives make up one of the major classes of disinfectants and are also used as preservatives and antibacterial agents in soaps and lotions.

In wine there exist several groups of phenolic compounds such as, hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, phenolic alcohols (non-flavanoids), and flavanoids (García-Ruiz and others 2011). These compounds are important antioxidants in wine (Radovanovic and others 2009; García-Ruiz et al., 2011). They contribute to the sensory characteristics of wine, including color, astringency, and bitterness (García-Ruiz et al., 2011)

Studies have shown that phenolic compounds present in wine have an effect on bacterial growth and metabolism (Ganan et al., 2009). The pH of wine has a substantial effect on the bactericidal activity of phenolic acids. The bactericidal activity increases with a decrease in pH (Wen et al., 2003; Kouassi and Shelef, 1998). The precise mechanisms of these compounds are not fully understood, primarily due to the large range of substitutions and configurations. Parasubstitutions of the phenolic ring of an alkyl chain of up to six carbon as well as halogenation and nitration has been shown to increase antimicrobial activity of phenolics (O'Connor and Rubino, 1991). Increasing the side chain length of phenolic compounds also enhance the antimicrobial activity (Kouassi and Shelef, 1998).

Phenolic compounds have been shown to inhibit the microbial cell in many ways. Effects to the cell include DNA, RNA, protein, and lipid synthesis inhibition as well as damage to bacterial membranes (Cushnie and Lamb, 2005). Scanning electron microscopy has illustrated the damage to the cell wall and release of cytoplasm material in the presence of phenols (García-Ruiz et al., 2011). It is thought

that the damage to membranes is caused by directly penetrating the layer (Cushnie and Lamb, 2005). By fusing into the membrane, phenolic acids also lead to ion leakage and proton influx (Campos and others 2009). Furthermore, by crossing the cell membrane by passive diffusion in their undissociated form, leads to acidification of the cytoplasm and causing proteins to denature (Kouassi and Shelef, 1998)

The effects of wine polyphenols on the growth of wine bacteria have been studied due to the important use of these bacteria in secondary malolactic fermentation and their potential contribution to medicine. Radovanovic and others (2009) showed that antimicrobial activity of six red wines increased as total phenolic increased against *E. coli* and *Staphylococcus aureus*.

Several other studies have investigated the antibacterial effect of individual phenolics. Ganan and others (2009) studied the effects of the major phenolic compounds of wine on the viability of *C. jejuni*. They found that gallic *p*-hydroxybenzoic acid, methyl gallate, epicatechin, synaptic acid, vanillic acid, and caffeic acid at concentrations normally found in wine or lower were effective (Ganan et al., 2009). Similarly Wen and others (2003) found that phenolics (cinamic, *p*-coumaric, ferulic and caffeic acids) had an antilisterial effect however they had synergistic effect when in the presence of each other. Resveratrol has been shown to be effective against pathogens *Staphylococcus aureus*, *Enterococcus faecalis* *Pseudomonas aeruginosa* at concentrations of 171-342 μg/L and was also effective against some fungal species (Chan, 2002). Hydroxycinammic acids proved to be effective against *E.coli* and *S.aureus* , (Herald and Davidson, 1983). Garcian-Ruiz

and others (2011) showed the flavanols and stilbenes had the greatest inhibitory effect followed by hydrocinnamic and hydroxybenzoic acid on lactic acid bacteria (LAB).

## Sulfur Dioxide

Sulfur dioxide is a weak acid that is often added in the winemaking process to control oxidation and the growth of yeast and bacteria (Hammond and Carr, 1976). Sulfur dioxide can be a byproduct of yeast metabolism which may contribute levels between 10 and 40 mg/L in finished wine (Usseglio-Tomasset, 1992).

The antimicrobial action of sulfite is pH dependent (Foegeding and Busta, 1991). As pH decreases, the proportion of sulfur dioxide or sulfurous acid increases. Due to the pH of wine, the majority of $SO_2$ is in the form of $HSO_3^-$ (Ough and Crowell, 1987). Molecular sulfur dioxide or undissociated sulfurous acid are more inhibitory than sulfite ions (Hammond and Carr, 1976). Only free $SO_2$ is an effective antimicrobial (Hammond and Carr, 1976). The antimicrobial effect is greatest at pH values below 4 and becomes ineffective at neutral pH (Foegeding and Busta, 1991; Hammond and Carr, 1976).

There are many factors that contribute to the antimicrobial properties of sulfur dioxide. Sulfur dioxide is highly reactive and interacts with many cell components (Foegeding and Busta, 1991). Sulfur dioxide creates stress on the plasma membrane (Carreté and others, 2002). It is widely accepted that sulfur dioxide enters cells through active transport (Hammond and Carr, 1976). Upon entering the cell

membrane sulfur dioxide disrupts metabolism to produce stasis or death (Hammond and Carr, 1976).

Sulfur dioxide has been shown to interfere with enzymes and proteins. Sulfur dioxide inhibits enzymes by binding enzyme intermediates or the end products, thus upsetting the reaction equilibrium (Foegeding and Busta, 1991). Sulfur dioxide cleaves disulfide bonds in proteins, which may change the molecular conformation of enzymes, thus modifying the active site (Foegeding and Busta, 1991). Furthermore disulfide bonds of proteins can be easily broken by sulfur dioxide, although this reaction is reversible (Hammond and Carr, 1976). Lipid peroxidation due to sulfur dioxide may interfere with membrane functioning, and evidence exists that the bisulfite ion may interact with pyrimidine bases (Foegeding and Busta, 1991). Sulfur dioxide has also been shown to break down thiamine which is a requirement for many microorganisms (Hammond and Carr, 1976).

Sulfur dioxide has been shown to be mutagenic. In an acidic environment sulfur dioxide can replace nucleophilic sulfur group of disulfide bonds of cystine or cystine peptides (Hammond and Carr, 1976). Bisulfate can convert cytosine to uracil, displaying a mutagenic effect (Hammond and Carr, 1976). Sulfur dioxide is also mutagenic due to interference in double helix formation, transcription and inactivation of RNA in coding for proteins (Hammond and Carr, 1976).

Waite and Daeschel (2007) reported that following pH, molecular sulfur dioxide concentration was the next most significant factor contributing to the efficacy of wine against *S. aureus*.

## Synergistic Effects

Recent studies indicate that the individual antimicrobial properties of specific compounds in wine have a synergistic effect (Boban et al., 2010; Weisse and others 1995; Møretrø and Daeschel, 2004). Møretø and Daeschel (2004) reported the combination of organic acid concentrations (malic and tartaric), ethanol (15%), and low pH (≤3.0) had stronger antimicrobial activity than the effects of these compounds individually (2004).

The synergistic effects shown in wine are largely due to the low pH (3.0-4.0). The pH of wine has significant effects on the antimicrobial parameters such as organic acids, phenolics, and sulfur dioxide. Wen and others (2003) reported that though the phenolic compounds in wine have antimicrobial activity, the nature of the effect was pH dependent (Wen et al., 2003). Fernandes and others (2007) found that a combination of ethanol and organic acids acted synergistically. Similarly it has been shown that by decreasing the pH and/or increasing the titratable acidity also causes an increase in the concentrations of molecular sulfur dioxide concentrations, thereby increasing antimicrobial activity (Waite and Daeschel, 2007). This was not true for *C. jejuni* which did not have a varying effect between pHs 3.2 and 7 (Ganan et al., 2009).

Synergistic antimicrobial effects in wine have also been demonstrated by Ganan and others (2009). Their research showed that red and white wines with the same ethanol content showed differing inhibitions suggesting that ethanol does not act alone against *C. jejuni*. Boban and others (2010) showed that ethanol and low pH on

their own had a limited antimicrobial effect. Together the two parameters were far more effective, but not to the extent as observed in wine.

# Chapter Two

## Investigation of the Inhibitory Effects of Diluted Wine against Bacterial Pathogens in Drinking Water

Evelyn R. Hartlerode and Mark A. Daeschel

## Abstract

Wine has been used since antiquity for medicinal purposes. It is known to have been used as an ointment by the ancient Greeks, Egyptians, and Romans to treat infections and other ailments. It has further been suggested that ancient Romans used wine as a means to treat stored water. It is well documented that wine in full concentration is highly effective at inactivating bacteria. The alcohol, pH, and organic acids in wine all contribute to the antimicrobial properties of wine. The aim of this study was to determine the efficacy of wine to disinfect water that had intentionally added bacterial pathogens in an attempt to determine if it was plausible for ancient societies to sanitize their water with wine.

This study determined the efficacy of wine to inactivate *Salmonella poona* NCTC 4840, *Escherichia coli* O157:H7 ATTC 43894, and *Escherichia coli* O157:H7 ATTC 43895 in bottled water at different exposure times. The effects were observed using wine both with and without added $SO_2$. This was compared to the inactivation of bacterial cultures in a prepared solution of bottled water and 0.1N HCl with pH of 3.0 and 2.5. The water was mixed with wine at concentrations between 0 and 20% wine. These and the HCl solutions were inoculated with ~ 5 log10 CFU/ml of bacterial cultures. After 1, 24, and 48 hours the treated waters were subject to microbial enumeration. A 100% kill was achieved against *S. poona* NCTC at 24 hours in water containing 20% wine and water adjusted to pH 2.5 with HCl. Water containing 10% wine with added $SO_2$ demonstrated 100% kill at 48 hours against *S. poona* NCTC

48480. All bacterial cultures exhibited growth both in treatments of 1% and 2% wine. *Escherichia coli* strains were resistant to inactivation in the wine and water treatments and were not subject to 100% inactivation under any of the wine concentrations. Concentrations between 5% and 20% had a bacteriostatic effect against *E.coli* O157:H7 43894 and 43895. It was concluded that white wine has the ability to disinfect water containing *S.poona* NCTC 4840 at a concentration of 20% however much higher concentrations were needed to eliminate *E. coli* O157:H7 43894 and 43895.

