

EFFECTS OF HYPOTAURINE ON THE BROWNING OF WHITE GRAPE JUICE AND
WHITE WINE

By

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To God and my dearly loved parents and brother

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LIST OF ABBREVIATIONS

ANOVA	ANALYSIS OF VARIANCE
AP	AFTER PASTEURIZATION
A REFL	A* VALUE IN REFLECTANCE MODE
A TRANS	A* VALUE IN TRANSMISSION MODE
BP	BEFORE PASTEURIZATION
B REFL	B* VALUE IN REFLECTANCE MODE
B TRANS	B* VALUE IN TRANSMISSION MODE
GRP	GRAPE REACTION PRODUCT
HT	HYPOTAURINE
L REFL	L VALUE IN REFLECTANCE MODE
L TRANS	L VALUE IN TRANSMISSION MODE
o-DPO	O-DIPHENOL OXIDASES
PPO	POLYPHENOL OXIDASES
PPM	PARTS PER MILLION
REFL	REFLECTANCE MODE
SO ₂	SULFUR DIOXIDE
TA	TITRATABLE ACIDITY
TRANS	TRANSMISSION MODE

Abstract of Thesis Presented to the Graduate School
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Browning is a significant concern in the production of white grape juice and wine, and the use of sulfite to inhibit browning is the most common practice in the industry. However, sulfite has been related to allergic reactions in sensitive individuals. A new potential browning inhibitor, hypotaurine, has been identified. This study investigated if hypotaurine would be as effective as sulfite in inhibiting browning in white wine and grape juice.

There were 4 treatments for Niagara and Thompson Seedless grape juices: control, hypotaurine (100ppm and 200ppm) and sulfite 100ppm; two treatments for wine made with Blanc du Bois grape: hypotaurine and sulfite (100ppm).

Significant differences were found among treatments. Browning reactions were evident in Niagara and hypotaurine treatments did not work in the same extent as sulfite, but they were lighter in color than the control. Thompson Seedless also showed browning reactions among their treatments except for the sulfite treatment that was lighter in color. Hypotaurine treatments were not different from the control meaning that they did not inhibit browning to the same extent as sulfite. Even though results for Blanc

du Bois wine are preliminary, since it will need a longer storage time in order to see the browning trend, the hypotaurine treatment showed lower L^* values meaning that hypotaurine treatment is darker than the sulfite treatment. The hypotaurine treatments did not inhibit browning to the same extent as the sulfite treatment.

CHAPTER 1 INTRODUCTION

According to the Food and Agriculture Organization (2009), there are approximately 7.5 million hectares of grapes grown in the world and of this production, approximately 71% is dedicated for wine and most of the remaining for grape juice production. Grapes are currently the highest valued fruit crop and the sixth highest valued of all crops produced in the United States (MKF Research LLC 2007).

During the production of wine and grape juice, one of the main quality issues or technical problems that occur in white grape juice and wine is browning. This event is essentially enzymatic; in the presence of oxygen, the polyphenol oxidase of the grape serves as a catalyst for the oxidation of certain phenolic compounds. The quinones thus formed polymerize to form brown pigments. This causes a radical change in the color of the juice and wine, and it greatly diminishes the quality of the final product (Sapis and others 1983).

Sulfite is one of the main components used during the processing of both white grape juice and wine. Small amounts of sulfite are added to inhibit the development of spoilage microorganisms and to prevent oxidation and browning. This compound is commonly used in the form of potassium metabisulfite and is typically added in concentrations between 50 and 100 parts per million (ppm) (Cooke and Lapsley 1988).

Sulfites have been demonstrated to be effective in inhibiting browning, but their safety has been questioned. Sulfites have been linked to allergic like reactions in some individuals. Even though sulfites appear as safe compounds for consumption of almost all subjects, several studies reported sulfite-sensitive individuals experiencing bad reactions upon ingestion of products with sulfiting agents (Stevenson and Simon 1981).

A new browning inhibitor, hypotaurine, was recently found in blue mussel and is currently being investigated. Hypotaurine is an amino acid found in millimolar concentrations in many animal tissues (Aruoma and others 1988). Fellman and Roth (1985) suggested that hypotaurine acts as an antioxidant in vivo by scavenging highly reactive hydroxyl radicals (OH). Besides its potential as a browning inhibitor, this compound shows some technical and physical properties that make it suitable for industrial uses.

The general objective of this project is to determine if hypotaurine can be as effective as sulfites in preventing browning in grape juice and wine. Specific objectives are to find an optimum concentration of hypotaurine that will work to prevent browning in grape juice and wine. The hypothesis to be tested is that hypotaurine is as effective as sulfites in preventing browning in grape juice.

CHAPTER 2 LITERATURE REVIEW

Economic Status of Grape Products Market

The production of wine and grape products, including wine, grape juice, raisins, and table grapes, as an industry represents about \$162 billion and supports the economy of the United States by providing more than one million jobs (as of 2005) (MKF Research LLC 2007). In 2005, the United States had approximately 935,000 grape bearing acres, giving a total crop value of \$3.5 billion, making grapes the highest value fruit crop in the country and the sixth highest value of all US crops (MKF Research LLC 2007). Of the total grape crop, 9% represents juice grapes, producing approximately 615,000 tons and providing a retail value of more than \$2.8 billion (MKF Research LLC 2007).

The product that has the most valuable impact in the American economy is wine, representing about \$11.4 billion of total revenues from wine sales by US wineries in 2005, including \$707 million in exports. However, the retail value of wine produced in the US in the same year was \$23.8 billion (MKF Research LLC 2007). For the grape juice market, the 2010 figures show that the tonnage of grapes crushed for concentrate production in California went down to approximately 536,000 tons compared with 2005 records. However, this decline does not affect the domestic juice consumption since processors increased their international grape juice purchases to supply their inventories (USDA 2011).

Characterization of Grape Varieties

The leading states in the production of grapes and grape related products are California, Washington, New York, Michigan, Pennsylvania and Oregon (MKF Research

LLC 2007). All these producers classically make white grape juice with Niagara grapes (Washington State University Viticulture and Enology). However, as mentioned before, sometimes the amount of juice demanded by the market exceeds the crop production of any individual fruit, in those cases the selection of other types of grapes is often used and Thompson Seedless is one of the varieties that is used to produce juice concentrate, especially for blending purposes (FAO 2001).

Niagara is a white variety from the species *Vitis labrusca* and is the result of the cross between the Concord and Cassady varieties, with medium-large clusters and large berries, and is considered an excellent grape for juice (Bordelon 2002). Thompson Seedless is a variety from the species *Vitis vinifera* and was introduced from Europe into California in 1872 by William Thompson. It is considered a multipurpose grape due to its wide use as a table, wine, concentrate and raisin grape (Christensen 2011). Blanc du Bois is a hybrid bunch grape variety created by the University of Florida in 1987 (Gray and Klein 1989). Blanc du Bois has shown a great potential in producing premium white wine providing a standard in wines for several southern states (Gray and Klein 1989).

Phenolic Compounds in Grapes and Grape Products

The content of phenolic compounds present in grapes depends on the variety and sometimes the climate may exert an influence on phenols composition (Zoecklein and others 1995). Wine phenolics depends on several factors such as phenolics present in the grape when the wine is made, the extraction and winemaking techniques and the different chemical reactions that take place during aging (Macheix and others 1991). Macheix and others (1990) showed that *Vitis vinifera* contain high concentrations and multiple varieties of phenolic compounds. Thus, grape juice is a great source of flavonoids and other phenolics in the human diet (Rice-Evans and others 1997).

Health Benefits of Phenolic Compounds

Positive health benefits have been attributed to grape juice; multiple studies reported some of these benefits as the increase of serum antioxidant capacity, protection of low-density lipoproteins (LDLs) against oxidation, decrease of native plasma protein oxidation, and reduction of platelet aggregation (Day and others 1997; Stein and others 1999; Keevil and others 2000; Chou and others 2001; O'Byrne and others 2002). Wine is also rich in polyphenols. Evidence of a negative association between coronary heart disease (CHD) mortality and wine consumption is one of protective effects of wine. Moderate consumption of ethanol reduces the incidence of CHD; Renaud and others (1992) attributed the beneficial effects of red wine to its content of phenolic compounds, which come from the grape.

Wine and grape juice have polyphenols at different concentrations (Scalbert and Williamson 2000) and they exhibit different biological activities; their antioxidant properties decrease platelet aggregation and endothelial adhesion, mediate nitric oxide production, suppress cancer cell growth, and reduce oxidative stress (Rice-Evans and others 1997; Andriantsitohaina 1999; Leikert and others 2002; Soleasa and others 2002).

Browning and its Relationship with Phenolic Compounds

One of the most important problems in the production of white grape juice and wine is browning, mainly enzymatic. In the presence of oxygen, polyphenol oxidases naturally present in grapes catalyze the oxidation of phenolic compounds (Sapis and others 1983). Polyphenoloxidases catalyze hydroxylation of monophenols to o-diphenols and the subsequent oxidation to o-quinones, which polymerize resulting in brown, red or black colored pigments, depending on the natural components in plant

tissues (Gasull and Becerra 2006). As a result, brown pigments affect the color of the juice and reduce the quality of the final product (Sapis and others 1983).

According to Sapis and others (1983), one of the most important aspects in the browning event is the oxidation of the substrate. Other studies confirmed this theory and report that browning is more related with the phenolic compounds than with the enzymes present in any given grape cultivar (Lee and Jaworski 1988). The same authors reported that each individual phenolic compound has their own degree of browning (Lee and Jaworski 1988). Under these assumptions, the oxidation of substrate is the main limiting factor in browning reactions.

Polyphenoloxidases (PPOs)

In general, enzymes that participate in these oxidation reactions are called polyphenoloxidases, however, the Enzyme Commission originally referred to two enzymes in this category: laccase also called p-diphenol: oxygen oxidoreductase (EC 1.10.3.1) and catechol oxidase or o-diphenol oxygen oxidoreductase (EC 1.10.3.2) (Macheix and others 1991). Laccase is not naturally found in grapes. It can be present in grapes, musts and wines owing to the secretion of a fungus called *B. cinerea* that causes different types of rot in grapes. O-diphenol oxygen oxidoreductase (catecholase and cresolase activity) is naturally present in grapes. All browning reactions in musts and wines may be the result of the combination of these three enzymes (Macheix and others 1991).

Usually the term polyphenoloxidases is used for the catecholase and cresolase activity, however, the term o-diphenol oxidase (o-DPO) distinguishes the last two from laccase. O-diphenol oxidases (o-DPO) have been found in several grape varieties including *Vitis vinifera*, *V. labrusca* and related hybrids. The catecholase activity is

higher than the cresolase activity. The absence of cresolase may be due to the considerable instability of the enzyme, which can be lost during some stages of juice or wine making (Macheix and others 1991). Enzyme activity is optimal at relatively low pH, varying from 4 to 6 depending on the cultivar, although the optimum pH depends or varies during grape maturation (Macheix and others 1991).

PPOs and Phenolics Oxidation

According to Zoecklein and others (1995), the enzymatic oxidation process starts in freshly crushed fruit. At this point, polyphenoloxidases (PPO), which are also referred to as phenolases or tyrosinases, catalyze the oxidation processes of dihydroxyphenols to their corresponding quinones; substrates in musts for these reactions are hydroxycinnamates and their derivatives (Zoecklein and others 1995). In the wine, browning reactions are mainly due to oxidation of phenolics such as catechins and leucoanthocyanidins and are generally by chemical means (Zoecklein and others 1995).

