EFFECTS OF SUPERCRITICAL CARBON DIOXIDE AND THERMAL PROCESSING CONDITIONS ON PHENOLICS, ANTIOXIDANT ACTIVITY, AND YEAST INACTIVATION IN MUSCADINE AND POMEGRANATE JUICE

by

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(Under the Direction of Karina G. Martino)

ABSTRACT

Muscadine juice and pomegranate juice were processed with two pasteurization methods, supercritical and thermal processing, to determine their effects on antioxidant activity (ORAC) and total phenolics (TPC) retention. The thermal processing conditions used were 50-80°C and 1-30 min. Supercritical conditions varied between 35-55°C, 20.7-34.5MPa, and 10-30 min. With thermal processing temperatures greater than 60°C, phenolics and ORAC values in muscadine juice began to decrease while pomegranate juice phenolics did not show any reductions until 70°C. By filtering particulates after thermal treatment, phenolics and ORAC retentions increased by at least 8% compared to unprocessed juice. The supercritical processing conditions of 45°C, 27.6MPa, and 20 min had the greatest phenolics and ORAC retentions. These conditions increased the phenolics and ORAC retentions in both muscadine and pomegranate juice by 1.2-1.9% compared to the unprocessed juice. In muscadine juice, supercritical processing achieved a 3-4 log reduction in *Zygosacchromyces bailii.*

INDEX WORDS: Supercritical carbon dioxide processing; thermal processing, pasteurization; filtration; antioxidants; phenolics; microbial inactivation; fruit juice; muscadine; pomegranate

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CHAPTER 1

LITERATURE REVIEW

1. Antioxidant and Phenolic Compounds

1.1 Importance of antioxidants

People are more aware of the health benefits of fresh foods, in part because the media is advertising published research findings. Some foods such as fruits have been tagged as 'functional foods' because of their physiological benefits such as slowing the onset of chronic diseases (Kaur and Kapoor, 2001). The main compounds that are responsible for these health benefits are believed to be polyphenolics (Liu, 2003)

Most auto-immune diseases such as cancer begin with free radicals. These reactive unpaired electrons react by acquiring electrons from other compounds. This electron transfer mechanism is the basic chain reaction step. Lipid peroxidation results in destruction of cell membranes or oxidation of proteins and DNA, leading in cellular disruption (Akoh and Min, 2008).

Free radicals can be generated during human metabolism. The body's immune system can also produce radicals to combat bacteria and viruses. Externally, free radicals can be caused by pollution, cigarette smoke, and insecticides. Antioxidants that are either naturally in the body or consumed, operate by scavenging these potentially harmful free radicals (Akoh and Min, 2008). Stimulating the amount of antioxidant compounds occurring naturally in the body has not been scientifically proven; thus, a possible way to increase the amount of exogenous antioxidants is through food intake.

Foods with the highest amounts of antioxidants are fruits and vegetables (Akoh and Min, 2008). Since the on-the-go consumer is increasingly demanding products that suit their lifestyle and have longer shelf lives, fruit juices are becoming more in demand (Sloan, 2009). With this added demand, a higher proportion of a person's daily antioxidant intake through fruit juices may result. Finding ways to increase or preserve endogenous antioxidants in juice through processing may not only improve the overall health of people who consume the products, but also stimulate even higher juice demand. Before determining how to increase the antioxidant activity retention during juice processing, the radical scavenging mechanism, phenolic characteristics, and the relationship between phenolics and antioxidant activity must be understood.

1.2 Scavenging mechanism of antioxidants

Free radicals are constantly produced through numerous biological reactions in the body such as mitochondrial respiratory reactions and any inflammatory condition. Humans can naturally protect against free radicals by enzymes, phenolic compounds, and other vitamins. Depleted antioxidant defenses can lead to oxidative stress, increasing the likelihood of cellular damage (Akoh and Min, 2008).

Antioxidants can inactivate free radicals by accepting a radical from an oxidizing species such as peroxyl (LOO·) and alkoxyl (LO·). Antioxidants can also quench singlet oxygen species. The effectiveness of antioxidants is determined by their chemical properties such as the hydrogen bond energy and resonance delocalization. Once the antioxidant molecule donates a hydrogen atom, the radical resulting is stabilized by delocalization of the unpaired electron or intramolecular hydrogen bonding around a phenol ring (Akoh and Min, 2008). Additionally, compounds that can exhibit a partially oxidized state have an increased ability to donate a

hydrogen atom from the aromatic hydroxyl group or to delocalize the ring structure (Akoh and Min, 2008).