## Introduction

Ancient Romans had knowledge of the value of wine as medicine however they were limited to empirical knowledge. Today, extensive literature exists describing the mechanisms of wine and its components, as an antimicrobial agent. The antimicrobial properties of wine may have made it possible for civilizations in antiquity to make additions of wine to their drinking water to render in safe from waterborne pathogens.

*Escherichia coli* and *Salmonella* are both Gram negative organisms that have the potential to cause severe illness. Certain strains of *E. coli* can cause gastroenteritis, urinary tract infections, neonatal meningitis and in more severe cases haemolytic-uremic syndrome, peritonitis, mastitis, and septicemia. *Salmonella* species have the potential to cause typhoid fever, and paratyphoid fever. Both organisms are facultative anaerobes. Though they are closely associated with the intestinal tracts of

animals they are ubiquitous and can survive freely in water (Soller and others ; Uyanik and others 2008). Today, despite medical advances there are 3,000 deaths caused by food borne pathogens per year in the United States (CDC, 2011). In the Greco-Roman era, food and water borne epidemics could have had tragic effects. By using wine to disinfect their potable water, sickness from pathogens could have potentially been avoided.

Ancient civilizations had several uses of wine including cultural and medicinal purposes. Wine is known to have been used as an ointment by the ancient Greeks, Egyptians, and Romans to treat infections and other ailments. Pliny the Elder and Hippocrates documented the ancient practices of using wine in medicine It has been suggested that ancient Romans used wine as a means to treat drinking water. It is well documented that wine in full concentration is highly effective at inactivating bacteria. The alcohol, pH, and organic acids in wine all contribute to the antimicrobial properties of wine. The objective of this study was to determine the efficacy of wine in disinfecting drinking water that had intentionally added bacterial pathogens.

## Material and Methods

### Bacterial Cultures

Strains used for these experiments were *Salmonella poona* NCTC 4840, *Escherichia coli* OH157:H7 43894 and *Escherichia coli* OH157:H7 43895. All strains were cultured in tryptic soy broth at $20^{o}$ C. Twenty four hours prior to experiments 0.1ml of culture was transferred to 10ml of tryptic soy broth (TSB).

## Wine Samples

Two Chardonnays produced at Oregon State University were used. One chardonnay was produced with no added sulfites whereas the other had sulfites added. The sulfite was added in the form of potassium metabisulfite to give a value of 50 mg/l total $SO_2$. The Chardonnays were added to bottled drinking water at v/v concentrations of 5, 10, 15, and 20 percent. Wines were heat treated in a water bath (Precision water bath, model 182, Precision Scientific Co., Chicago, IL.) at $65^{o}$ C for 15 minutes to eliminate any residual yeast.

## pH Adjusted Treatments

For all experiments, control samples of bottled water prepared with HCl 1N were used to adjust the pH. The pH was adjusted to 2.5 and 3.0.

## Wine Analysis

The wines were analyzed for pH, ethanol, titrayable acidity, and free and total $SO_2$. The pH of all treatments was measured with an Orion model 501 ion analyzer (Thermo Electron Corp.; Waltham, Massachusetts) pH meter and an Orion pH electrode (Thermo Electron Corp., Model 91-O2; Waltham, Massachusetts). Ethanol was measured using standard procedures for the Dujardin-Salleron Model #360 ebulliometer (Laboratoires Dujardin-Salleron, Paris, France). Total acidity was measured potentiometrically using an Orion model 501 ion analyzer (Thermo Electron Corp.; Waltham, Massachusetts) pH meter and titrating with sodium hydroxide to a pH of 8.2. Titratable acidity was reported as g/l tartaric acid. Free bound and total

$SO_2$ was measured by Allison O'Neal (OSU, Osborne Lab) by means of aeration oxidation method as recommended by the organization international de la vigne et du vin (OIV).

## Microbial Analysis

*Escherichia coli* strains were centrifuged using a Sorvall Superspeed SS1 centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at 8000 rpm for 15 minutes and re-suspended in sterilized bottled water. *Escherichia coli* and *Salmonella* strains were used to inoculate 99ml volumes of wine and bottled water at concentrations of 0, 1, 2, 5, 10, 20 % (v/v) as well as bottled water adjusted with HCl to pH 2.5 and 3.0. All strains for all treatments were serially diluted using Butterfield's phosphate buffer (Lombard, Ill.). For all experiments dilutions were plated in duplicate on plate count agar (Neogen Corp,, Lancing, Mich.) after 1, 24, and 48 hours. Bacterial count were determined by pour plate method using plate count agar (Difco, Detroit, Mich.). All plates were counted after incubation for 48 hours at $37^{o}$ C. Initial inocula levels were verified by diluting and plating the test cultures at time zero. All experiments were performed in duplicate.

## Results and Discussion

Wine at diluted levels was shown to have an inhibitory effect against *E. coli* O157:H7 ATTC 43894, 43895, and *S. poona* NCTC 4840. Results were similar to

those of Ganan and others (2009) in which wine (11.5% ethanol) diluted to 25% (v/v) were shown to significantly reduce the viability of *Campylobacter jejuni.* The study was also found to be in agreement with the finding of Fernandes and others (2007); they reported that *Listeria* in a model stomach was more sensitive to wine as concentrations increased.

We studied the effect of two diluted chardonnay wines, one with added $SO_2$ (8 mg/l free, 33.28 mg/l bound) the other with no added $SO_2$ (4mg/l bound) on the viability of *E.coli* O157:H7 ATTC 43894, 43895701, and *S. poona* NCTC 4840 (Table 2.1). Both wines contained approximately 13% ethanol. Wine with no added $SO_2$ had a total acidity of 5.69g/l whereas wine with no added $SO_2$ had a total acidity of 6.76g/l (Table 2.1). There was slight variation in pH; 2.9 for wine with added $SO_2$ and 2.78 for wine with no added $SO_2$ (Table 2.2). The pH for all water and wine samples was acidic. The pH of samples ranged from 4.47 to 2.58. The pH for water with no wine was 6.65. This was as expected due to the weak buffering capacity of mineral water (Azrak and others 2003).

Table 2.1. Wine Sample Parameters.

| Wine Sample | Free $SO_2$ (mg/L) | Bound $SO_2$ (mg/l) | Total $SO_2$ (mg/L) | Titratable Acidity (mg/L Tartaric Acid) | Ethanol (%) | pH |
|---|---|---|---|---|---|---|
| With $SO_2$ | 8 | 33.28 | 41.28 | 5.69 | 13.2 | 2.90 |
| No Added $SO_2$ | --- | --- | 4 | 6.76 | 13.1 | 2.78 |

Table 2.2. pH of Treatments.

| pH of Water and Wine Solutions | | | |
|---|---|---|---|
| Wine with added $SO_2$ | | Wine with no added $SO_2$ | |
| Percent Wine | pH | Percent Wine | pH |
| 0 | 6.93 | 0 | 6.93 |
| 1 | 5.70 | 1 | 5.15 |
| 2 | 4.37 | 2 | 4.20 |
| 5 | 3.59 | 5 | 3.52 |
| 10 | 3.35 | 10 | 3.26 |
| 20 | 3.10 | 20 | 3.03 |

The three bacterial strains demonstrated different sensitivities to the various treatments over time (Table 2.3). *Salmonella poona* NCTC 4840 was the most sensitive to the treatments, followed by *E. coli* O157:H7 43895 and then *E. coli* O157:H7 ATTC 43894*700.* Enteric organisms such a *Salmonella* have developed mechanisms to survive acidic environments (Gorden and Small, 1993; Bearson and others 1997). *Salmonella* species have been shown to be more acid sensitive than *E. coli* species (Gorden and Small, 1993). Furthermore Just and Daeschel (2003) reported that *E. coli* is more tolerant to wine than *Salmonella.*

*Salmonella poona* NCTC 4840 was the only strain that was 100% inactivated. This kill for *S.poona* was achieved with the 20% wine (v/v) treatment either with or without $SO_2$. The 10% wine (v/v) solutions had a bactericidal effect on *S.poona* for treatments with and without $SO_2$. However, the 10% (v/v) wine solution only achieved 100% kill for *S.poona* in the solution with added $SO_2$. The 10% (v/v)

solution was relatively bacteriostatic for both *E.coli* strains with $\log_{10}$ CFU/ml reduction ranging from 0.069 to 0.756.

For all three strains there was continued growth for the 1% and 2% wine (v/v) treatments. *E.coli* O157:H7 ATTC 43894 also sustained growth in the treatment of 5% wine (v/v) with added $SO_2$ treatment. The sustained growth for these treatments suggests that there was too low a concentration of antimicrobials to inhibit growth as well as the potential of nutrients to support growth.

The water adjusted to pH 2.5 treatments was effective against all strains used. After 48 hours, 100% kill was observed for the three strains. Furthermore, a 100% kill was achieved after 24 hours for all strains with the exception of the *E.coli* O157:H7ATTC 43895. There appeared to be a difference in the effectiveness of the pH 2.5 treatment between *E.coli.*O157:H7 ATTC 43894 and *E.* coli O157:H7 ATTC 43895. *Escherichia coli* O157:H7 43894 was more sensitive to the pH 2.5 treatment; achieving total kill at 24 hours rather than 48 hours. The water adjusted to pH 3.0 showed to be less effective than the pH 2.5 treatments. The pH 3.0 was relatively bacteriostatic with the exception of the *E. coli* O157:H7 ATTC 43894 strain. Results varied for *E.coli* O157:H7 ATTC 43894 between the average log reductions of the pH 3.0 treatments.