According to Lee and Jaworski (1990) the most common phenolic compounds found in white grapes are esters of hydroxycinnamic acid, catechins and procyanidins. In musts, the phenolic compounds that can be found in greater proportions are caffeoyl tartaric (caftaric) acid and *p*-coumaroyl tartaric (coutaric) acid and both compounds are oxidized, in presence of air and phenoloxidases, to caftaric acid *o*-quinones (Cheynier and others 1990). For wines, the phenolic composition would depend on several factors such as the phenolic composition of the grapes from which the wine was made, the extraction process and winemaking techniques, and finally the chemical reactions that occur in wine while aging (Macheix and others 1991).

The period that the white grape pomace stays in contact with the skins before pressing influences the phenolic content of wines by increasing astringency, caftaric

acid amount, and sensitivity to browning. The oxidation reactions and browning occur primarily in the initial stages of the wine making process as phenolics diffused in the juice react with the enzymes present naturally in grapes. The physical damage of the grapes in crushing causes cell disruption, which facilitates phenolics diffusion into the must and the beginning of deterioration reactions. The extraction of compounds depends on contact time, berry crushing, temperature, homogenization of must, addition of sulfite, among others.

Browning reactions that begin in the first stages of must preparation, even before any treatment, are basically the result of oxidation reactions between naturally present phenolic compounds and polyphenoloxidases that catalyze these reactions. There are four major compounds that are the most rapidly oxidized by grape *o*-DPO: (+)catechin, (-)-epicatechin, caffeic acid and catechol or 4-methylcatechol. Carboxyl group esterification from caffeic and *p*-coumaric acids with tartaric acid resulted in an increase in catechol oxidase activity (Macheix and others 1991). The esterification mechanism yields caftaric acid. Caftaric acid is a compound that is readily oxidized, forming caftaric acid quinones that may react with other phenolic compounds either by coupled oxidation mechanisms regenerating caftaric acid or by forming a substance between caftaric acid quinone and other phenolic compounds such as flavonoids (Macheix and others 1991). Cheynier and others (1990) also support the theory that caftaric and coutaric acids oxidize to form caftaric acid *o*-quinones, which can undergo coupled oxidations, where other phenolic compounds are oxidized to their quinones, reducing some caftaric acid quinones back to caftaric acid and being able to oxidize again by *o*-

DPO. The quinones generated by these oxidation reactions polymerize to form brown pigments in the product.

Other Factors Affecting Enzymatic Oxidation

Besides the substrate, there are other factors that also influence the enzymatic oxidation in musts. Among these parameters, the pH of the must, the amount and type of phenolic compound, temperature, and the amount of dissolved oxygen are important factors influencing browning (Zoecklein and others 1995).

The loss of a hydrogen ion from the hydroxyl group from phenols forms a negative phenolate ion and its salts are called phenolates. The oxidation velocity will depend on this phenolate ion and this in turn depends on pH. The phenolate ion can transfer an electron to an oxygen molecule, creating superoxide anion, meanwhile the phenol is converted into a quinone (Paladino and others 2008). This creates a chain reaction which accelerates oxidation velocity. The phenol ionization reaction that produces phenolate has a pKa between 9 and 10, meaning that as the pH increases, the amount of phenolate molecules increases, and therefore oxidation susceptibility (Paladino and others 2008). Moreover, pH affects the equilibrium between the different fractions of sulfur dioxide: at higher pH, lower free molecular sulfur dioxide concentration, and consequently lower protection against oxidation (Paladino and others 2008). The optimum pH range where PPOs will achieve peak activity is between acid and neutral. For the majority of fruits and vegetables, the optimum activity is between a pH of 6.0 and 6.5 while below 4.5 the activity is reduced (Lamikanra 2002). It has been reported that for pHs below 3, the enzymatic activity is irreversibly inactivated (Lamikanra 2002).

Oxidation reactions are at a maximum at 35 to 45°C. Refrigeration of musts after pressing is necessary in white grape juice and wine making. The oxygen consumption

will stop when the temperature reaches 0°C (Macheix and others 1991). The disadvantage of lowering the temperature is that it just blocks the activity of PPOs but does not eliminate PPO. Additionally, lower temperatures enhance the oxygen dissolution in the musts, which can cause oxidation when it reaches higher temperatures (Macheix and others 1991). Likewise, *o*-DPO and laccase can be denatured by increasing temperatures, thus obtaining a wine enzymatically stable since all the enzymes are inactivated (Macheix and others 1991). To achieve total inactivation of enzymes, temperatures between 60 - 70°C are needed, and this treatment is usually performed after crushing and destemming. The temperature needed should be reached in a very short time and kept it for 30 to 60 minutes (Macheix and others 1991). In white musts, almost complete destruction of enzymes, less than 1% of initial activity, can be achieved by heating to 70°C for 3 to 5 minutes, depending on the pH of the medium, and in fact, the lower the pH of the must, the faster the denaturation (Macheix and others 1991).

Oxygen uptake is another factor that influences degradation reactions. The uptake in musts is mainly due to enzyme activity; however, some residual oxygen uptake can be present even after polyphenoloxidases disappear. Oxygen uptake also depends on grape variety and temperature. The major uptake takes place at 35 to 45°C and it seems to increase very rapidly at 20 to 35°C (Macheix and others 1991). This increase in uptake at that temperature is related to the increase in PPO activity in musts. There is also oxygen uptake in wines. There is no PPO present in wine and the uptake is mainly by chemical reactions. The phenolic content determines the oxygen uptake potential of a wine. Oxidation of phenolic compounds is affected by the pH of the product, and

under acid conditions, the oxidation reaction will be slow but the total oxygen uptake high. Conversely, in alkaline conditions, oxygen uptake will be faster but limited (Macheix and others 1991). These reactions can be explained by regenerative polymerization, in which a dimer of the original vicinal diphenol can be subjected to reoxidation, leading to the high oxygen uptake in acidic conditions (Macheix and others 1991).

Glutathione and its Anti-browning Role

Glutathione is a peptide that can be found naturally in grapes and wines; it is a very strong antioxidant and it has been suggested as an anti-browning agent in white wines (Moreno-Arribas and Polo 2009). Glutathione is a compound that interferes with the oxidation mechanism of caftaric acid by reacting with the caftaric acid quinone and forming 2-S-glutathionyl caftaric acid, also called grape reaction product (GRP) (Cheynier and others 1990). GRP is a non-substrate compound for PPO and can prevent oxidation reactions and limit browning. However, when the glutathione is all used up, caftaric acid quinones are involved in coupled oxidation reactions and thus all the remaining phenolic compounds still present can be oxidized (Macheix and others 1991).

Berry disruption during crushing leads to the combination of caftaric acid quinones with the glutathione present in grapes resulting in GRP, which is not brown and will not brown enzymatically (Macheix and others 1991). The caftaric acid/glutathione ratio also plays an important role in browning reactions. At 1 mol of glutathione per mole of caftaric acid, the GRP conversion yield is high, demonstrating high affinity between the caftaric acid quinone for glutathione. With less than 1 mol of glutathione, GRP conversion depends on glutathione available for the reaction. Nevertheless, GRP can

also be oxidized by laccase resulting in brown polymers (Ribereau-Gayon and others 2000). In conclusion, the caftaric acid:glutathione ratio is often a determinant in browning reactions (Macheix and others 1991).

Sulfur Dioxide in Oxidation Prevention

Sulfur dioxide (SO₂) has been used for centuries to protect juices and wines from oxidation reactions and browning (Macheix and others 1991). It has many properties which make it indispensable in juice and wine making, with both antimicrobial and antioxidant activities. SO₂ inhibits microbial growth and is more effective against bacteria than yeasts. At low concentrations this inhibition can be transitory, but at high concentrations SO₂ is able to destroy certain microbial populations (Ribereau-Gayon and others 2000). As an antioxidant, SO₂ can bind with dissolved oxygen; the reaction is slow and its protection is against chemical oxidations of wine phenolic compounds (Ribereau-Gayon and others 2000). SO₂ also works by inhibiting enzyme activity (*o*-DPO and laccase) and can inactivate these enzymes over time (Ribereau-Gayon and others 2000).

Grape juice and wines contain many compounds that are very oxidizable. Sulfur dioxide's main mode of action is to compete with oxygen for susceptible chemical groups available for reactions. As mentioned before, browning reactions are a result of polyphenoloxidases activity catalyzing the oxidation of certain phenolics to their corresponding quinones, but SO₂ works by destabilizing disulfides bridges that keep enzymes in their native or active form (Zoecklein and others 1995).

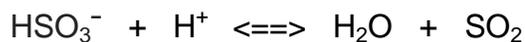
Bound and Free Sulfur Dioxide

Sulfur dioxide can be present in two forms: bound and free, and their sum represents total SO₂. Bound SO₂ is the result of the formation or reaction of different

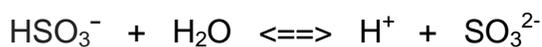
compounds, such as aldehydes, anthocyanins, proteins, sugar, etc., and bisulfite ion.

The amount of bound SO₂ depends on a pH-dependent reversible reaction in which the lower the pH, the slower the addition. This bound SO₂ will have less inhibition towards deleterious reactions, in comparison with free SO₂ that would have the most antimicrobial effect (Zoecklein and others 1995).

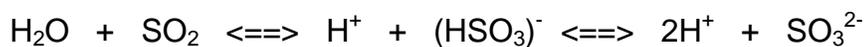
The main way to add sulfite to grape juice or wine is in the salt form as potassium metabisulfite. This salt dissociates, giving two moles of SO₂ for each mole of salt, providing a yield of 57.6% from potassium metabisulfite (Rotter 2011). Potassium metabisulfite dissociates into potassium ions (K⁺) and singly ionized bisulfite (HSO₃⁻). Sulfur dioxide dissociates into three fractions depending on the pH and the fractions that can be found in the solution are: molecular SO₂ (SO₂), sulfite (SO₃⁼) and bisulfite (HSO₃⁻). The dissociation of these fractions is almost immediate (Rotter 2011). Wine is acidic so bisulfite (HSO₃⁻) can transform into sulfur dioxide according to this equation



Additionally, another reaction occurs:



The complete reaction is shown below:



According to Figure 1-1, the predominant form of free SO₂ in wine and juice is bisulfite since the pH is typically between 3 and 4. This form causes inactivation of PPO and binds with quinones that can be present in juice. Bisulfite can also bleach brown pigments and slows polymerization reactions of certain phenols (Rotter 2011).

The most active form of sulfite is the molecule SO_2 . It has anti-microbial activity in juices and wines, and is about 500 times more active than bisulfite in anti-microbial activity (Rotter 2011). Molecular SO_2 also possesses antioxidant activity against deterioration reactions; it is volatile and responsible for odor and sulfurous taste in wines (Rotter 2011). The bisulfite fraction is the form that binds with other compounds. These bound products are mainly unstable and may provide a reserve for free SO_2 ; however these will depend on binding kinetics of the bound products (Rotter 2011). The binding rate will depend on the dissociation constant (k) for each individual binding reaction, the lower the k value of the reaction, the more favored is the bisulfite addition reaction (Rotter 2011). For that reason, acetaldehyde (with a low k value) binding reaction is very fast and strong.