1.3 Fruit phenolic compounds

Phenolic compounds are secondary plant metabolites, usually occurring in cell vacuoles. They are distinguished by the number of rings, hydroxyl and methoxyl groups, and sugar attachments.

Most naturally occurring phenolic compounds in fruits are in conjugated forms, accounting for 12-76% of all phenolics (Imeh and Khokhar, 2002). Phenolics are mostly found in the fruit's seeds followed by the skin, flesh, and juice (Striegler et al., 2005). The distribution and composition of the phenolic compounds in juice products are dependent on the juice processing application which affects the rupture of the vacuoles and cell wall differently (Bengoechea et al., 1997).

Phenols in fruits are usually categorized into two major categories, flavonoids and nonflavonoids. Flavonoids, which are larger molecules, include flavanones, flavanonols, flavones, and anathocyanins (Kaur and Kapoor, 2001). Nonflavonoids consist mostly of benzoic acids, hydroxycinnamic acids, stilbenes, and tannins (Waterhouse and Ebeler, 1998). Flavonoids account for a higher percentage of the phenolics in fruits so they will be discussed in detail (Hounsome et al.,, 2008).

1.3.1 Flavonoids

Many kinds of flavonoids exist, but anthocyanins make up the highest proportion of flavonoids in fruits (Williams and Grayer, 2004). Anthocyanins are responsible for the blue, purple, and red color of many fruits. These compounds can exist in four equilibrium structure

states. Two of the states, the flavylium cation and the quinonoidal base, are responsible for the red and blue colors in fruits (de Pascual-Teresa and Sanchez-Ballesta, 2008).

There are more than 500 compounds found in the anthocyanin family. Anthocyanins are glucoside forms of their counterpart, anthocyanidins. Anthocyanins are centered on the flavylium cation and have hydroxyl, methoxyl, acyl groups at positions around the three rings. Glucose, galactose, rhamnose, arabinose, and xylose are the most common sugars attached to an anthocyanin molecule (de Pascual-Teresa and Sanchez-Ballesta, 2008). These different sugars along with the number of hydroxyls on the three rings determine the specific type of anthocyanin (de Pascual-Teresa and Sanchez-Ballesta, 2008). Figure 1.1 shows the base structure of an anthocyanin molecule. R groups can be either methoxyl or hydroxyl groups.

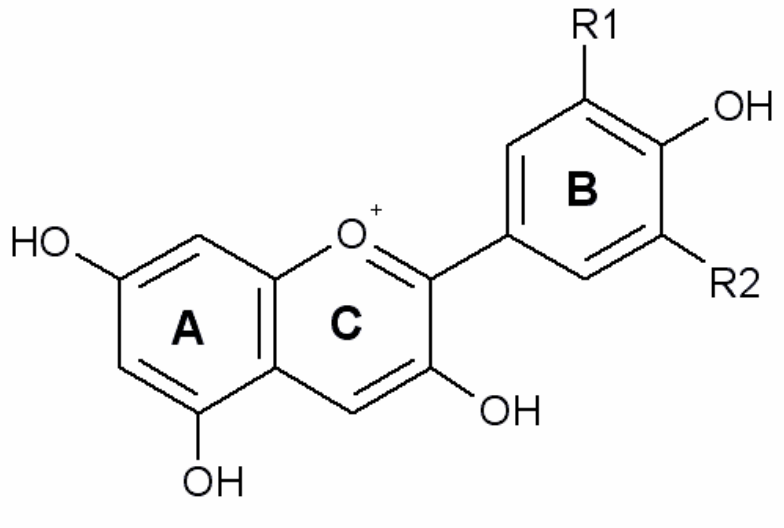

Figure 1.1. Anthocyanin base structure

The most widespread fruit anthocyanin is cyanidin-3-glucoside. However, malvidin glucosides are most common in grapes. The most six common anthocyanidins in fruits are pelargonidin, cyanidin, delphinidin, petudnidin, peonidin, and malvidin (Williams and Grayer, 2004).