The presence of sulfur dioxide did not seem to have any additional effect for either of the *E. coli* strains used. Sulfur dioxide did appear to contribute to the inhibitory effects against *S. poona* with the 10% wine (v/v) treatment. Overall, the presences of $SO_2$ did not appear to have an effect on the bactericidal nature of the treatments. This was not surprising due to the low level of free $SO_2$ in the wine used

for the treatments (8mg/L). It is not uncommon for finished wine to have concentrations of free $SO_2$ of up to 50 mg/L (Dott and Trüper, 1976).

Considering the low levels of ethanol in the diluted wines, it can be concluded that ethanol was not a major contributor towards the inhibitory effects of wine. This is in accordance with the finding of Boban and others (2010).

Table 2.3. Average log reduction of bacterial pathogens in wine with and without $SO_2$ diluted to different levels over time.

| Treatment | Exposure Time (hour) | | | | | |
|---|---|---|---|---|---|---|
| | Log Reduction | | | | | |
| | With added $SO_2$ | | | With no added $SO_2$ | | |
| *Salmonella poona* NCTC 4840 | 1 | 24 | 48 | 1 | 24 | 48 |
| 0% Wine | -0.05 | 0.25 | 0.45 | -0.11 | 0.02 | 0.38 |
| 1% Wine | 0.05 | -2.88 | -3.17 | -0.17 | -2.61 | -2.25 |
| 2% Wine | 0.00 | -1.69 | -2.51 | -0.10 | -1.16 | -1.74 |
| 5% Wine | 0.06 | 0.50 | 1.14 | -0.08 | 0.61 | 0.79 |
| 10% Wine | 0.06 | 2.27 | 4.26[1] | 0.01 | 1.26 | 2.40 |
| 20% Wine | 0.37 | 4.26[1] | 4.26[1] | 0.54 | 4.21[1] | 4.21[1] |
| Water Adjusted to pH 3.0 | 0.16 | 3.52 | 1.33 | -0.01 | 0.50 | 0.71 |
| Water Adjusted to pH 2.5 | 0.95 | 3.52 | 4.26[1] | 1.04 | 4.21[1] | 4.21[1] |
| | | | | | | |
| *Escherichia coli* O157:H7 ATTC 43894 | 1 | 24 | 48 | 1 | 24 | 48 |
| 0% Wine | -0.03 | -0.70 | -1.20 | -0.95 | -0.93 | -0.80 |
| 1% Wine | -0.02 | -3.41 | -3.68 | -0.95 | -2.48 | -3.90 |
| 2% Wine | -0.08 | -3.62 | -3.75 | -0.95 | -1.36 | -1.66 |
| 5% Wine | -0.06 | -3.58 | -4.00 | -0.89 | -0.09 | -1.35 |
| 10% Wine | -0.04 | -0.36 | 0.11 | -0.93 | -0.82 | -0.76 |
| 20% Wine | 0.03 | 0.14 | 0.12 | -0.92 | -0.46 | 1.47 |
| Water Adjusted to pH 3.0 | 0.16 | 1.10 | 3.63 | 3.19 | 3.19 | 3.19 |
| Water Adjusted to pH 2.5 | 0.01 | 4.11 | 3.96[1] | 3.19 | 3.19 | 3.19 |
| | | | | | | |
| *Escherichia coli* O157:H7 ATTC 43895 | 1 | 24 | 48 | 1 | 24 | 48 |
| 0% Wine | -0.07 | 0.21 | -3.43 | 0.11 | -0.24 | 0.10 |
| 1% Wine | -0.25 | -3.26 | -3.43 | 0.03 | -2.81 | -2.95 |
| 2% Wine | -0.11 | -1.28 | -3.12 | 0.04 | -0.22 | -1.62 |
| 5% Wine | -0.05 | -0.04 | -0.04 | 0.03 | -0.20 | 0.13 |
| 10% Wine | -0.03 | 0.00 | 0.07 | 0.07 | 0.08 | 0.23 |
| 20% Wine | -0.02 | 0.58 | 1.55 | 0.08 | 0.32 | 1.96 |
| Water Adjusted to pH 3.0 | -0.01 | 0.01 | 0.17 | 0.07 | -0.03 | 0.55 |
| Water Adjusted to pH 2.5 | -0.06 | 2.08 | 3.89 | 0.11 | 1.61 | 3.03 |

[1] 100% kill

## Conclusion

Although the study found that higher concentrations (>20%) are needed to disinfect water that does not mean that it wasn't possible for ancient civilization to sanitize their water with wine. It has been reported that ancient wines probably contained higher levels of acidic acid if not turned entirely to vinegar. Ancient Roman wines frequently contained herbs such as thyme and oregano. Continued studies should be conducted using wine treated with acetic acid as well as herbal extracts. Further studies should also consist of different wines and pathogens. Different results would be expected using different wines. The properties of different wines vary greatly and it would be expected that different results would be obtained using different wines.

The information garnered from this study supports the potential for a wine based disinfectant. Knowledge of minimum concentrations of wine could lead to a more economic formulation for such disinfectants. From the data discussed it can be assumed that higher concentrations of wine (above 20%) would be needed.

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# Chapter Three

## Investigation of Sensory Attributes of Diluted Wine Used as Disinfecting Agent for Drinking Water

Evelyn R. Hartlerode and Mark A. Daeschel

## Abstract

Sensory analysis was conducted to determine the detection threshold of wine in water as well as to determine the consumer preference based on refreshing attributes of various samples consisting of wine diluted with water. The detection level was determined utilizing standard methods of the triangle test. The refreshing attribute was determined with a forced choice preference test. Both studies consisted of 20 students and staff from the University as being representative of consumers.

The sample concentrations of white wine in water for the triangle test were 0.00%, 0.005%, 0.01%, 0.05%, and 0.1% (v/v). For each set of samples panelists were asked to identify the sample that was different. Statistical analysis was carried out based on number of correct choices made by participants. Samples within each set were determined to be significantly different at an $\alpha$ risk of $\leq 0.05$. The assessors were not able to determine a difference between water and 0.005% wine in water (v/v) samples ($\alpha \geq 0.5$). For all other sample sets there was a significant ($\alpha \leq 0.5$) detectable difference.

For each sample set in the triangle test the assessor was asked to rank their feeling of confidence in their decision using a 7-point hedonic scale; one representing "no confidence" and seven representing "extremely confident". The mean overall confidence choice in triangle test choices for samples ranging 0%-0.1% wine was 4.31. The confidence choice between samples was significantly different ($p<0.0.5$) between samples containing correct choice concentration of, 0.00 - 0.100, 0.005 - 0.050, 0.005 - 0.100, and 0.010 - 0.100. Although the difference between the lower

concentration samples was statistically different for the triangle test the response from the consumer in regards to their reported confidence was statistically insignificant.

The sample sets for the preference test were; 0% vs. 1%, 1% vs. 5%, 5% vs. 10%, and 10% vs. 20% wine. The five sets of samples were presented to the panelists in order of increasing concentration. For each set the panelists were asked to decide which of the two samples they thought was the most refreshing. Hypothesis testing was carried out to determine whether the response frequencies between each pair of samples were significantly different ($p \leq 0.05$). The 5% water in wine (v/v) solution was found to be more refreshing than water alone ($p=0.04$). There was no statistically significant difference between the other sample sets.

## Introduction

Refreshing is a term that's been used to describe beverages of wine mixed with water in ancient times (Singleton, 1997). The first objective of this study was to determine the minimum detection level of wine mixed with water. The second objective was to determine whether or not wine mixed with water would be considered a refreshing drink compared to water.

It is generally accepted that refreshing drinks are beverages that help to alleviate symptoms experienced during water deprivation, including thirst, mouth dryness and mental fatigue (Labbe and others 2009a). The concept of refreshing is closely tied to the physiological mechanisms of thirst. The feeling of thirst is part of a complex physiological system regulated primarily by the brain in the hypothalamus that controls the volume of the extracellular liquid (Labbe et al., 2009a).

Studies show that two components that appear to contribute consistently to refreshing perception are energizing and thirst-quenching and mouth wetting (Labbe et al., 2009a; Ramsay and Booth, 1991).The sensory attributes of food and beverage can either have a positive or negative relation to 'refreshing'. It has been determined that a products' likeness to water is the best indicator of refreshing. Cold and clear attributes have been shown to have a significant impact on refreshing (Labbe et al., 2009a; Labbe et al., 2009b; Zellner and Durlach, 2002). Sourness also relates to refreshing due to its mouth wetting effects. Sour flavors increase salivary flow (Ramsay and Booth, 1991). Alternatively astringency and bitterness can take away from refreshing (Ramsay and Booth, 1991).

To our knowledge, no study exists that analyzes the refreshing quality of wine. Although little is known about the refreshing characteristic of wine, the sensory attributes are well known. Sensory attributes that may affect wine in terms of refreshing are ethanol, acidity, and astringency.

Ethanol adds texture and gustative sensations and interacts with other wine components making it and important sensory attribute to wine (Meillon et.al., 2009). It was determined by Meillon and others (2009) that a decrease in ethanol concentration reduces the consumer's perception of heat. Ethanol also contributes to the perception of bitterness and astringency. As ethanol levels are decreased bitterness decreases and astringency increases. On the other hand, as the pH of wine decreases so does astringency (Fontoin and others 2008). Based on the knowledge of consumers' definition of refreshing it can be foreseen that as wine is diluted ethanol and pH decrease and may indeed be considered a refreshing drink.

Although a subjective term, ‘refreshing’ can be studied using qualitative methods. In this study we analyzed diluted wine using the triangle test, hedonic scale, and paired preference testing.

## Materials and Methods

### Samples

Two wines were used in this study. For the triangle test Hogue Cellars Columbia Valley Fumé Blanc 2008 was used while Barnard Griffin Columbia Valley Fumé Blanc 2008 was used for the consumer preference test. Both wines were purchased at a local grocery store and stored at $20^{o}$ Celsius. Water used in these tests was commercial bottled water stored at $20^{o}$ Celsius.