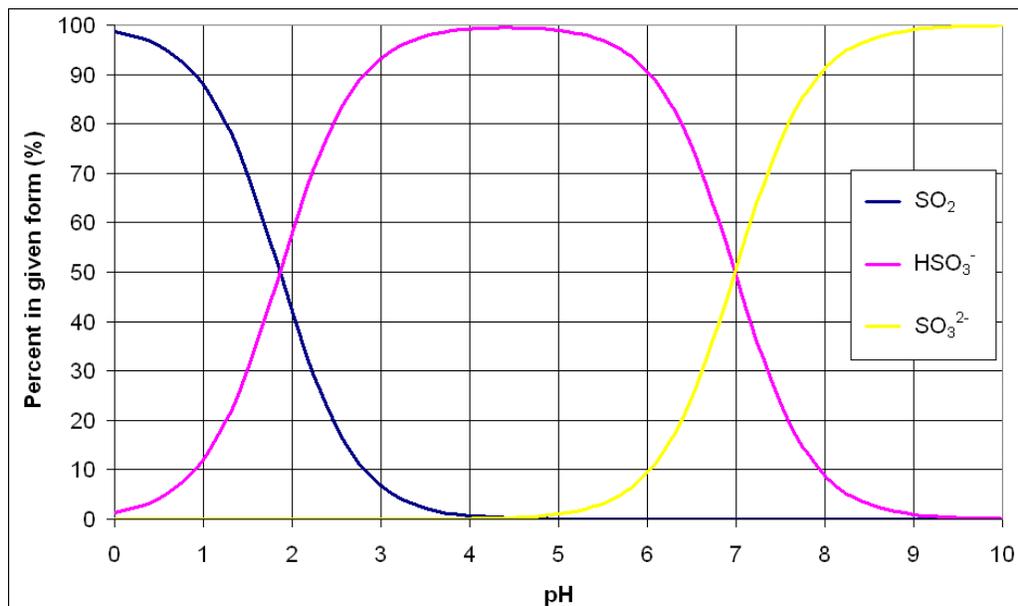


Figure 1-1. Representation of different forms for sulfite at different pH values.

Sulfur dioxide consumes oxygen and decreases the oxidation of other compounds (Ribereau-Gayon and others 2000). Without sulfite, oxygen consumption is very fast and is complete within an average of 4 to 20 minutes, demonstrating the high oxidation

rate of grape juice. However, in wines, chemical reactions play a more important role since oxidation enzymes are no longer present or active. The sulfur dioxide reacts with the dissolved oxygen to protect the wine (Ribereau-Gayon and others 2000). Rotten grapes also can generate a great impact on oxidation reactions because laccase is more active and stable than *o*-DPO. Appropriate sulfiting can prevent laccase activity (Ribereau-Gayon and others 2000).

To obtain effective results in wines, grapes and musts should be rapidly sulfited before fermentation in order to avoid any oxidative reaction. Since oxidation in grapes and especially in musts starts very fast, sulfite is normally added during the crushing or pressing stages, where effective sulfiting will protect the product from future deterioration reactions (Ribereau-Gayon and others 2000). The amount of sulfites used in grape juice and wine varies, ranging typically from 50 to 150 ppm. At 25 ppm, enzymatic activity will be reduced and at 50 to 100 ppm the activity will be totally inhibited (Macheix and others 1991). The maximum sulfite usage for wines in the USA is 350 ppm (Ribereau-Gayon and others 2000). Winemakers usually presume that about 50% of their sulfite addition becomes bound when the total addition was below 30-60 ppm. Above this level, subsequent additions are thought not to bind, giving free SO₂ almost exclusively (Rotter 2011).

Disadvantages of Sulfur Dioxide Usage

High levels of sulfite should be avoided for health and sensory reasons. High sulfite levels in wines impart a metallic and harsh character and high levels of free sulfur dioxide give a pungent aroma, sharpness in the nose and a “soapy” smell (Zoecklein and others 1995). Sulfites neutralizes wine aroma and at high concentrations imparts a burning sensation in the aftertaste (Ribereau-Gayon and others 2000).

Sulfites have been classified as generally recognized as safe by the U.S. Food and Drug Administration (FDA), but recently this status was adapted by rescinding it on raw fruits and vegetables and is required to be labeled in packaged food when residual sulfite levels are greater than 10 ppm as total SO₂ (Taylor and others 1988). Even though sulfites are apparently safe for most individuals, some studies have raised concerns of adverse reactions due to its consumption. Taylor and others (1988) mentioned that the most common reaction is asthma. Acute toxicity has been reported in studies in animals and depending on the animal species, it was found that the median lethal dose (LD₅₀) is between 0.7 and 2.5g of sulfites per kilogram body weight, comparing its toxicity to compounds such as sodium bicarbonate or potassium chloride (Ribereau-Gayon and others 2000). During a chronic toxicity study, it was found that three kinds of health complications were generated: thiamine deficiency due to its destruction by SO₂, histopathological modification of the stomach and slowed growth, giving enough supporting evidence for the World Health Organization to set a recommended daily value of 0.7 mg of SO₂ per kilogram body weight (Ribereau-Gayon and others 2000). Allergic reactions were proven even at very low ingested levels (around 1mg) and the primary concern is the asthmatic group (4-10% of the human population).

Hypotaurine

Despite years of enological research, no satisfactory alternative has been found for sulfite in preventing microbiological spoilage and oxidation in wine. Finding a substitute or a PPO inhibitor that acts as well as sulfite, while avoiding the adverse reactions sulfite causes to the consumers, could be beneficial to many food industries.

A new possible inhibitor, hypotaurine (2-aminoethane sulfinic acid), a compound that can be found in blue mussel, clams, squid, and octopus, was recently discovered and its properties are currently being identified. It shows similar properties to sulfite and it can be useful for the prevention of enzymatic browning or oxidation. According to Schulbach and others (2011), hypotaurine can be added to foods at a level of 500 ppm or less in order to prevent browning, although levels less than 100 ppm are considered adequate. Hypotaurine can be added to vegetables or fruit surfaces, to solid or liquid foods, such as juices or wine, and also can be added to shrimp or other seafood to inhibit browning reactions (Schulbach and others 2011). It has a very high water solubility having a dissolving capacity up to 100 mg/ml, although it is soluble in other solvents such as ethanol or vegetable oil (Schulbach and others 2011).

Hypotaurine is an amino acid found in millimolar concentrations in many animal tissues and has been suggested to act as an antioxidant in vivo by scavenging highly reactive hydroxyl radicals (OH) (Aruoma and others 1988), although the scavenging mechanisms of hydroxyl radicals have not been explored quite well (Green and others 1991). The metabolic precursors of hypotaurine, such as cysteamine, cysteinesulphinic acid and cysteic acid, have also been proposed to act as antioxidants in the brain.

Hypotaurine is a compound that can be formed naturally by the catabolism of cysteine reacting with cysteine dioxygenase CDO, converting it into cysteine sulfinic acid and then by decarboxylation it is transformed into hypotaurine (Schulbach and others 2011).

Hypotaurine is readily oxidized to form taurine. Taurine is also another component that is considered a potent antioxidant. Both taurine and hypotaurine are abundant in

the retina and have been considered to be potential antioxidants in the retina. Pasantes-Morales and others (1985) showed that these two components inhibit light-induced lipid peroxidation and thus, protect rod outer segment structures in frogs' retina. Between taurine and hypotaurine, the latter is the more potent antioxidant (Penfold and Provis 2005). Several studies have demonstrated the importance of taurine, hypotaurine and its precursor in the antioxidant activity in the human body. It has also been shown that these compounds, especially hypotaurine, have antioxidant properties in food.

Bent (2008) hypothesized that the inhibiting compounds present in blue mussel can be isolated and can be used to inhibit plant PPO. However, it was predicted that the inhibitor might not have the same effect for all types of PPOs due to the differences in their structures. Bent (2008) showed the improved performance of hypotaurine to inhibit the action of PPO in avocado, banana, red delicious apple and potato.

Hypotaurine can be used in combination with other compounds that assist in browning inhibition. It can be used with sulfites in order to decrease the amount of this compound used in foods and reach a safe level (Schulbach and others 2011). Hypotaurine can also be combined with other compounds such as ascorbic acid, erythorbic acid, synthetic antioxidant (butylated hydroxyanisole (BHA)), plant based phenolic compounds (tocopherols, flavonoid compounds, coumarins), acidulants (citric, malic, phosphoric acids), chelators (sorbic acid), polyphosphates, EDTA, and enzyme inhibitors (Schulbach and others 2011).

In addition to being an inhibitor of PPO in some food samples, other advantages over conventional PPO inhibitors are that it is found in nature, is colorless, odorless, tasteless, and is able to provide high levels of PPO inhibition at relatively low

concentrations (Bent 2008). One of the disadvantages is its high cost, but with further work a new purification method could be developed to obtain hypotaurine at a lower cost.

CHAPTER 3 MATERIALS AND METHODS

Grapes

Niagara grapes were obtained from Geneva, New York. The grapes were hand harvested and shipped via Fedex – 1 day delivery. The condition of the grapes upon arrival was very bad. They were crushed and brown and had lost a lot of juice.

Thompson Seedless grapes were obtained from California and they were shipped to Tampa where they were picked up. The grapes were in very good condition upon arrival. Blanc du Bois grapes were harvested from the Florida A&M University vineyards in Tallahassee, Florida. The fruit were very ripe and contained some rotten fruit at the time of the harvest. All grapes were kept in a cold room at 2°C until processed, approximately 24 hours.

Grape Juice and Wine Production

Juice Production

Grapes were divided in groups of approximately 18 to 22 kg and each one used for one treatment. Each group was crushed and de-stemmed one at a time. The crushed grapes were weighed and the amount of sulfite or hypotaurine to add was calculated and added to each treatment and well stirred. For sulfite addition, an 18% w/v stock potassium metabisulfite solution was used and for hypotaurine a 15% w/v stock solution was used. After the addition of the treatment, the crushed grapes were pressed in a bladder press, obtaining approximately 9.5 L of juice per treatment. Finally, juice was bottled in one plastic gallon containers and frozen at -29°C until filtration. After about 7 weeks, juices were thawed and filtered. The filtration process was performed with the Buon Vino Super Jet Filter using # 1 coarse filtration filter pads. Filtered juice

was bottled again in three gallon containers and held at 2°C for 1 day until pasteurization. For pasteurization, juices were heated to 88°C and filled at that temperature into 590 ml Polyethylene terephthalate (PET) bottles, capped, and then turned upside down to sterilize the caps. After one minute they were put in a cold wash of water and ice (approximately 0°C) to avoid juice degradation. Bottles were placed at room temperature, approximately 20°C, and away from sunlight for storage.

To evaluate the action of the hypotaurine in comparison with sulfite, the following treatments were established with 2 replications: control (without the addition of sulfite or hypotaurine), hypotaurine 100 ppm (HT100), hypotaurine 200 ppm (HT200) and sulfite 100 ppm. These treatments were established according to the following criteria: hypotaurine 100 and 200 ppm were established by a previous study in where the levels of hypotaurine used were 25, 50, 100 and 200 ppm, in where levels of 100 and 200 ppm showed protection against browning reactions. For this reason was decided to use the levels of 100 and 200 ppm as reference points for this study. And for the sulfite treatment was established the 100 ppm because the maximum amount of sulfite that can be used in grape juice and wine is between 50 – 150 ppm, so it was chosen the amount of 100 ppm as a reference point for this study.