1.4 Antioxidant activity and phenolic compound relationship

The antioxidant potential of phenolics depends on the number of hydroxyl groups. Hydroxyl group that are held more loosely on the ring exert a higher antioxidant capacity. Additionally, the number of hydroxyl substitutions is proportional to antioxidant activity (Cao et al., 1997). Oxidation and polymerization reactions can reduce the number of hydroxyl groups which decrease antioxidant activity. The arrangement and location of hydroxyl groups on the phenolic rings impact antioxidant activity. The 3 hydroxyl position and an ortho-dihydroxy substitution in the B ring of flavonoids result in higher ORAC (Noda et al., 2002). Additionally double bonds in the C ring accelerate electron delocalization, producing higher antioxidant activity. Finally, methylation and glycosylation around the phenolic ring also positively influence the antioxidant activity. However, methylation showed higher stability to oxidative and thermal conditions compared to compounds that exhibited glycosylation (Lee and Talcott, 2004).

These studies clearly show how the structure of phenolic compounds impact ORAC. To determine the overall impact, studies often report the correlation or relationship of the phenolic compounds to the ORAC. Existing literature shows that the correlation between average values of phenolics and ORAC in unprocessed fruits and juice ranges between 0.73 and 0.99 (Lee and Talcott, 2004; Pacheco-Palencia et al., 2007; Yildirim et al., 2005).

1.5 Quality impact of phenolic compounds

In addition to the ORAC of phenolic compounds, they are also associated with sensory quality, in both a negative and positive way. Phenolics of grapes are the main compounds responsible for color, taste, mouth feel, oxidation and other chemical reactions in juice. Phenolic levels can be affected by numerous juice processing conditions, including crushing, pressing, and heat pasteurization (Wulf and Nagel, 1976). At high concentration, they may participate in food discoloration, and interact with proteins, carbohydrates, and minerals (Imeh and Khokhar, 2002).

Most flavonoids have a bitter or astringent taste with sweet aftertaste (Drewnowski and Gomez-Carneros, 2000). Of the flavonoids, flavanones (eg. naringenin) are the most bitter. Additionally, food astringency is associated with phenolic content, especially tannins (Tomás-Barberán, 2001). It has been has been reported that bitterness increases with polymerization of flavonoids up to tetrameter molecules (Robichaud and Noble, 1990).

In addition to taste, phenolic compounds are highly associated with the visual appearance of fruits. Generally, an increase in anthocyanin pigmentation is considered a positive attribute because of the vibrant blue and red colors (Tomás-Barberán, 2001). Phenols can also negatively impact the visual appearance, especially when oxidized (Main and Morris, 1991). Oxidation reactions involving phenolics can lead to undesirable changes such as loss of flavor and color, development of off-flavors and brown colors. However, some oxidation reactions can also lead to improved color, color stability and sensory characteristics (Nagel and Wulf, 1979). Color stability closely corresponds to the degree of polymerization of these compounds. Sometimes, phenolic compounds are not soluble in acidic aqueous solution, leading to the formation of precipitates and juice haze that also affects food quality (Tomás-Barberán, 2001).

2. Muscadine Grapes and Pomegranates

Muscadine and pomegranate juice were both processed and analyzed together in this study because they are both Georgia commodities which show promising harvests in the future.

2.1 Muscadine grapes: their phenolic compounds and antioxidant activity

Aside from oranges, grapes are the world's largest fruit crop with more than 60 million tons produced annually (Schieber et al., 2001). Muscadine grapes (*Vitis routndofolia)* are primarily grown in the southeastern United States because of warm, humid conditions. They grow as small, loose clusters of large berries on vines (Vaughan, 2003). Over 70 different

cultivars of this unique flavored fruit exist. The fruit can be processed into value-added products such as wines, juices, jams, and jellies. Recent economic analysis has shown muscadine production to be a profitable venture for growers (Noguera, 2005).

Muscadine grapes have an abundance of phenolic compounds. The major antioxidant compounds in muscadine grape are phenolic acids, flavonols, anthocyanins, and ellagic acid (Lee et al., 2005). The predominance of anthocyanin-3,5-diglucosides distinguish muscadine grapes from other grape varieties. These compounds may be more resistant to thermal degradation compared to monoglucosides (Lee and Talcott, 2002). The major anthocyanin is malvidin-3-glucoside and the major flavan-3-ol is (±) catechin. Flavonoids, shown to be potent scavenging antioxidants, exist at a concentration of 1000-1800mg/L (Yildirim et al., 2005). On average, 65% of the polyphenols are found in the seeds; 22% in the stems; 12% in the skins and only 1% in the pulp (Vine, 2002). In the seed and skin, flavanoids and flavan-3-ols are mostly found (Sun 2002). These grape components have anticancer (Yi et al., 2006) and anti-inflammatory (Greenspan et al., 2005) properties.