### Panelists

For both the triangle test and the preference test untrained consumer panelists were used. The panelists for both tests included 20 students and staff from the Department of Food Science and Technology at Oregon State University. All panelists were volunteers.

### Sample Preparation

#### *Triangle Test*

Aliquots of wine were added to samples of bottled drinking water. The sample concentrations of wine in water were 0.005%, 0.01%, 0.05%, and 0.1% (v/v). One

ounce (30ml) samples were served to each panel member in nine ounce plastic cups at room temperature.

### *Paired Difference Test*

Aliquots of wine were added to samples of bottled drinking water. The sample concentrations of wine in water were 5%, 10%, 15%, 20%, and 25% (v/v). One ounce (30ml) samples were served to each panel member in nine ounce plastic cups at room temperature.

## Experimental Design

### *Triangle Test*

Five sets of samples were presented simultaneously for the triangle test. The sample sets included the following odd samples 0.005%, 0.01%, 0.05%, and 0.1% wine (v/v). The order of sets of samples was presented in order of increasing concentration of wine. Samples were arranged in random order. The sample arrangements included the following configurations; ABB, BAA, AAB, BBA, ABA, and BAB. Random numbers were assigned to each sample using the random number generator in Microsoft® Excel. Panelists were given water to rinse between each sample set. For each set of samples panelists were asked to identify the sample that was different. Using a hedonic scale ranging from one to seven they were asked to rank their level of confidence in their decision; one representing “no confidence” and seven representing “extremely confident”.

### *Paired Difference Test*

Five sets of samples were presented to the panelists. Each sample set included two samples of different concentrations of wine in water. The sample sets included; 0% vs. 5%, 5% vs. 10%, 10% vs. 20%, and 20% vs. 25%. The two samples for each set were placed randomly. The sample sets were presented in order of increasing wine concentration. For each set the panelists were asked to decide which of the two samples they thought was the most refreshing.

## Data Analysis

### *Triangle test*

Data for the triangle test was analyzed using the Test Sensitivity Analyzer (Meilgaard and others 1999) in Microsoft excel to test for the null ($H_0$: A = B) and the alternative hypothesis ($H_0$: A≠B). The level of significance accepted was predetermined at an alpha-level of 0.05. A one way ANOVA was also performed on the data retrieved from the hedonic scale rating the consumers' confidence in their chosen sample. From the ANOVA analysis a post hoc Tukey Honestly Significant Difference (HSD) test was performed to check for significance across means. For the Tukey HSD difference was accepted at a *p*-value of 0.05.

### *Paired Difference Test*

Analysis of the preference test was conducted with a one proportion z test using Minitab 15. The two-tailed proportion z test was conducted for each set of

samples. The null hypothesis ($H_0$: A = B) was rejected at a *p*-value of > 0.05 and the alternative hypothesis ($H_0$: A ≠B) was accepted at a *p*-value of ≤ 0.05.

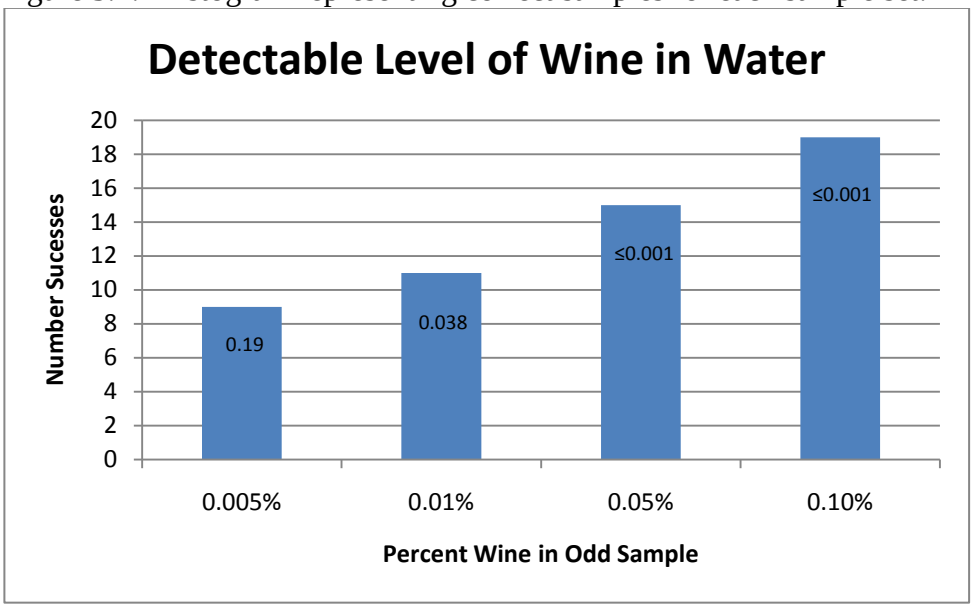
## Results and Discussion

### *Triangle Test*

The triangle test is a common method used in difference testing to identify the presence or absence of a significant difference between samples. In this study, a panel of untrained consumers determined if there was a significant difference between plain water and water mixed with low concentrations of fumé blanc wine. Samples within each set were determined to be significantly different an α risk of ≤ 0.05.

The assessors were not able to determine a difference between water and 0.001% wine in water (v/v) samples (α ≥ 0.5). For all other sample sets there was a detectable difference. It can be said with 95% confident that there was a detectable difference between water and the samples with a concentration 0.005% or higher. As concentrations of wine in the samples increased the α risk decreased dramatically indicating a significant difference between samples.

Figure 3.1. Histogram representing correct samples for each sample set.



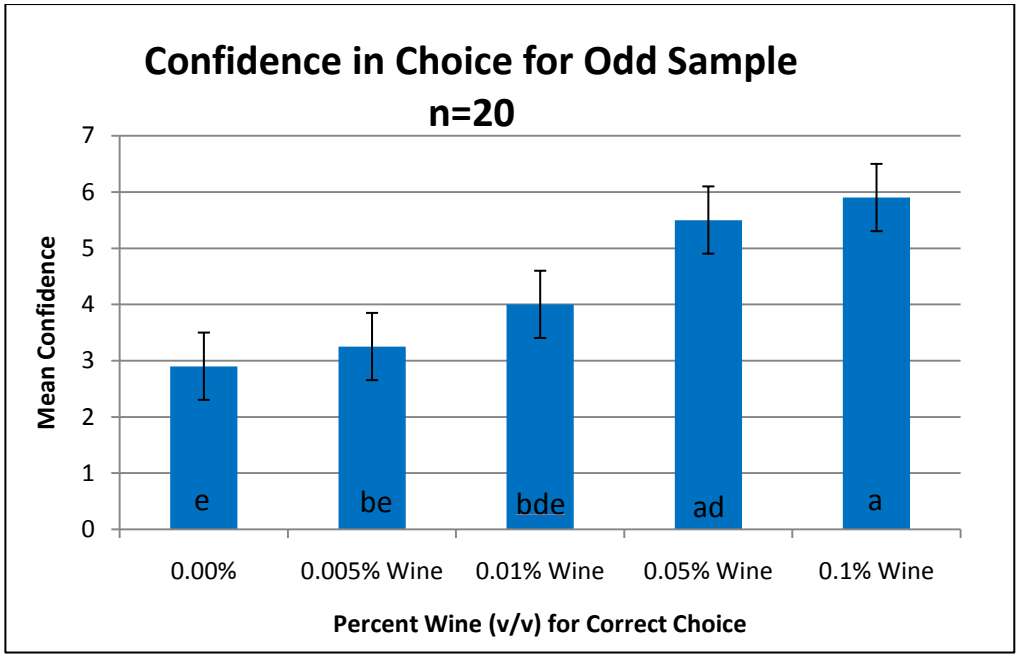

Data labels in chart represent p-values

## *Hedonic Scale Test*

In addition to the triangle test, each assessor was asked to rate their level of confidence in their selection using a seven point hedonic scale. Figure 3.2 shows the distribution of consumer responses for their level of confidence evaluated on a 7-point hedonic scale, with 1= "no confidence" and 7= "extremely confident". Single factor ANOVA was used to determine whether the response frequencies for the level of confidence among samples were significantly different along with a Tukey HSD (Honestly Significant Difference) test. The mean overall confidence choice in triangle test choices for samples ranging 0%-0.1% wine was 4.31. Each sample set was evaluated separately for the level of confidence. The confidence choice between samples was significantly different ($p<0.0.5$) between samples containing correct choice concentration of, 0.00 - 0.050, 0.00 - 0.100, 0.005 - 0.050, 0.005 - 0.100, and

0.010 - 0.100. Although the difference between the lower concentration samples was statistically different for the triangle test the response from the consumer in regards to their reported confidence was statistically insignificant. This further shows that as the percentage of wine in samples increases the perceived difference increases.

Figure 3.2. Consumer response distribution of confidence in choice for each sample set based on a 7-point hedonic scale. Different superscripts represent a significant difference at the $p<0.05$ level, Tukey's HSD




## *Preference Test*

A series of preference tests were used to determine if wine added to water is more refreshing compared to plain water and to determine if the perceived level of refreshing increases along with wine concentration. The response frequencies for the consumers participating in the preference test are given in figures 3.3-3.7.

A one variable two-tailed proportion z-test was conducted for each sample set using Minitab 15.1 statistical software. The one variable proportion z-test was used to

determine whether the response frequencies between each pair of samples were significantly different. Significance was determined based on a *p*-value $\leq 0.05$.

The test statistic for the 5% water in wine (v/v) vs. water alone was statistically significant (p=0.04), there for we reject the null hypothesis that there was no difference between the two population proportions. The results indicate that a 5% wine in water (v/v) solution was considered to be more refreshing than water alone. The preferences between samples containing wine were not significantly different (p>0.05). Similarly Meillon and others (2009) reported the existence of slight differences in wine that was dealcoholized.