Wine Production

Grapes were divided into 8 groups of 15 to 20 kg each, using 2 groups for each treatment. Grapes were first crushed and de-stemmed. After crushing, the grapes were weighed and the amount of sulfite or hypotaurine was calculated and added to each treatment and well stirred. For sulfite and hypotaurine addition, an 18% w/v and 15% w/v stock potassium metabisulfite and hypotaurine solutions, respectively, were used. Solution additions were used to deliver 50 mg/L of sulfite and 100 mg/L of hypotaurine.

To obtain 50 mg/L of sulfite, 0.23ml of stock solution per pound (0.45 Kg) of juice were added, and for 100 mg/L of hypotaurine, 0.3ml of stock solution were added per pound (0.45 Kg) of juice.

The treatments were then pressed in bladder press to yield 11 liters of juice and placed into 3 gallons carboys with fermentation locks and left at 2°C for the weekend. Then juice was racked and sugar and pH were checked. Sugar was added to increase the content to 20% soluble solids. Finally, the juice was inoculated with *Saccharomyces cerevisiae* Premier Cuvée. Approximately 5 g of yeast, previously rehydrated in 100 ml of warm water, were added. The carboys were placed at 13°C for fermentation. After 1 month, the fermentation was complete (no residual sugars). All treatments were racked again and transferred to clean 1 gallon containers. For the sulfite treatments, 25 mg/L more sulfite was added. After about 4-5 months at 13°C, wine was transferred into 375ml clean glass bottles and added 25mg/L as final sulfite addition before storage, giving a total addition of 100mg/L of potassium metabisulfite in the entire winemaking process. The 2 treatments with 2 replications used were 100ppm of sulfite and 100ppm of hypotaurine.

Chemical Analysis of Samples

The parameters were measured before pasteurization (BP) and after pasteurization (AP) and every month of storage for juice samples. For wine samples the parameters were measured every month of storage. The parameters were color (L^* , a^* , b^*), pH, titratable acidity (TA) and soluble solids. Two separate bottles were sampled at each storage time, and duplicate samples were taken for each bottle. Color analysis was performed using the HunterLab ColorQuest XE, a dual beam xenon flash

spectrophotometer, to measure L*, a* and b* values, in total transmission (trans) and reflectance (refl) modes.

The pH was measured on each sample using the Fisher Scientific Accumet Basic AB15 pH meter with 13-620-631 probe. Titratable acidity was assayed by titrating 5 ml of sample diluted to 150 mL with deionized water to a pH end point of 8.2 using 0.1N sodium hydroxide. To obtain the tartaric acid value in g/L based on TA values, the following formula was used (Zoecklein and others 1995):

$$\frac{g}{L} \text{ Tartaric acid} = \frac{(ml \text{ base})(N \text{ base} \frac{mol}{L})(75 \frac{g}{mol})}{ml \text{ sample}}$$

Soluble solids in each juice sample were measured with the Fisher Thermo Scientific ABBE-3L refractometer set to read Brix from 0 to 85%.

Additional Experiments

Some additional experiments were conducted in conjunction with the main experiments in order to have a better understanding of what was happening with the hypotaurine.

Before Crushing and After Pressing Grapes

This experiment was conducted to see if hypotaurine was binding with grape skins or other compounds and this was limiting its browning inhibition capacity. Grape juice was prepared on a small scale. White grapes were obtained from a local store, crushed by hand each 1 kg, placed in a Ziploc bag, and pressed with cheesecloth to obtain the juice. Hypotaurine (200 ppm) was added either before crushing or after pressing the grapes. A control sample was made without hypotaurine.

Different Hypotaurine Concentrations

An additional experiment was conducted to determine if higher levels of hypotaurine would inhibit browning. Grape juice was prepared on a small scale as described above. White grapes were obtained from a local store and were crushed by hand, placed in a Ziploc bag, and pressed with cheesecloth to obtain the juice. Three concentrations of hypotaurine were added to the juice: 0, 200 and 1000 ppm.

Different Heating Temperatures

An experiment was conducted to see if high temperatures affected hypotaurine browning inhibition capacity. Grape juice was prepared on a small scale. White grapes were obtained from a local store and were crushed by hand, placed in a Ziploc bag, and pressed with cheesecloth to obtain the juice. The experiment heated a hypotaurine stock solution to 3 different temperatures: 40, 60 and 85°C prior to addition to grape juice. A no heat hypotaurine and control samples were also included. The hypotaurine concentration used in this experiment was 200ppm for all treatments.

Statistical Analysis

Data were subjected to analysis of variance using SAS. The data were analyzed as a factorial with the GLM procedure with 4 treatments and 5 storage times (for juice) and 2 treatments with 3 storage times (for wine). There were a total of 16 observations for juice (4 treatments, 2 bottles per treatment, 2 reps per bottle). For wine there were a total of 8 observations (2 treatments, 4 bottles per treatment). The mean separation for the main effects of treatment and storage time were analyzed using Tukey's Honestly Significantly Different (HSD) Test ($p < 0.05$) for multiple comparisons. The means of the interaction between treatments and storage were plotted in graphs.

CHAPTER 4 RESULTS AND DISCUSSION

Niagara

Color results are divided into L*, a* and b* values with 2 modes: transmission and reflectance. Total transmission is the combination of regular transmission and diffuse transmission. Regular transmission is light transmitted straight through the material. Diffuse transmission takes into account surface texture or internal scattering of the material that can cause the light to scatter and also contains part of the color of the material and haze. For reflectance measurements, reflectance with specular included was used and this eliminates the differences due to surface differences and provides measurements that correspond to changes only to pigment color.

The main effects of treatments were significantly ($p < 0.05$) for L trans, a trans, b trans, L refl, a refl, b refl, soluble solids, acidity and pH. The treatment x time interaction effect were significant for a trans, b trans, a refl, b refl and acidity. Results from the main effects, both in transmission and reflectance mode are shown in Table 4-1.

The main effects of treatment (averaged over all storage times) for L trans show that the control treatment (77.57) is different from HT100 (82.96) and HT200 (81.45) treatments and in turn these are different from sulfite treatment (87.53). This clearly shows that the sulfite treatment is lighter in color than hypotaurine and control treatments, but hypotaurine treatments are lighter than the control.

The a* values also shows differences between treatments. Control, with a value of 4.08, is significantly different from the hypotaurine treatments (2.19 and 2.37) and also from the sulfite treatment. The average Δa^* values of the difference between control and each treatment shows that control treatment contains more red color in comparison

with hypotaurine and sulfite treatments. An increase in red color denotes or confirms browning in samples (Krokidaa and others 2007).

There were also differences in b^* values between treatments. The control, with a b^* value of 30.32, is different from the hypotaurine treatments (20.46 and 21.97) and these are different from the sulfite treatment with b^* value of 13.52. Positive values for the b^* value demonstrates that samples contain yellow in their hue. Changes in yellow color may be due to brown pigments (Mohammadi and others 2008). The average Δb^* values of the difference between control and each treatment are positive, meaning that each treatment is less yellow (brown) than the control.

Reflectance L^* values also show significant differences among the treatments. The control is different from the hypotaurine treatments and these are different from the sulfite treatment. The highest L value was from the sulfite treatment with a value of 33.80 and the lowest value was the control with a value of 30.55. The ΔL^* values of the difference between the control and each treatment are positive, demonstrating that hypotaurine and sulfite treatments are lighter than the control, but the sulfite treatment is lighter than the hypotaurine treatments.

Reflectance a^* values are significantly different from each other. The control treatment has the highest a^* value of 1.21, indicating more redness in their hue. HT100 and HT200 have higher values (0.64 and 0.50) than the sulfite treatment (-0.09) indicating more browning. Δa^* values of the difference between control and each treatment are positive demonstrating that hypotaurine and sulfite treatments are less red (brown) than control.

There were also significant differences in reflectance b^* values between treatments. The control treatment has the highest value of 3.96 and the sulfite treatment the lowest value of 0.38. The Δb^* values for each treatment with respect to the control are positive, showing that all treatments are less yellow (brown) than the control.

There were significant differences in soluble solids (S.S) between the treatments. The treatment with the highest soluble solids was the sulfite treatment with 13.60% and the lowest value was the control treatment with 12.14%. This drop in the soluble solids in the control treatment may be due to the pasteurization process. During the transition between the recirculation water and the samples, the first and last samples could contain water because it was difficult to see when the sample starts coming out. The low mean value for the control treatment may be due to that the first and last samples could be diluted with some of the recirculation water of the pasteurization process. These differences among the treatments may also be due to the slightly different maturity of grapes selected for each treatment. The control treatment (12.14%) was significantly different from the other treatments. The HT100 and HT200 treatments (12.91 and 13.25) were not significantly different from each other. The HT100 treatment was significantly different from the sulfite treatment but HT200 was not significantly different from the sulfite treatment.

There were also slight differences in acidity values between treatments. The control treatment shows the lowest acidity value (2.88 g/L tartaric acid) and this may be due to the same explanation for the soluble solids. Some dilution of the sample could happen with recirculation water from the pasteurization process. The HT100 and sulfite treatments are similar to each other but are significantly different from the other

treatments. The acidities of the HT200 and sulfite treatments were significantly different from the other treatments.

Significant differences between treatments were also found for the pH. The sulfite treatment was similar to the HT200 treatment but significantly different from the other treatments. The HT200, HT100 and control treatments were similar to each other and the control and HT100 are similar to each other but significantly different from the sulfite treatment.

Figure 4-1 shows the treatment x storage time interaction for L trans values. All treatments show a decrease in L* values through 1 month and after that tend to stabilize. The control sample was already brown after processing and the L* values changed very little through storage time. After 4 months, the sulfite treatment remains the lightest in color but the hypotaurine and control treatments had similar color. This indicates that the hypotaurine treatments continued to brown some during storage.

Figure 4-2 shows the behavior of the a* values of each treatment over time. The control treatment had the highest a* value initially and decreased after the pasteurization and the first month. This decrease in brownness may be caused by some precipitation of the browning particles that sank to the bottom of the container and were not taken into account in the measurements. The a* values for treatments HT100 and HT200 do not change much over time but their final a* value is very close to the control value. The sulfite treatment had the lowest a* values throughout storage.

Figure 4-3 shows the b* values for the different treatments. This value represents the yellowness or brownness present in the hue of samples. As was expected, the control treatment shows the higher b* values in comparison with the other treatments

throughout storage. Hypotaurine treatments show similar behavior through time, increasing slightly during storage. The sulfite treatment had the lowest b^* values throughout storage meaning that it contains less brown hue. Even though the b^* values did not change greatly over time, the hypotaurine treatments finished with b^* values closer to the control treatment.

The behavior of soluble solids over time among the treatments is variable but slight and is shown in figure 4-4. The soluble solids of the sulfite treatment are steady going from approximately 14°Brix to 13.5°Brix. The HT treatments vary some during storage but show no trends. The soluble solids of the control treatment decreases at the last storage time. This could be caused by the addition of some recirculation water during the pasteurization process in these bottles sampled.