Muscadine juice has been analyzed for its ellagic acid, total anthocyanins, total phenols, and antioxidant activities for several varieties (Lee and Talcott, 2004). Total ellagic acid ranged from 105-322mg/L. Total anthocyanins varied from 180-610mg/L. Total phenols had values of 1040-2900mg/L. The ORAC was 15.5-26.7μmol Trolox equivalents/ml. This study found that ripened fruits had significantly higher values than unripened fruits.

2.2 Pomegranates: their phenolic compounds and antioxidant activity

Pomegranates and the juice have a complex variety of anthocyanins and phenolic compounds. The main pomegranate phenolics are anthocyanins including the glucosides and diglucosides of gallic acid, ellagic acid, cyanidin, and delphinidin. One extremely important

phenolic compound is punicalagin, a hydrolyzable tannin. This compound exhibited the highest antioxidant activity of the fruit's phenolic compounds because of its 16 phenolic hydroxyls per molecule. The other compounds mentioned earlier have only 3-4 hydroxyl groups. Punicalagin consists of 62.8% of the total phenolics content while anthocyanins and ellagic acids amount for 15.6% and 4.9% respectively (Gil et al., 2000). Its structure is shown in Figure 1.2.

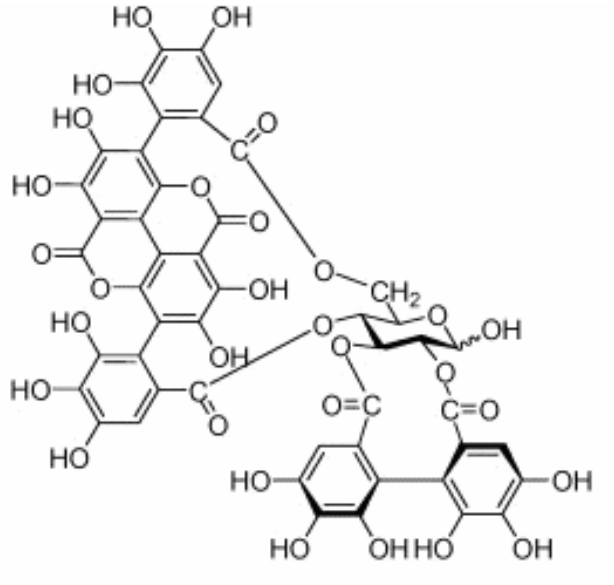

Figure 1.2. Punicalagin structure

Pomegranate juice had the highest antioxidant capacity of all commonly consumed commercial juices. Commercial pomegranate juice even showed an antioxidant activity three times higher than that of red wine and green tea (Seeram et al., 2008). This commercial juice also has a 50% higher antioxidant activity than juice extracted only from the fruit's arils. Punicalagin is found in much higher content in commercial juice as this tannin gets incorporated into the juice from the fruit rind during industrial processing (Gil et al., 2000). Commercial juice had a punicalagin concentration of 421.3mg/L, while juice from fresh arils only had a content of 12.7mg/L. The juicing method for commercial pomegranate juice extracts bitter phenolic

compounds from the fruit's pith. Juice processors need to decide if taste or phenolic content is more important (Seeram et al., 2008).

3. Juice Processing

3.1 Antioxidant changes due to juice processing steps including filtration

Fruit juice has lower concentrations of total phenolics, anthocyanins, and ORAC values compared to the whole fruit. Only low levels are extracted from fruit skins during the initial juice processing steps, and the majority stays in the skins and seeds (Striegler et al., 2005).

Once the fruit begins the initial juicing process, degradative reactions begin to turn natural polyphenols into new compounds. Temperature is the key factor that determines this degradation rate. Fruit must be either cold or hot pressed. During cold pressing, enzymes can be added to the juice to facilitate clarification and filtration. Typically, muscadine grapes are cold pressed. Hot pressing involves heating the must to 60°C, with the addition of pectolytic enzymes to break down natural pectins. Hot pressing usually results in higher juice, tannin, and pigment (color) yields than cold pressing (Threlfall, 2005). For example, muscadine juice that was hot pressed produced a higher free ellagic acid and ellagitannin concentrations (10.14 and 22.66mg/L, respectively) compared to cold pressed juice (3.85 and 0.64mg/L, respectively). Heat was able to release a higher proportion of these condensed tannins from the grape skins in this case as larger polyphenolics are cleaved; but too much heat could produce negative impacts on juice yield, color, and nutraceutical levels (Lee and Talcott, 2004). Another study showed that hot pressing (43°C) yielded 50% higher phenolics and antioxidant activity than cold pressing (22°C) (Carlson, 2003).