Figure 3.3. Preference test results for sample set 0% vs. 5% wine

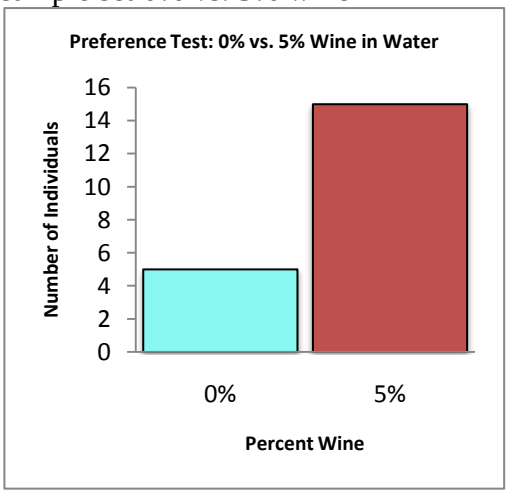



Figure 3.4. Preference test results for sample set 5% vs. 10% wine

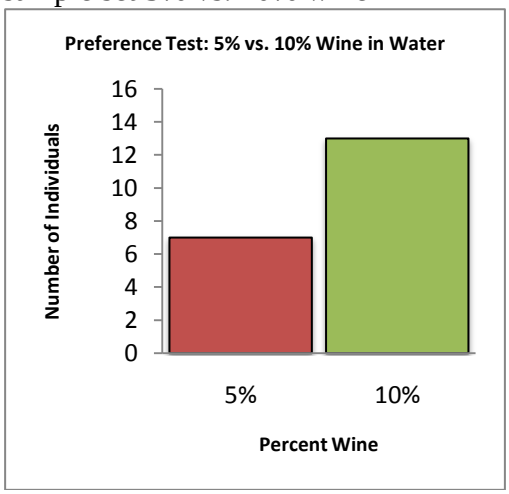
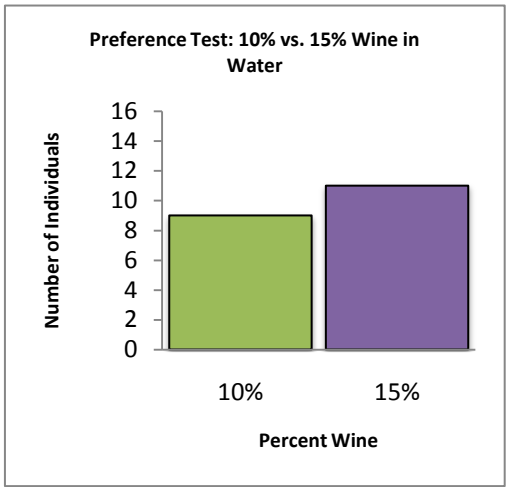



Figure 3.5. Preference test results for sample set 10% vs. 15% wine

Figure 3.6. Preference test results for sample set 15% vs. 20% wine



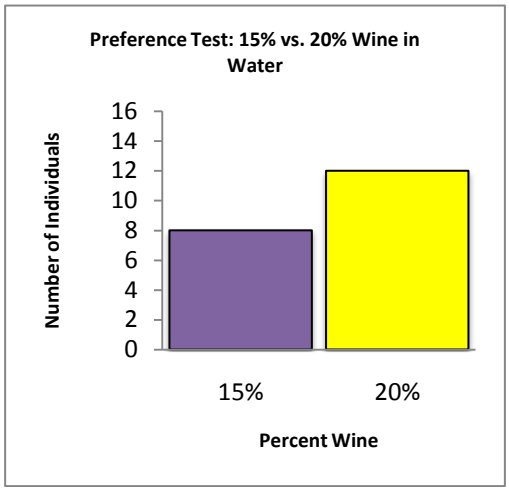
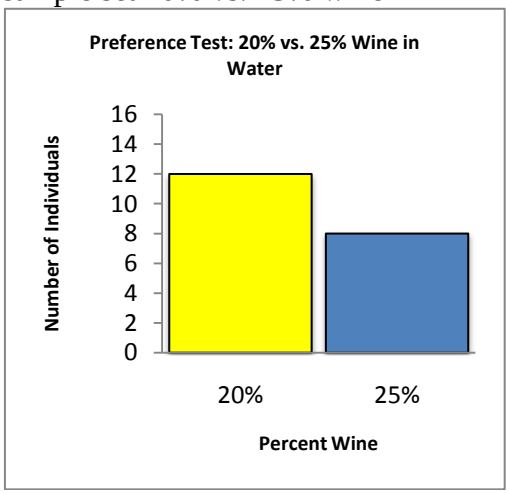




Figure 3.7. Preference test results for sample set 20% vs. 25% wine




## Conclusion

The aim of this study was to determine the minimum detection level of white wine mixed with water and to determine if this mixture would be considered refreshing by a panel of consumer representatives. Overall, this study showed that wine mixed in water had a very low detection level (< 0.01%). We administered a hedonic scale along with the triangle test to determine how confident panelists were with their choice of odd sample. The results of the hedonic scale suggested that the panelists were guessing at the lower concentration levels. Furthermore we determined that a concentration of 5% wine in water was more refreshing compared to water alone. The preference test was limited to the comparisons of individual trials. Further studies should incorporate a factorial design to compare higher concentrations of wine and water against wine alone.

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# Chapter Four

# Conclusions

Ancient Romans knew of wines healthful properties and its ability to ward off disease. Recorded history shows that it was common practice during ancient times to add water to wine. In this study we demonstrated that this ancient practice may have inhibited the growth of pathogenic bacteria. This study concludes that wine mixed with water at a concentration above 5% wine (v/v) had the ability to inhibit growth of *Escherichia coli* O157:H7 ATTC 43894, 43895 and *Salmonella* NCTC 4840. Concentrations of 20% wine (v/v) eliminated *Salmonella,* however higher concentrations were needed to eliminate *E. coli* species. *Escherichia coli* were found to be more tolerant to wine than *S. poona.* It was determined that neither pH nor $SO_2$ in wine had a significant contribution to the inactivation of these pathogens.

Further studies should investigate the efficacy of wine to disinfect water with the addition of acetic acid. It is likely that the addition of acetic acid would greatly impact the ability of wine to disinfect at low concentrations. Furthermore there would also be a benefit from utilizing wine that was prepared similarly to ancient Roman wines.

The study also determined that a solution of 5% wine (v/v) mixed with water was more refreshing than water alone. This finding is in agreement with the belief that the mixtures made by the Romans was thought to be a refreshing drink. Further studies should investigate the refreshing attribute of wine mixed with water at concentrations above 10%. Sensory studies, such as descriptive analysis should be carried out to

further determine the refreshing attributes of wine.

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# Appendices

Appendix I. Raw data of log reduction

| ***Salmonella poona* NCTC 4840, Wine with $SO_2$** | | | |
|---|---|---|---|
| Treatment | 1 Hour | 24 Hours | 48 Hours |
| 0% Wine | 1.93E+04 | 8.15E+03 | 2.95E+03 |
| 1%Wine | 1.79E+04 | 1.34E+07 | 2.47E+07 |
| 2%Wine | 1.70E+04 | 1.10E+06 | 5.80E+06 |
| 5% Wine | 1.36E+04 | 5.95E+03 | 1.45E+03 |
| 10% Wine | 1.36E+04 | 1.13E+02 | 0.00E+00 |
| 20% Wine | 7.80E+03 | 0.00E+00 | 0.00E+00 |
| pH3.0 | 1.18E+04 | 1.05E+03 | 4.50E+02 |
| pH2.5 | 2.15E+03 | 6.50E+00 | 0.00E+00 |
| | | | |
| Treatment | 1 Hour | 24 Hours | 48 Hours |
| 0% Wine | 2.13E+04 | 1.30E+04 | 1.38E+04 |
| 1%Wine | 1.44E+04 | 1.39E+07 | 2.97E+07 |
| 2%Wine | 1.95E+04 | 7.15E+05 | 6.00E+06 |
| 5% Wine | 1.86E+04 | 5.45E+03 | 1.20E+03 |
| 10% Wine | 1.84E+04 | 8.25E+01 | 0.00E+00 |
| 20% Wine | 7.55E+03 | 0.00E+00 | 0.00E+00 |
| pH3.0 | 1.31E+04 | 5.00E+02 | 1.60E+03 |
| pH2.5 | 1.95E+03 | 4.50E+00 | 0.00E+00 |
| | | | |
| ***Salmonella poona* NCTC 4840, Wine with no added $SO_2$** | | | |
| Treatment | 1 Hour | 24 Hours | 48 Hours |
| 0% Wine | 1.94E+04 | 2.03E+04 | 2.26E+04 |
| 1%Wine | 2.64E+04 | 7.75E+06 | 1.12E+07 |
| 2%Wine | 2.23E+04 | 1.55E+05 | 6.00E+05 |
| 5% Wine | 1.81E+04 | 4.15E+03 | 2.50E+03 |
| 10% Wine | 1.63E+04 | 9.85E+02 | 7.40E+01 |
| 20% Wine | 3.90E+03 | 0.00E+00 | 0.00E+00 |
| pH3.0 | 1.43E+04 | 6.50E+03 | 4.45E+03 |
| pH2.5 | 8.00E+02 | 0.00E+00 | 0.00E+00 |

| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 2.33E+04 | 1.23E+04 | 2.10E+03 |
| 1%Wine | 2.27E+04 | 5.65E+06 | 7.70E+05 |
| 2%Wine | 1.94E+04 | 3.65E+05 | 1.35E+06 |
| 5% Wine | 2.17E+04 | 3.85E+03 | 2.80E+03 |
| 10% Wine | 1.56E+04 | 8.10E+02 | 5.80E+01 |
| 20% Wine | 5.75E+03 | 0.00E+00 | 0.00E+00 |
| pH3.0 | 1.95E+04 | 4.15E+03 | 2.30E+03 |
| pH2.5 | 2.85E+03 | 1.00E+00 | 0.00E+00 |