Changes in acidity of all treatments for Niagara variety during storage time are shown in figure 4-5. In general, acidity did not change much during storage. The control had lower acidity than the other treatments after 4 months of storage, which agrees with the lower soluble solids of this treatment after 4 months.

Figure 4-6 shows the pH values for all the treatments over time. The pH of all treatments increased during storage, with the pH of the sulfite treatment remaining higher throughout storage. It is not clear why the pH increased during storage but it possibly was due to precipitation of potassium bitartrate.

Thompson Seedless

The main effects of treatments were significant ($p < 0.05$) for L trans, a trans, btrans, L refl, a refl, b refl, soluble solids, acidity and pH. The treatment x time interactions were significant for L trans, a trans, b trans, L refl, a refl, and b refl. Results from the main effects, are shown in Table 4-2.

Although there were significant main effects of treatments on all color values, the treatment x storage time interaction was very significant for color. Significant differences were also found for soluble solids (S.S), titratable acidity and pH. Soluble solids were similar in the control, HT100 and HT200 treatments, but the sulfite treatment had slightly higher soluble solids. The HT200 treatment had higher acidity and lower pH than the other treatments.

Figure 4-7 shows the treatment x time interaction for L refl values. All treatments show a decrease in their lightness by time points AP and 1. There was very little browning in the juice initially, but browning began during storage. By 2 months the sulfite treatment was lighter than the other treatments and remained lighter. The control treatment had similar levels of browning as the hypotaurine treatments (L value) at 1-3 months of storage.

According to the mean separation of the treatments for every time point, there were no significant differences ($p < 0.05$) between treatments for the time points BP, AP and 1. At 3 months of storage there were significant differences among some treatments. The control treatment is significantly different (darker) from the sulfite treatment with a p value of 0.0006. The HT100 treatment is close to being significantly different from the sulfite treatment with a p value of 0.0521, but this value denotes no significant difference even the mean values for the HT100 do not change that much from time point 2 to time point 3 and this could indicate that there are differences between both treatments.

Figure 4-8 shows the behavior of the a^* values for each treatment over time. All treatments show an increase in their a^* values through 1 month, then either decrease or

remain the same. Overall the control treatment shows the highest a^* values throughout storage, meaning it contains the most browning among the treatments.

There were no differences in time point AP but there are differences in time points 1, 2 and 3. After 1 month, the control treatment is significantly higher than the HT100, HT200 and sulfite treatments with p values of 0.0437, 0.0079 and <0.0001 , respectively. Also the hypotaurine treatments were significantly different from the sulfite treatment. After 3 months, there are significant differences between the control and the sulfite treatment.

Figure 4-9 shows the behavior of the b^* values during storage. The control, HT100 and HT200 treatments steadily increased in b^* value over time, showing an increase in browning. However, the sulfite b^* value decreased slightly during storage. This shows that the sulfite treatment did not brown during storage while the other treatments browned and behaved similarly.

For time points BP and AP there were no significant differences in b^* values among the treatments. However, for points 1, 2 and 3 there are significant differences among the treatments. The sulfite treatment is significantly different from the HT100, HT200 and control treatments.

Figure 4-10 shows the behavior of the soluble solids of all treatments over time. All treatments show a slight decrease in their soluble solids values but overall they show a similar trend through the entire storage time. The HT100 treatment has a decrease in soluble solids at 1 month.

The interaction between the treatments through time for acidity values is shown in figure 4-11. The HT200 had the highest acidity throughout storage, and in general, the

acidity showed no trends during storage. Figure 4-12 shows the pH of all treatments over time. pH of all treatments tended to remain stable or increase slightly during storage. The HT200 treatment had lower pH values than the other treatments throughout storage.

Blanc du Bois

Results from the main effects, both in transmission and reflectance mode are shown in Table 4-3. The main effects of treatments were significant ($p < 0.05$) for L trans, btrans, L refl, b refl, acidity and pH. There were no significant differences for treatment x time interactions. The main effects of treatments (for all storage times) show that there were significant differences in all variables except for a^* values. Results show that the sulfite treatment, with higher L^* values (trans and refl), is lighter in color than the hypotaurine treatment. The sulfite treatment had lower b^* values than the hypotaurine treatment which means that it contains less yellow or brown hue. The presence of yellow color in samples may be due to the formation of brown pigments (Mohammadi and others 2008). In general, the b^* values are very small which could mean that there is not that much yellow color in samples being more close to neutral hues. There were also significant differences in acidity and pH. The sulfite treatment shows higher acidity and lower pH in comparison with the hypotaurine treatment.

Matzdorf and others (1994) reported that the L^* and a^* values were the most relevant browning signs in their studies. Theory confirmed by Lee and Nagy (1988) that reported that Hunter L^* values is an indicator of browning and it generally decreased as a function of storage time and temperature. Changes in b^* value may be due to several reasons such as decomposition of pigments (chlorophyll, carotenoid or anthocyanins)

and non-enzymatic browning reactions resulting in the formation of brown pigments (Mohammadi and others 2008).

Niagara and Thompson underwent in browning reactions over the entire storage time except for the Thompson Seedless sulfite treatment. The a^* and b^* values increased overall for both varieties indicating the presence of brown pigments in the juice. This was also an exception for the sulfite treatment in Thompson Seedless that had very low the a^* and b^* values.

Since Blanc du Bois wine had only been bottled for a few months and are relatively “new” for a wine, they had not browned to any extent. Longer storage times will be needed to see the browning trends, but it appears the hypotaurine treatments are already slightly darker than the sulfite treatment. Hypotaurine treatment showed higher L^* , a^* and b^* values overall in comparison than with the sulfite treatment meaning that browning reactions start to occur.

The sulfite treatments in Thompson Seedless and Blanc du Bois show the expected behavior on how sulfite protects the juice and wine from browning. Sulfite inhibits PPOs present in grapes avoiding the formation of quinones that at some point could polymerize and transform into brown pigments. After the juice was pasteurized and wine fermented, this should eliminate any PPOs trace that might remain in juice and wine, but sulfite also protected against chemical oxidations after pasteurization and fermentation during storage. Sulfite competes with oxygen to protect the oxidation of phenolic compounds present in juice and wine.

The hypotaurine treatments did not show the browning protection that was expected. Bent (2008) found that hypotaurine works successfully in apples, bananas,

potatoes and avocados, not just only by decreasing the amount of browning but also by increasing the time before the browning was observed. He stated that hypotaurine inhibits PPO directly (Bent 2008). However, the measurements that he developed were for short periods of time, where the maximum measurement time period was 2 weeks. This was not the case in this experiment where the measurements were performed over 3 or 4 months.

Also differences in inhibition can exist due to the differences among the structures of the different types of PPOs present in plants. PPOs has varying affinities to different substrates, and can also have differences in the affinity or mechanisms for inhibitors. Variation in PPOs has been observed suggesting a possible variation in the size/shape between grapes PPOs and hypotaurine binding site (Bent 2008). This could be one of the reason why the hypotaurine is not preventing the browning in juice as was expected.

The lack of oxygen scavenging during storage was another possible reason why hypotaurine did not prevent browning. According to Aruoma and others (1988), hypotaurine has been suggested to act as an antioxidant in vivo by scavenging highly reactive hydroxyl radicals, although its pathway has not been explored quite well yet.

According to Ribéreau-Gayon and others (2000), if the crushed grapes undergo fermentation before being sulfited, the protective action of sulfite is reduced. If the addition of sulfite is made before or during grapes crushing, it increases the risk that part of the sulfite will be bound to the solid fragments of grapes (Ribereau-Gayon and others 2000).

The results of whether the addition of hypotaurine before crushing or after pressing affects its anti-browning characteristics are shown in Table 4.4. Table 4-4

shows that the L*, a* and b* values do not change drastically for before crushing and after pressing treatments, meaning that it does not matter at what point the hypotaurine solution is added to grapes. Also these results show that all the hypotaurine treatments prevented browning in this white grape juice compared to the control. This agrees with Bent (2008) results that show that hypotaurine prevents browning in short periods of time. Color in samples was measured after the treatments were added and they were not store to measure their change in color over time.

According to Schulbach and others (2011), hypotaurine at levels of 500ppm or less can prevent or inhibit browning in food, but levels less than 100ppm are generally sufficient. In this study, hypotaurine levels of 100 and 200ppm showed similar results but neither prevented browning. Table 4-5 shows that the L*, a* and b* values are similar between the 200ppm and 1000ppm treatments, and both prevented browning. These results confirm that the 200ppm hypotaurine level that was used in this study should have been sufficient to browning.

Bent (2008) showed that hypotaurine prevents browning during short periods of time at room temperature, but in this experiment the juice was subjected to pasteurization, and the hypotaurine effectiveness could have been altered with heat. According to Bucak and others (2009), hypotaurine was not affected by very low temperatures, but no studies on the effects of heat on hypotaurine have been reported. Results on heating hypotaurine to different temperatures are shown in Table 4-6.

Table 4-6 shows that the L*, a* and b* values were similar among the different temperatures. Temperature does not seem to have a major affect on the browning inhibition capacity of hypotaurine. All of the hypotaurine treatments inhibited browning.

If heat had affected the structure of hypotaurine and had oxidized into taurine, the latter has been shown to be a powerful radical scavenger, which means that it would also protect samples from browning (Green and others 1991).

Table 4-1. Main effects of treatment on the color and quality of Niagara grapes.

Treatment	L trans	a trans	b trans	L refl	a refl	b refl	Soluble Solids (%)	Acidity (g/L tartaric acid)	pH
Control	77.57 c	4.08 a	30.32 a	30.55 c	1.21 a	3.96 a	12.14 c	2.88 c	3.49 bc
HT100	82.96 b	2.19 b	20.46 b	32.75 b	0.64 b	1.57 b	12.91 b	3.17 b	3.52 bc
HT200	81.45 b	2.37 b	21.97 b	32.99 b	0.50 c	1.25 c	13.25 ab	3.41 a	3.56 ab
Sulfite	87.53 a	0.14 c	13.52 c	33.80 a	-0.09 d	0.38 d	13.60 a	3.15 b	3.61 a

*Mean separation executed with Tukey's test for Multiple Comparison. HT100 and HT200 are hypotaurine samples at 100 and 200 ppm, respectively.

Table 4-2. Main effects of treatment on the color and quality of Thompson Seedless grapes.

Treatment	L trans	a trans	b trans	L refl	a refl	b refl	Soluble Solids (%)	Acidity (g/L tartaric acid)	pH
Control	92.99 ab	-0.26 a	4.12 ab	32.84 b	0.19 a	0.44 a	16.24 b	2.90 b	3.59 b
HT100	93.87 a	-0.55 b	3.42 bc	33.10 a	0.06 b	0.23 b	15.23 b	2.65 b	3.65 b
HT200	91.76 bc	-0.27 a	4.54 a	32.95 b	0.08 b	0.27 b	15.75 b	3.57 a	3.39 c
Sulfite	91.51 c	-0.32 a	3.09 c	33.18 a	0.009 c	-0.43 c	17.61 a	2.90 b	3.75 a

*Mean separation executed with Tukey's test for Multiple Comparison. HT100 and HT200 are hypotaurine samples at 100 and 200 ppm, respectively.

Table 4-3. Main effects of treatment on the color and quality of Blanc du Bois wine.

Treatment	L trans	a trans	b trans	L refl	a refl	b refl	Acidity (g/L tartaric acid)	pH
Sulfite	93.55 a	-0.71 a	6.34 b	32.91 a	0.05 a	1.56 b	5.34 a	4.00 b
HT	92.09 b	-0.93 a	10.06 a	32.50 b	-0.02 a	2.77 a	4.75 b	4.11 a

*Mean separation executed with Tukey's test for Multiple Comparison. HT100 and HT200 are hypotaurine samples at 100 and 200 ppm, respectively.

Table 4-4. L*, a* and b* values of white grape juice comparing the addition of hypotaurine (200ppm) before crushing and after pressing.

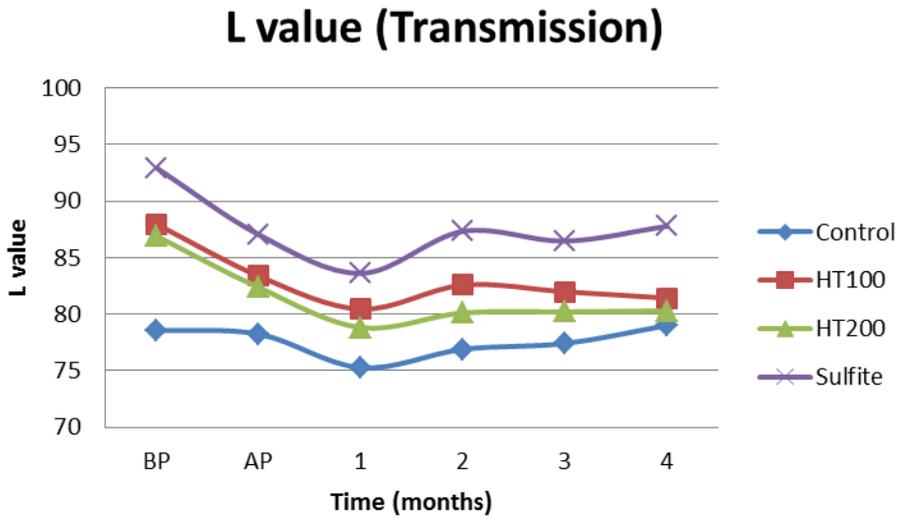
ID	L	a*	b*
Control	32.27	0.04	2.71
Before	33.44	-1.14	-0.62
After	33.49	-1.15	-0.37

Table 4-5. L*, a* and b* values of white grape juice for the different concentrations of hypotaurine.

ID	L	a*	b*
Control	31.01	0.68	2.42
200ppm	33.96	-0.7	0.1
1000ppm	33.64	-0.55	-0.24

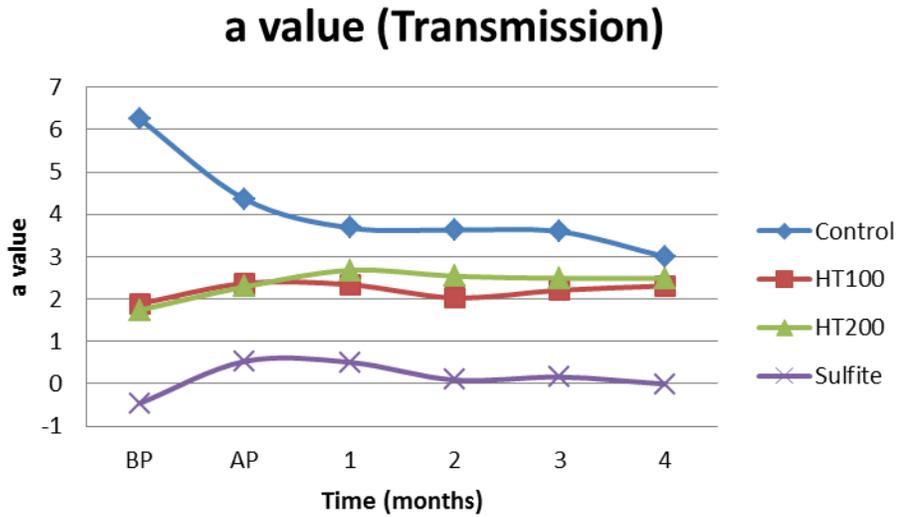
Table 4-6. L*, a* and b* values of white grape juice as affected by heating hypotaurine prior to addition.

ID	L*	a*	b*
Control	32.27	0.04	2.71
No Heat	33.64	-1.13	-0.41
40°C	33.44	-1.03	-0.64
60°C	34.3	-1.18	-0.63
85°C	33.4	-1.11	-0.68



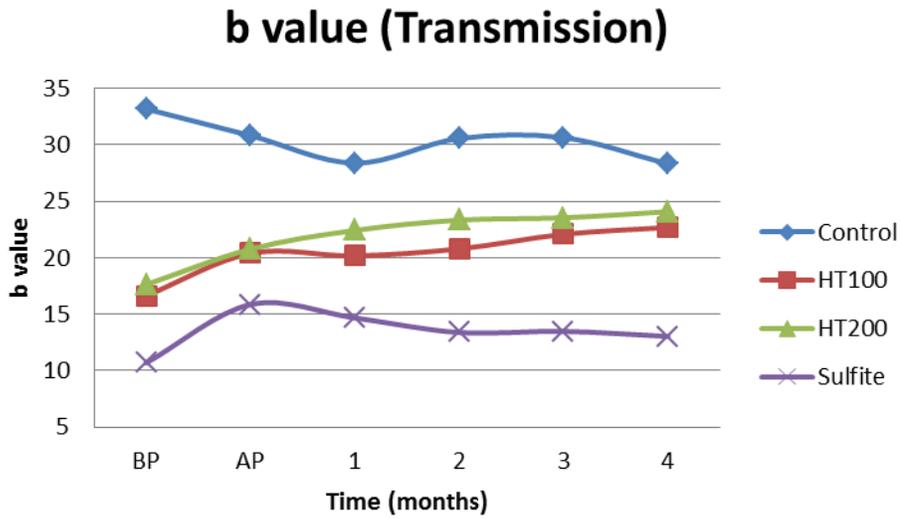
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-1. Interaction of Treatment x Time for L* values in Transmission Mode for Niagara.



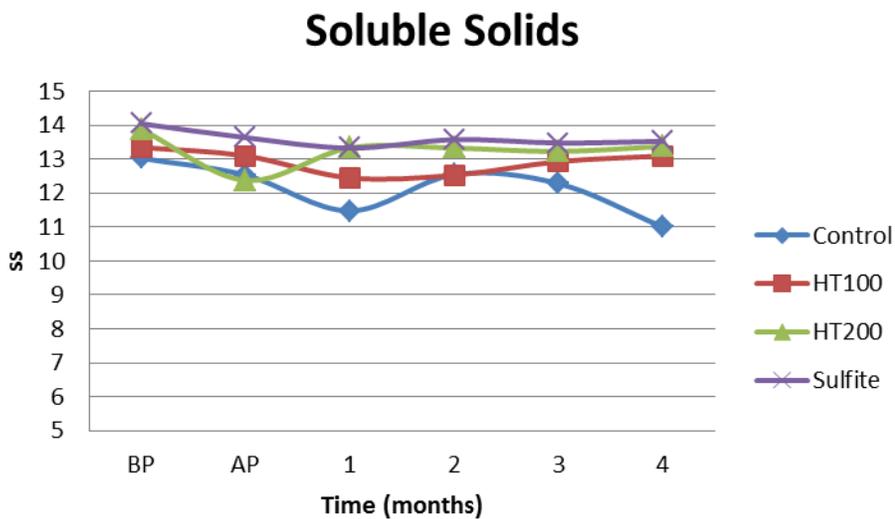
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-2. Interaction of Treatment x Time for a* values in Transmission Mode for Niagara.



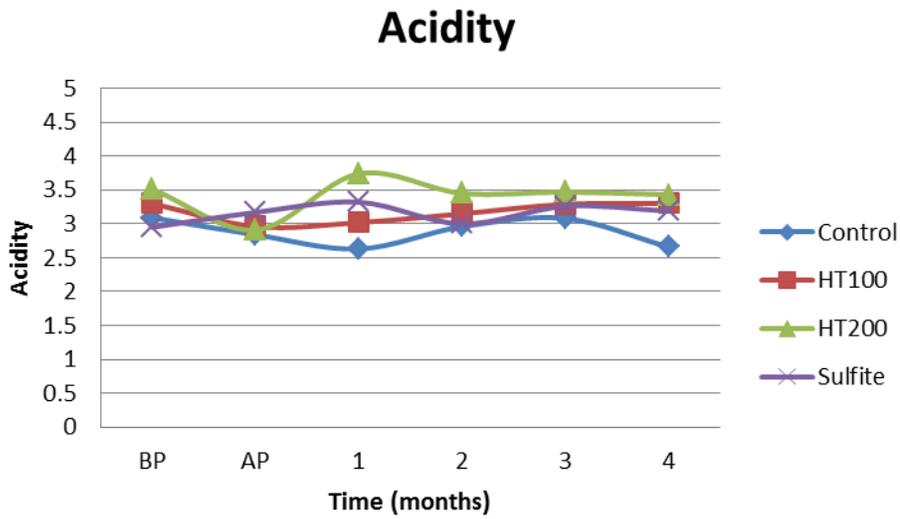
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-3. Interaction of Treatment x Time for b* values in Transmission Mode for Niagara.



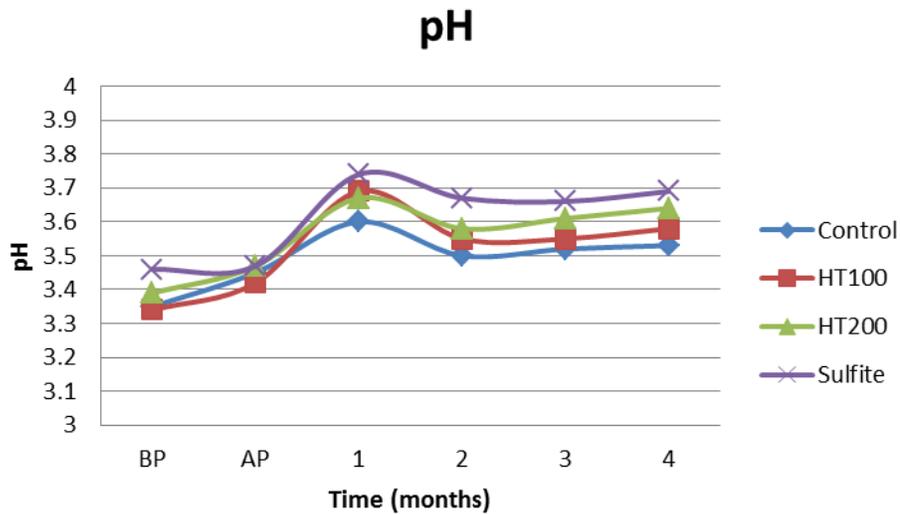
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-4. Interaction of Treatment x Time for Soluble Solids values for Niagara.