***Escherichia coli* O157:H7 ATTC 43895, Wine with added $SO_2$**

| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 1.12E+04 | 5.50E+03 | 5.05E+03 |
| 1%Wine | 1.17E+04 | 1.68E+07 | 2.85E+07 |
| 2%Wine | 1.10E+04 | 1.60E+05 | 1.54E+07 |
| 5% Wine | 1.20E+04 | 1.08E+04 | 1.18E+04 |
| 10% Wine | 1.11E+04 | 9.65E+03 | 7.80E+03 |
| 20% Wine | 9.45E+03 | 2.95E+03 | 2.60E+02 |
| pH3.0 | 9.00E+03 | 9.75E+03 | 5.45E+03 |
| pH2.5 | 1.07E+04 | 8.00E+01 | 2.50E-01 |

| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 1.09E+04 | 6.15E+03 | 8.05E+03 |
| 1%Wine | 2.37E+04 | 1.73E+07 | 2.26E+07 |
| 2%Wine | 1.35E+04 | 2.02E+05 | 1.01E+07 |
| 5% Wine | 9.40E+03 | 1.00E+04 | 9.25E+03 |
| 10% Wine | 9.35E+03 | 9.20E+03 | 8.25E+03 |
| 20% Wine | 1.02E+04 | 2.05E+03 | 2.75E+02 |
| pH3.0 | 1.02E+04 | 8.65E+03 | 7.35E+03 |
| pH2.5 | 1.11E+04 | 7.55E+01 | 1.50E+00 |

**Escherichia coli O157:H7 ATTC 43895, Wine no added $SO_2$**

| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 1.18E+04 | 1.43E+04 | 1.22E+04 |
| 1%Wine | 1.40E+04 | 9.25E+06 | 1.41E+07 |
| 2%Wine | 1.29E+04 | 1.31E+04 | 1.02E+06 |
| 5% Wine | 1.36E+04 | 1.37E+04 | 8.50E+03 |
| 10% Wine | 1.14E+04 | 1.23E+04 | 9.55E+03 |
| 20% Wine | 1.17E+04 | 4.45E+03 | 2.55E+00 |
| pH3.0 | 1.25E+04 | 1.28E+04 | 2.85E+03 |
| pH2.5 | 9.95E+03 | 3.75E+02 | 2.30E+01 |

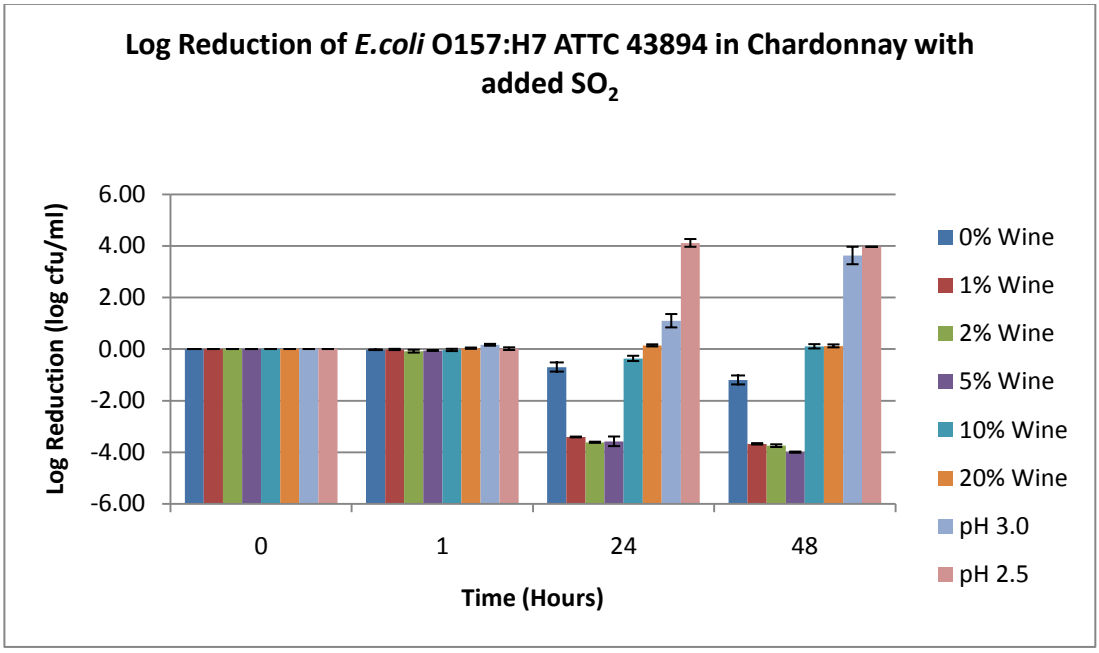
| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 1.10E+04 | 4.65E+04 | 1.14E+04 |
| 1%Wine | 1.37E+04 | 9.60E+06 | 1.23E+07 |
| 2%Wine | 1.36E+04 | 4.55E+04 | 3.60E+05 |
| 5% Wine | 1.41E+04 | 4.00E+04 | 1.38E+04 |
| 10% Wine | 1.36E+04 | 1.22E+04 | 7.95E+03 |
| 20% Wine | 1.26E+04 | 1.08E+04 | 1.03E+04 |
| pH3.0 | 1.23E+04 | 1.89E+04 | 6.00E+03 |
| pH2.5 | 1.31E+04 | 3.45E+02 | 8.00E+00 |

***Escherichia coli* O157:H7 ATTC 43894, Wine with added $SO_2$**

| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 9.55E+03 | 3.05E+04 | 9.75E+04 |
| 1%Wine | 1.01E+04 | 2.28E+07 | 4.10E+07 |
| 2%Wine | 1.27E+04 | 4.00E+07 | 5.80E+07 |
| 5% Wine | 1.01E+04 | 2.27E+07 | 8.70E+07 |
| 10% Wine | 1.11E+04 | 2.69E+04 | 8.75E+03 |
| 20% Wine | 9.05E+03 | 6.05E+03 | 7.95E+03 |
| pH3.0 | 5.80E+03 | 4.05E+02 | 4.25E+00 |
| pH2.5 | 1.01E+04 | 0.00E+00 | 0.00E+00 |

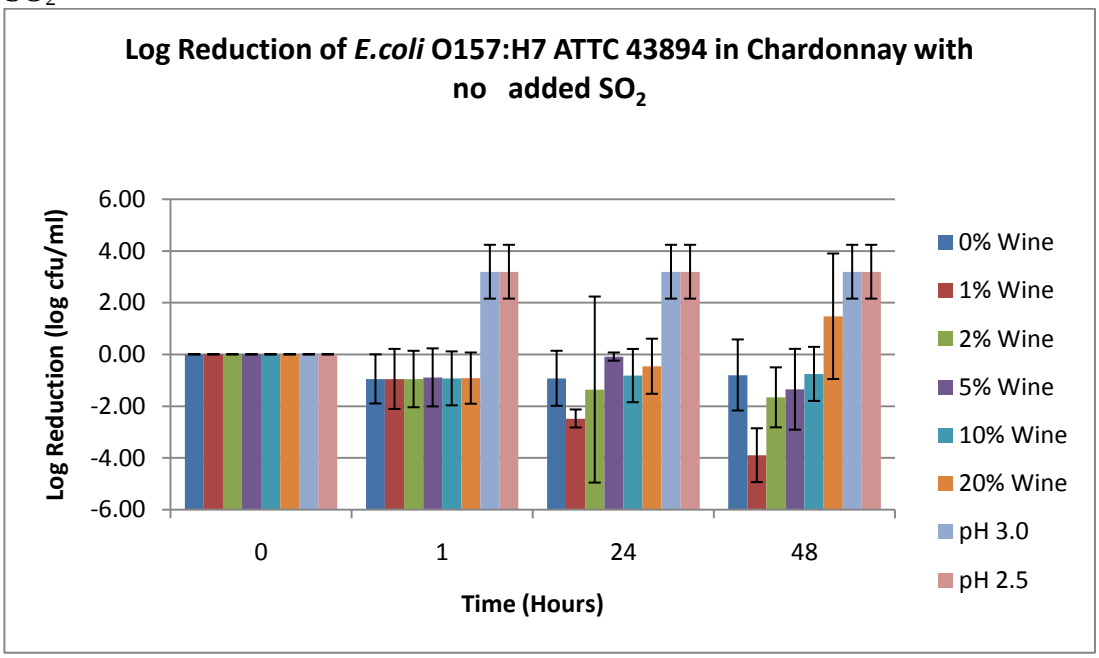
| Treatment | 1 Hour | 24 Hours | 48 Hours |
|---|---|---|---|
| 0% Wine | 1.01E+04 | 6.90E+04 | 2.17E+05 |
| 1%Wine | 9.05E+03 | 2.45E+07 | 4.65E+07 |
| 2%Wine | 9.80E+03 | 3.60E+07 | 4.55E+07 |
| 5% Wine | 1.09E+04 | 5.35E+07 | 9.65E+07 |
| 10% Wine | 9.00E+03 | 1.68E+04 | 5.95E+03 |
| 20% Wine | 8.05E+03 | 7.30E+03 | 6.10E+03 |
| pH3.0 | 6.85E+03 | 1.34E+03 | 4.75E+00 |
| pH2.5 | 7.90E+03 | 5.00E-01 | 0.00E+00 |
| | | | |
| ***Escherichia coli* O157:H7 ATTC 43894, Wine with no added $SO_2$** | | | |
| Treatment | 1 Hour | 24 Hours | 48 Hours |
| 0% Wine | 1.29E+04 | 1.92E+04 | 2.11E+04 |
| 1%Wine | 1.83E+04 | 1.29E+07 | 1.23E+07 |
| 2%Wine | 1.58E+04 | 2.50E+02 | 9.40E+04 |
| 5% Wine | 1.47E+04 | 1.30E+04 | 1.16E+05 |
| 10% Wine | 1.33E+04 | 1.01E+04 | 8.95E+03 |
| 20% Wine | 1.15E+04 | 4.75E+03 | 1.28E+03 |
| pH3.0 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| pH2.5 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | |
| Treatment | 1 Hour | 24 Hours | 48 Hours |
| 0% Wine | 1.50E+04 | 1.25E+04 | 4.55E+03 |
| 1%Wine | 1.06E+04 | 1.15E+07 | 1.23E+07 |
| 2%Wine | 1.25E+04 | 1.00E+02 | 5.45E+04 |
| 5% Wine | 1.01E+04 | 1.45E+04 | 1.05E+04 |
| 10% Wine | 1.32E+04 | 1.06E+04 | 8.80E+03 |
| 20% Wine | 1.46E+04 | 4.25E+03 | 8.70E+02 |
| pH3.0 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| pH2.5 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | |
| | | | |