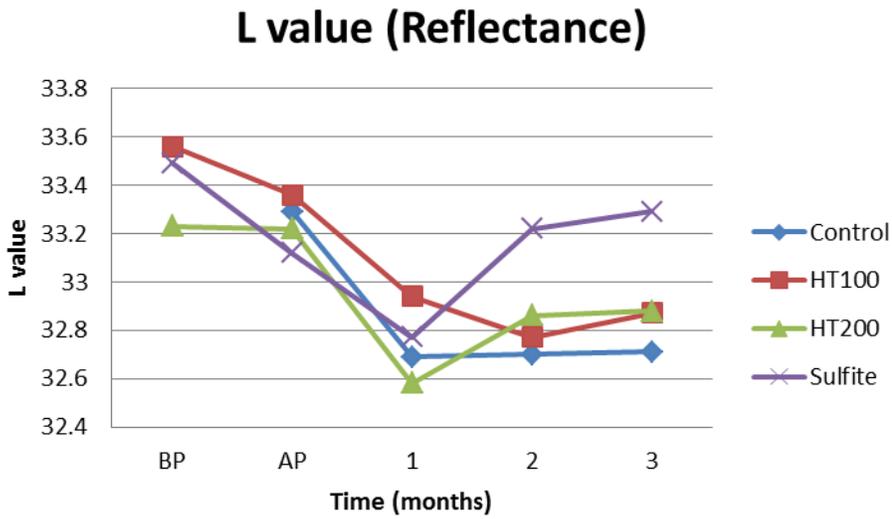
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-5. Interaction of Treatment x Time for Acidity values for Niagara.



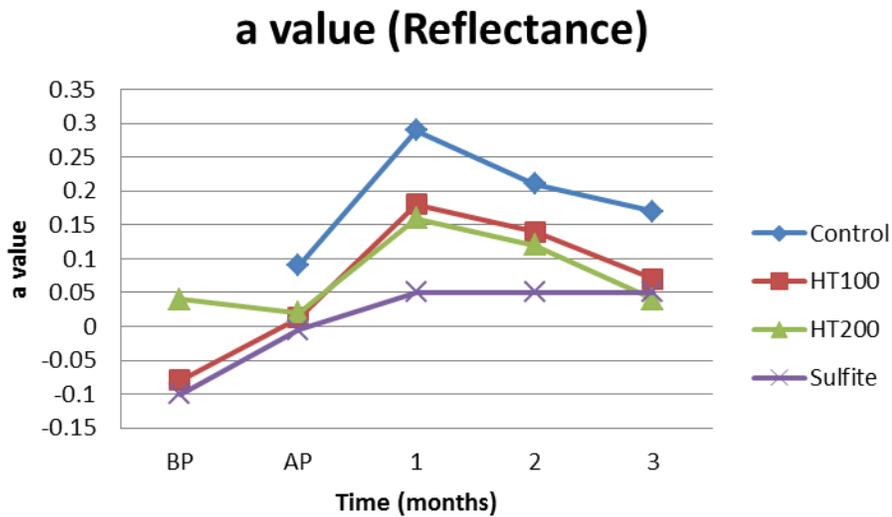
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-6. Interaction of Treatment x Time for pH values for Niagara.



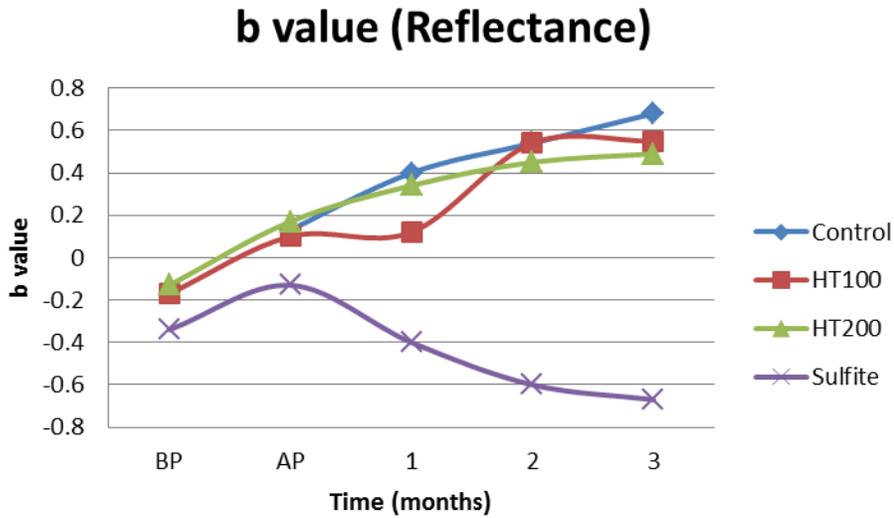
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-7. Interaction of Treatment x Time for L* values in Reflectance Mode for Thompson Seedless.



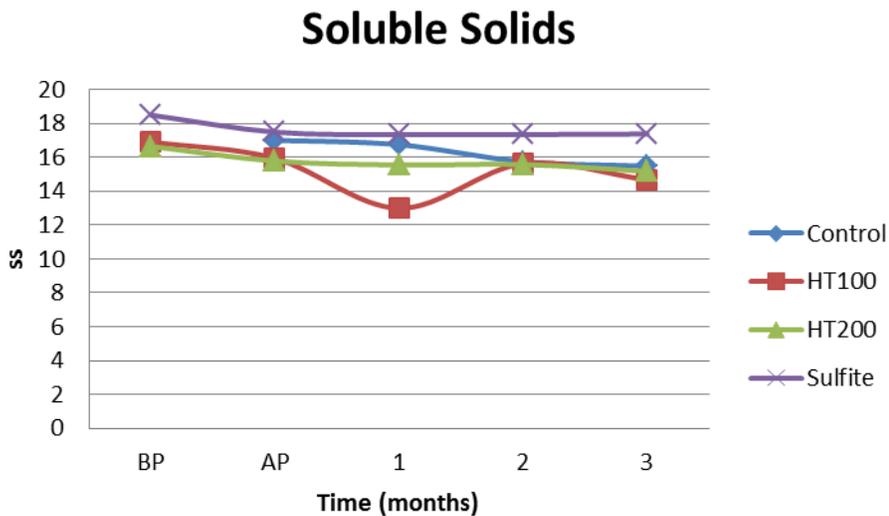
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-8. Interaction of Treatment x Time for a* values in Reflectance Mode for Thompson Seedless.



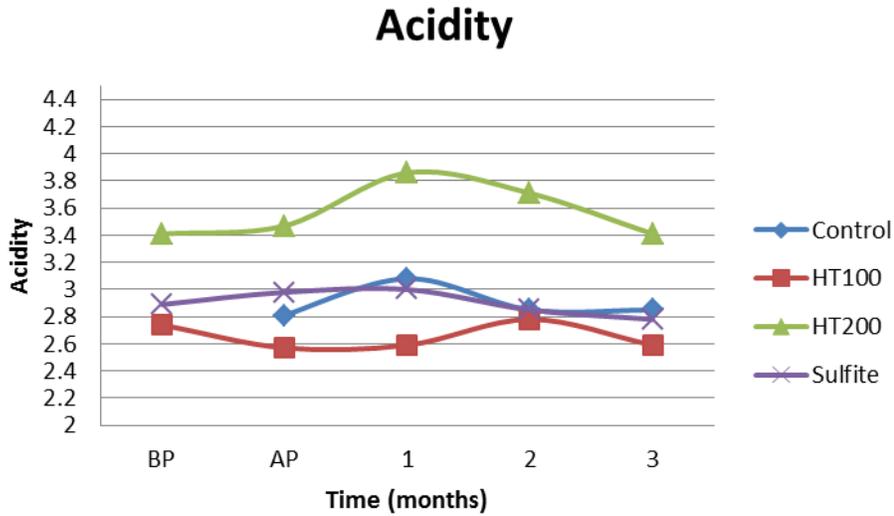
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-9. Interaction of Treatment x Time for b* values in Reflectance Mode for Thompson Seedless.



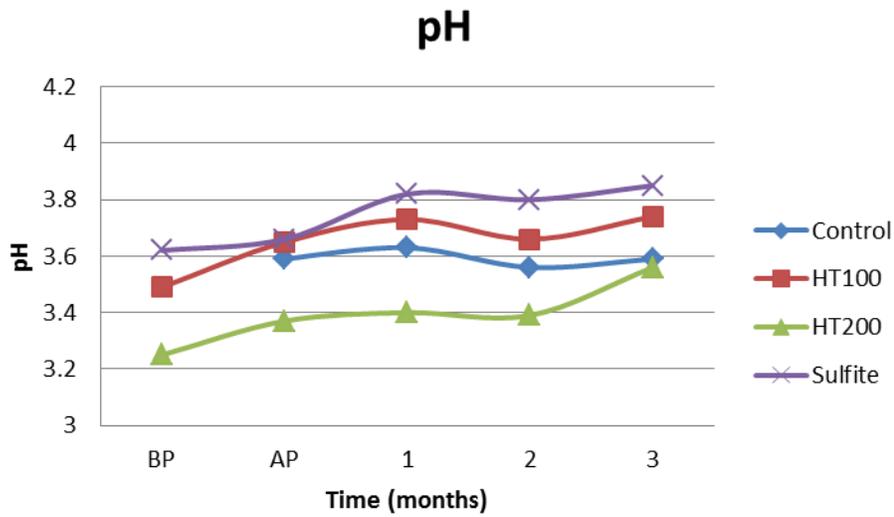
BP: Before Pasteurization, AP: After Pasteurization

Figure 4-10. Interaction of Treatment x Time for Soluble Solids for Thompson Seedless.



BP: Before Pasteurization, AP: After Pasteurization

Figure 4-11. Interaction of Treatment x Time for Acidity for Thompson Seedless.



BP: Before Pasteurization, AP: After Pasteurization

Figure 4-12. Interaction of Treatment x Time for pH for Thompson Seedless.

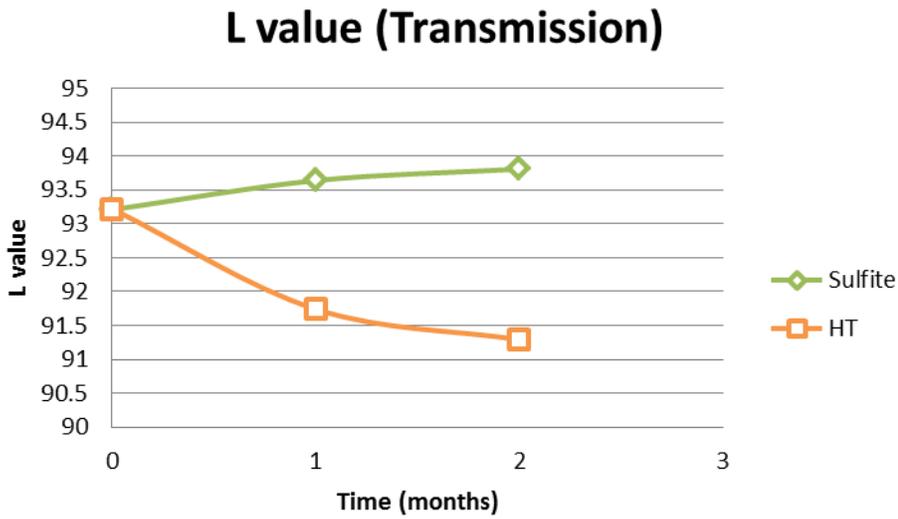


Figure 4-13. Interaction of Treatment x Time for L* values in Transmission Mode for Blanc du Bois.