Appendix II. Log Reductions of Chapter *E. coli* O157:H7 ATTC 43894 in Wine with added $SO_2$



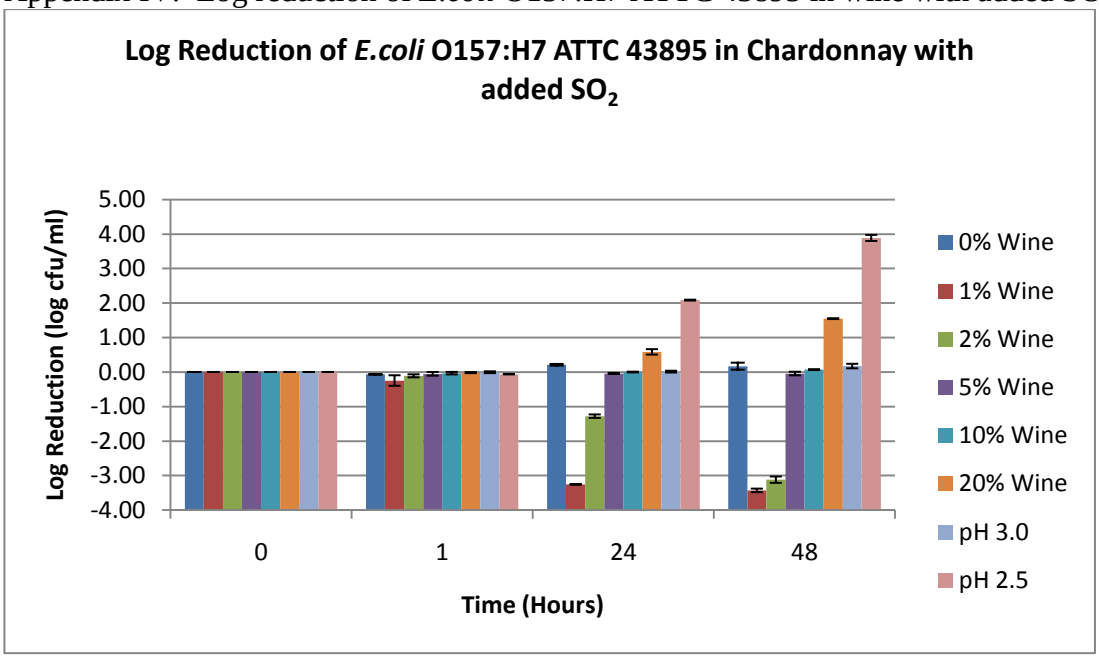

Error bars indicate max and min points

Appendix III. Log reduction of *E. coli* O157:H7 ATTC 43894 in wine with no added $SO_2$



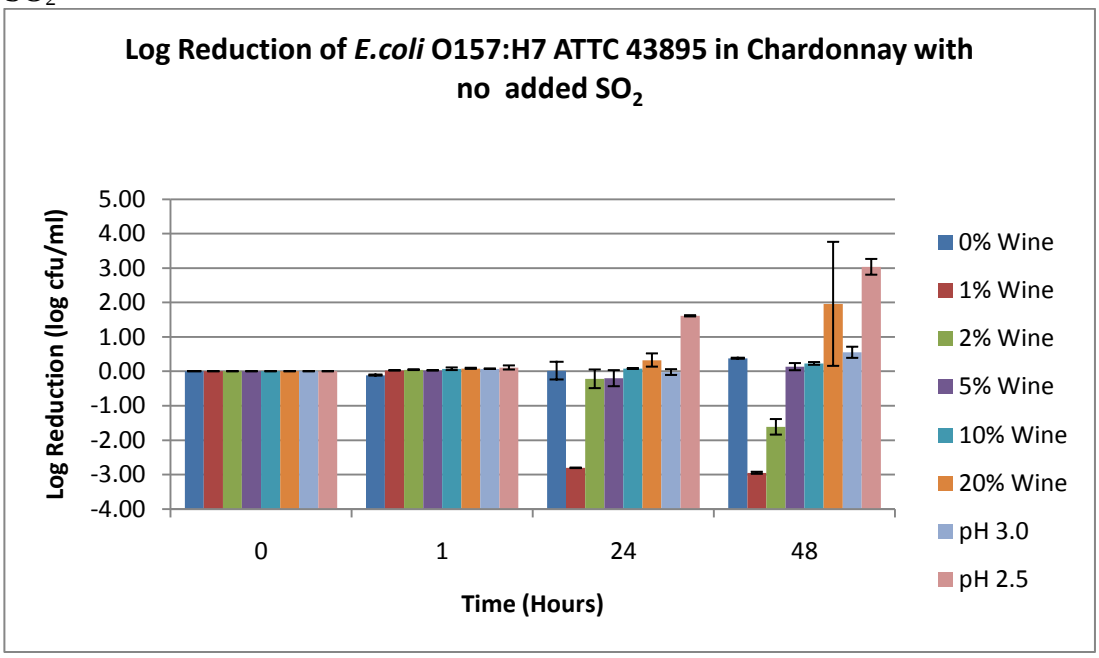

Error bars indicate mad and min values

Appendix IV. Log reduction of *E.coli* O157:H7 ATTC 43895 in wine with added $SO_2$



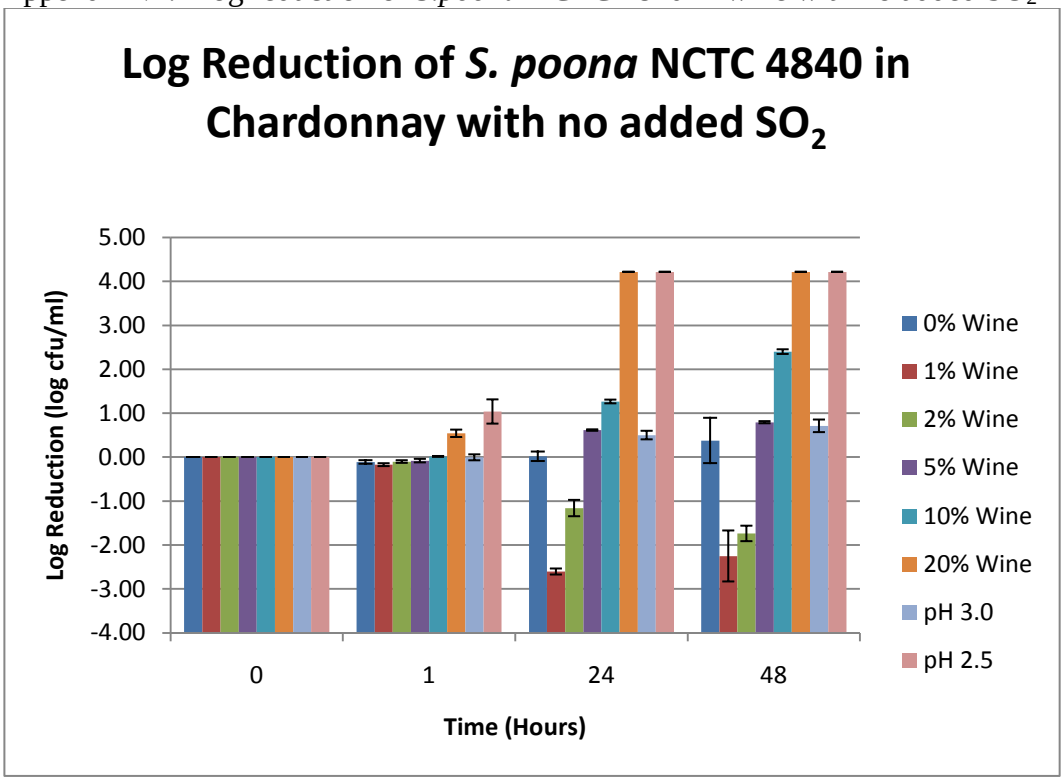

Error bars indicate max and min values

Appendix V. Log reduction on *E.coli* O157:H7 ATTC 43895 in wine with no added $SO_2$