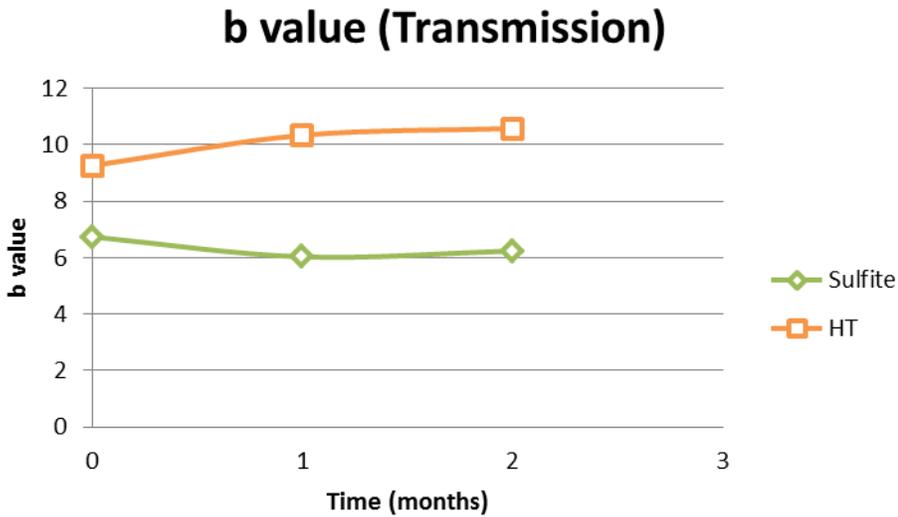


Figure 4-14. Interaction of Treatment x Time for b* values in Transmission Mode for Blanc du Bois.

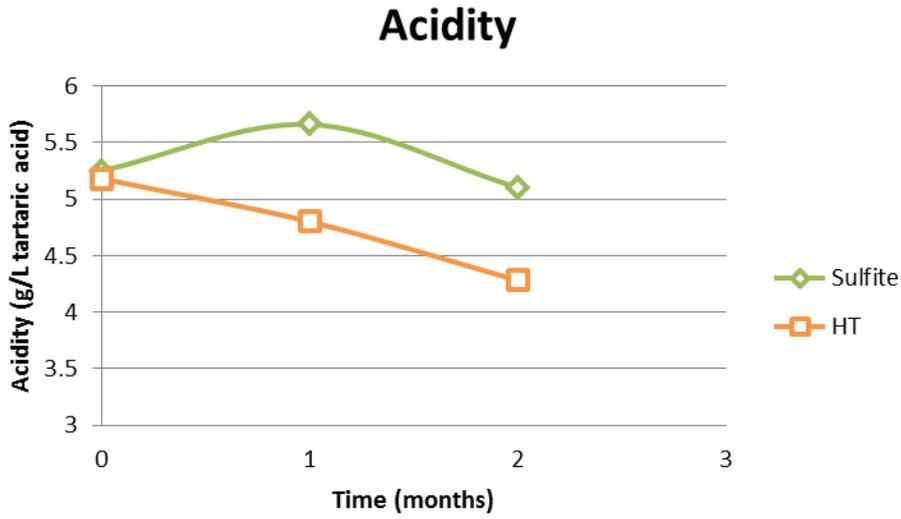


Figure 4-15. Interaction of Treatment x Time for acidity for Blanc du Bois.

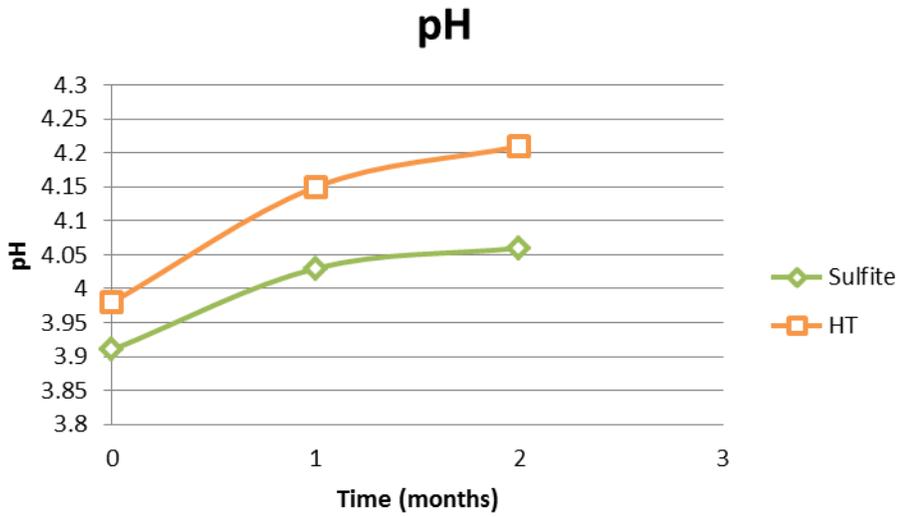


Figure 4-16. Interaction of Treatment x Time for pH for Blanc du Bois.

CHAPTER 5 CONCLUSION

There were significant differences among treatments. Browning took place in both Niagara and Thompson Seedless juice samples except for the sulfite treatment in Thompson Seedless juice that showed browning protection over time. The hypotaurine treatments did not inhibit browning to the same extent.

There were differences in all Niagara treatments. Even though Niagara was received in a less than optimal level of quality, which made it more difficult to see difference among samples, it can be stated that the control was the treatment with the lower L^* values and higher a^* and b^* values, meaning that it was darker in color. The hypotaurine treatments were significantly different from the sulfite treatment which means that hypotaurine did not work the same as sulfite, but the samples treated with hypotaurine were lighter than the control. Hypotaurine did not show comparable browning inhibition in samples as sulfite at any time point throughout storage.

Thompson Seedless also presented differences among the treatments. The interaction between treatments and storage time was significant and demonstrated more clearly the differences between treatments through time. For the L^* value, sulfite was the only treatment with significant difference (lighter in color) from the control. Hypotaurine treatments were not different from the control meaning that the hypotaurine had the same lightness in color as the control treatment and was not working as the sulfite treatment in preventing browning in samples. The control and hypotaurine treatments were significantly different from the sulfite treatment in their a^* values. But the hypotaurine treatments were also significantly different from the control treatment having lower a^* values. Overall, all treatments had positive a^* values

indicating the presence of redness in their hue. Sulfite treated samples had the lowest a^* values. The b^* values for the sulfite treatment were significantly lower from the other treatments. The sulfite treatment was the best treatment in inhibiting browning among all the treatments.

There were significant differences in the Blanc du Bois wine variables except for the a^* value. The treatment by storage time interaction was not significant. The sulfite treatment showed to have the highest L^* value and the lowest b^* value in comparison with the hypotaurine treatment, meaning that it was lighter in color and contained less brown in its hue respectively. Once again, the sulfite treatment had better browning protection than the hypotaurine treatment over time in the wine.

The addition of hypotaurine before crushing or after pressing, the increase in the hypotaurine concentration and heating the hypotaurine to different temperatures did not affect its browning inhibition capacity. The stage in which the hypotaurine is added did not affect its performance. Levels of 1000ppm did not show better browning protection than 200ppm, therefore levels of 200ppm should be enough to prevent oxidation in the juice. Heating the hypotaurine solution did not affect its inhibition capacity. In general, the L^* , a^* and b^* values show similar behavior for all the additional experiments which confirms the browning inhibition capacity of the hypotaurine for short storage times as was proven in previous studies.

Recommendations for this study are to develop the experiment with sound Niagara grapes in order to see how the hypotaurine works in the browning process. Also it would be good to measure the color in wine in a more extended shelf life in order to see the browning behavior in longer periods of time. Finally, further work should be

done in using mixes of hypotaurine with sulfite or other browning inhibitors at different concentrations to see if there is synergism between compounds and this can help to decrease the amount of sulfite used. Also enzyme kinetics study should be done in grapes with the usage of hypotaurine in order to see the type of inhibition that this compound can cause in grape PPOs. Sensory acceptability panels should be conducted to see how hypotaurine treated samples will be compared to commercial white grape juice. Studies to find a lower cost purification of hypotaurine is also of great importance because the current cost of the compound is prohibitive for industrial use.

APPENDIX
COLOR CHANGES DURING THE STORAGE TIME



Figure A-1. Niagara treatments before pasteurization process.



Figure A-2. Niagara treatments after pasteurization process.



Figure A-3. Niagara treatments after 4 months of storage.

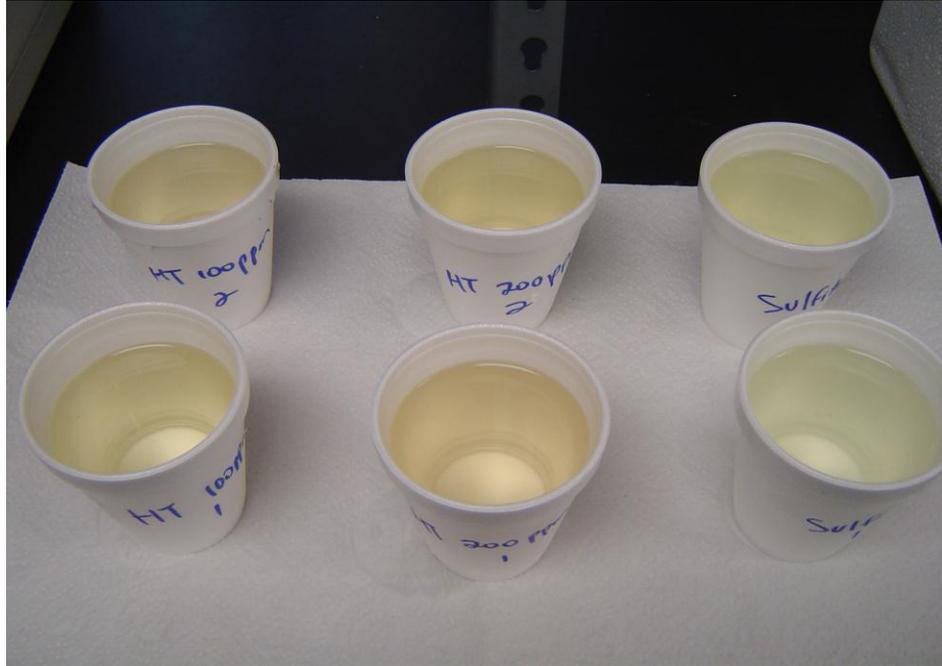


Figure A-4. Thompson Seedless treatments before pasteurization process.



Figure A-5. Thompson Seedless treatments after pasteurization process.



Figure A-6. Thompson Seedless treatments after 3 months of storage.



Figure A-7. Blanc du Bois treatments at time 0.



Figure A-8. Blanc du Bois treatments after 2 months of storage.

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BIOGRAPHICAL SKETCH

Betty Coello was born in Guayaquil, Ecuador in January of 1986. After graduating as the fifth top of her class from “La Moderna Sergio Perez Valdez” high school, she continued with her studies at Escuela Superior Politecnica del Litoral (ESPOL) in Guayaquil, Ecuador from 2004 to 2010 where she obtained a bachelor in science in food engineering. After graduating in February of 2010, she decided to pursue her master’s degree in the Food Science and Human Nutrition Department at the University of Florida. Betty was offered an assistantship and worked with Dr. Charles Sims and Dr. Maurice Marshall in a study that involved sensory changes in white wine and white grape juice due to browning reactions. She graduated in August 2012 with a master’s degree in food science with a minor in packaging science and is prepared for new challenges and opportunities in the food career.