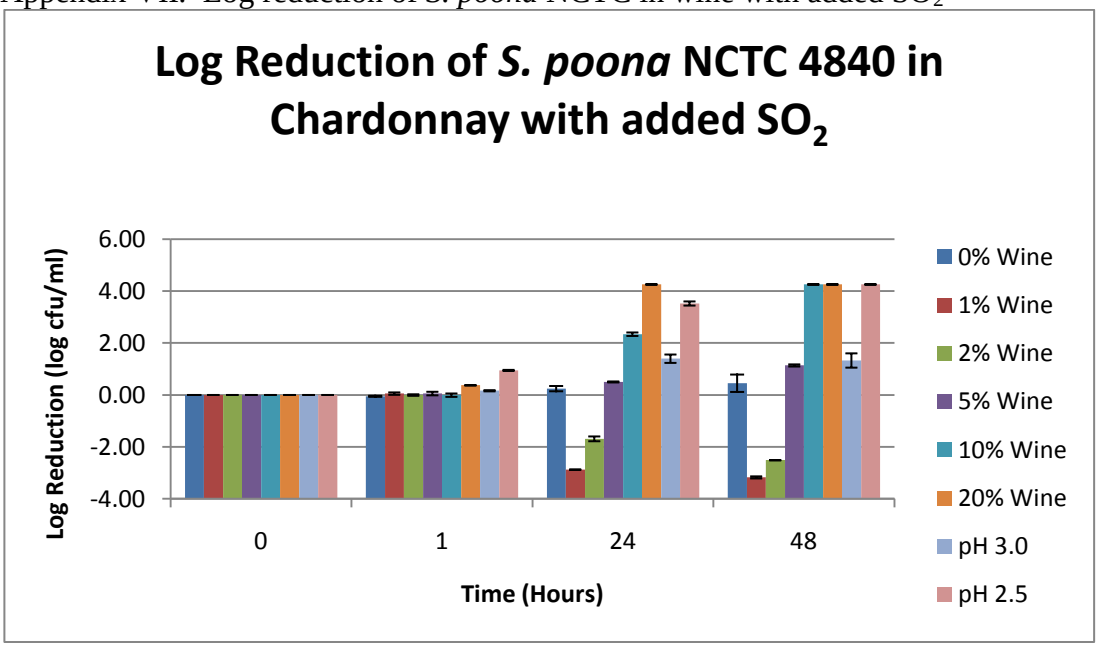

Error bars indicate max and min values

Appendix VI. Log reduction of *S.poona* NCTC 4840 in wine with no added $SO_2$




Error bars indicate max and min values

Appendix VII. Log reduction of *S. poona* NCTC in wine with added $SO_2$




Error bars indicate max and min values

Appendix VIII. Questionnaire for triangle test

Triangle Test Name _____

Instructions: There are five sets of trays, each with three samples. For each tray write the number of each sample in the space provided. Taste each sample. Two are identical; determine which is the odd sample and write the number in of the sample. If no difference is apparent, you must guess. After determining which is the odd sample, indicate how confident you are in your choice. Circle the number that represents your level of confidence in your choice, 1 being not confident at all and 7 being complete confidence.

**Tray 1**

Set of 3 samples which is the odd sample? Comments

_____ _____ _____ _____ _____

1 2 3 4 5 6 7

**Tray 2**

Set of 3 samples which is the odd sample? Comments

_____ _____ _____ _____ _____

1 2 3 4 5 6 7

**Tray 3**

Set of 3 samples which is the odd sample? Comments

_____ _____ _____ _____ _____

1 2 3 4 5 6 7

**Tray 4**

Set of 3 samples which is the odd sample? Comments

_____ _____ _____ _____ _____

1 2 3 4 5 6 7

**Tray 5**

Set of 3 samples which is the odd sample? Comments

_____ _____ _____ _____ _____

1 2 3 4 5 6 7

Appendix IX. Raw data for triangle test

| Odd Sample | Number of Correct Choices |
|---|---|
| All Samples O% | N/A |
| 0.005% | 9 |
| 0.010% | 11 |
| 0.050% | 15 |
| 0.100% | 19 |

Appendix X. Data Analyzer Results of triangle test

| | Inputs | | | Output |
|---|---|---|---|---|
| | number of respondents | Number of Correct Responses | Probability of a Correct Guess | Type I Error |
| | n | x | po | alpha-risk |
| 0.005 % | 20 | 9 | 0.33333 | 0.19055 |
| 0.010 % | 20 | 11 | 0.33333 | 0.03764 |
| 0.050 % | 20 | 15 | 0.33333 | 0.00017 |
| 0.100 % | 20 | 19 | 0.33333 | 1.2E-08 |

Appendix XI. Raw data for hedonic scale

| Assessor | 0.00% | 0.005% Wine | 0.01% Wine | 0.05% Wine | 1% Wine |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 3 | 1 | 1 |
| 2 | 1 | 2 | 3 | 6 | 7 |
| 3 | 3 | 6 | 7 | 7 | 7 |
| 4 | 2 | 5 | 6 | 7 | 7 |
| 5 | 3 | 3 | 2 | 4 | 5 |
| 6 | 4 | 4 | 5 | 6 | 7 |
| 7 | 3 | 6 | 6 | 7 | 7 |
| 8 | 2 | 2 | 6 | 7 | 6 |
| 9 | 1 | 1 | 2 | 6 | 6 |
| 10 | 6 | 7 | 7 | 7 | 7 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 5 | 2 | 4 | 5 | 6 |
| 12 | 1 | 1 | 1 | 3 | 2 |
| 13 | 6 | 7 | 3 | 7 | 7 |
| 14 | 2 | 3 | 2 | 3 | 3 |
| 15 | 2 | 1 | 5 | 7 | 7 |
| 16 | 2 | 3 | 2 | 4 | 6 |
| 17 | 6 | 6 | 6 | 7 | 7 |
| 18 | 1 | 2 | 1 | 7 | 7 |
| 19 | 5 | 1 | 4 | 5 | 7 |
| 20 | 2 | 2 | 5 | 4 | 6 |

Appendix XII. ANOVA Results for Hedonic Scale Data

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| 0.00% | 20 | 58 | 2.9 | 3.2526316 |
| 0.005% Wine | 20 | 65 | 3.25 | 4.6184211 |
| 0.01% Wine | 20 | 80 | 4 | 3.8947368 |
| 0.05% Wine | 20 | 110 | 5.5 | 3.2105263 |
| 1% Wine | 20 | 118 | 5.9 | 3.2526316 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 143.04 | 4 | 35.76 | 9.8085751 | 1.099E-06 | 2.4674936 |
| Within Groups | 346.35 | 95 | 3.6457895 | | | |
| Total | 489.39 | 99 | | | | |

Appendix XIII. Questionaire for the preference test

| |
|---|
| Name _____<br><br>Instructions: There are five sets of trays, each with two samples. For each tray, taste both samples. Determine which is more refreshing and circle the number of the sample |
| **Tray 1**<br>Set of 2 samples<br><br>313 972 |
| **Tray 2**<br>Set of 2 samples<br><br>349 556 |
| **Tray 3**<br>Set of 2 samples<br><br>231 128 |
| **Tray 4**<br>Set of 2 samples<br><br>615 883 |
| **Tray 5**<br>Set of 2 samples<br><br>464 864 |

Appendix XIV. Raw Data for Preference test

Preference choice for each set of samples

| Assessor | Tray 1 | Tray 2 | Tray 3 | Tray 4 | Tray 5 |
|---|---|---|---|---|---|
| 1 | 0% | 10% | 10% | 20% | 25% |
| 2 | 5% | 5% | 10% | 15% | 20% |
| 3 | 5% | 5% | 10% | 15% | 20% |
| 4 | 5% | 10% | 15% | 20% | 20% |
| 5 | 5% | 10% | 15% | 20% | 25% |
| 6 | 5% | 10% | 15% | 20% | 25% |
| 7 | 5% | 10% | 15% | 20% | 20% |
| 8 | 5% | 10% | 15% | 20% | 20% |
| 9 | 5% | 10% | 15% | 15% | 20% |
| 10 | 5% | 10% | 15% | 20% | 25% |
| 11 | 0% | 5% | 10% | 15% | 20% |
| 12 | 0% | 10% | 15% | 15% | 20% |
| 13 | 5% | 10% | 10% | 20% | 25% |
| 14 | 5% | 10% | 15% | 20% | 25% |
| 15 | 5% | 5% | 10% | 15% | 25% |
| 16 | 0% | 5% | 10% | 15% | 20% |
| 17 | 5% | 5% | 15% | 15% | 25% |
| 18 | 5% | 5% | 10% | 20% | 20% |
| 19 | 5% | 10% | 15% | 20% | 20% |
| 20 | 0% | 10% | 10% | 20% | 20% |

Appendix XV. Minitab data for preference test

———— 5/25/2011 1:17:15 PM ————————————————

Welcome to Minitab, press F1 for help.

Test and CI for One Proportion: Tray 1

Test of p = 0.5 vs p not = 0.5

Event = 5.00%

| Variable | X | N | Sample p | 95% CI | P-Value |
|---|---|---|---|---|---|
| Tray 1 | 15 | 20 | 0.750000 | (0.508954, 0.913429) | 0.041 |

Test and CI for One Proportion: Tray 2

Test of p = 0.5 vs p not = 0.5

Event = 10.00%

| Variable | X | N | Sample p | 95% CI | P-Value |
|---|---|---|---|---|---|
| Tray 2 | 13 | 20 | 0.650000 | (0.407811, 0.846091) | 0.263 |

Test and CI for One Proportion: Tray 3

Test of p = 0.5 vs p not = 0.5

Event = 15.00%

| Variable | X | N | Sample p | 95% CI | P-Value |
|---|---|---|---|---|---|
| Tray 3 | 11 | 20 | 0.550000 | (0.315278, 0.769422) | 0.824 |

Test and CI for One Proportion: Tray 4

Test of p = 0.5 vs p not = 0.5

Event = 20.00%

| Variable | X | N | Sample p | 95% CI | P-Value |
|---|---|---|---|---|---|
| Tray 4 | 12 | 20 | 0.600000 | (0.360543, 0.808810) | 0.503 |

Test and CI for One Proportion: Tray 5

Test of p = 0.5 vs p not = 0.5

Event = 25.00%

| Variable | X | N | Sample p | 95% CI | P-Value |
|---|---|---|---|---|---|
| Tray 5 | 8 | 20 | 0.400000 | (0.191190, 0.639457) | 0.503 |