Filtering or clarification is performed either before or after pasteurization. Filtering removes the pomace to produce a clear juice. The pomace includes the skin, seeds, and rind.

The wine industry produces 5-7 million tons of pomace per year. After juicing and before filtration, pomace represents approximately 20% of the weight in grapes (Schieber et al., 2001). This important health by-product is a rich source of phenolics especially anthocyanins, catechins, and flavonol glycosides which can be absorbed by the body (Schieber et al., 2001).

Pomace clarification of açaí, a fruit rich in antioxidants, resulted in a 27% loss in total polyphenolics, 20% reduction in both total anthocyanins and antioxidant activity, and 15% loss in non-anthocyanin polyphenols. Clarification also reduced the monomeric anthocyanins which contribute to the red color and aesthetic quality. Another study found an 18.1% decrease in antioxidant activity after clarification in grapes (Yildirim et al., 2005).

The pomace, left over from the grape juice clarification, exhibited higher antioxidant values compared to the whole fruit (82.3% to 68.9%). Additionally, phenolic compounds extracted from the pomace showed higher antioxidant capacity than red wine phenols. Thus, grape pomace could be highly desirable as a health-byproduct or to prevent the formation of off-flavor and toxic compounds resulting from lipid oxidation (Vaughan, 2003).

3.2 Juice Pasteurization Alternative Methods and their Effects on Antioxidants

The industry's current standard for juice pasteurization is a high temperature short time (HTST) process**.** Some juice processors are using a type of HTST process called flash pasteurization. Depending on the juice processor, juice is heated to a very high temperature and then chilled to refrigeration conditions within 30s. A typical pasteurization process that meets FDA's Juice HACCP regulations is 90°C for 2 seconds, followed by filling at 85°C and holding at that temperature for 1min. Juice is then cooled as quickly as possible (Mazzotta, 2001). This process achieves the minimum 5-log pathogen reduction. Juice processors can target either *E.*

coli O157:H7, *Salmonella*, or *Listeria monocytogenes* or any other pathogenic organism that is most problematic.

Depending on the type of heat treatment applied to the juice, the high energy transmitted may trigger unwanted reactions in the food, leading to undesirable products or the loss of health-promoting compounds (Barbosa-Canovas, 2005). The following section discusses studies that have determined the effects of these compounds with varying processing temperatures. Since product quality and health are very important to consumers, the concept of preservation by nonthermal or alternative methods has been studied. During nonthermal processing, the temperature of the juice is held below the temperatures necessary for thermal pasteurization. Therefore, phenolic compounds are expected to undergo minimal degradation during nonthermal processing. Fruit juices can be processed nonthermally using high hydrostatic pressure, pulse electric fields, ohmic heating, ultraviolet light, and supercritical carbon dioxide (Barbosa-Canovas, 1998).

High hydrostatic pressure uses pressures of 405.3-911.9MPa to inactivate microorganisms. This treatment is not dependent on residence time, thus reducing processing time. The threshold pressure for microbial inactivation is dependent on the specific organism, yet most cannot survive past 50.7MPa (Barbosa-Canovas, 2005). A high pressure treatment (400MPa/40°C/min) on orange juice resulted in a 54% increase in carotenoids and a 39% increase in vitamin A. This process also resulted in 20-40% increases in various flavanones and no decrease in radical scavenging capacity. A flash pasteurization treatment (90°C/min) led to a decrease in both carotenoids and vitamin A, as well as a reduction in most flavanones. This thermal treatment also decreased radical-scavenging capacity by 7% (Sanchez-Moreno et al., 2005). One negative aspect of high hydrostatic pressure is the inability to oxidative and

hydrolytic enzymes. This inability results in greater degradation of anthocyanins and quality during storage (Gimenez, 2001).

Pulse electric field (PEF) processing can be another alternative to thermal processing for fruit juices which occurs in the form of short pulses. PEF uses a short processing time because of the high intensity fields. These short pulse fields cause cell membrane damage. PEF showed no flavanone or radical scavenging degradation while thermal pasteurization showed decreases in both, as described previously (Sanchez-Moreno et al., 2005).

In 2000, the FDA approved UV-light at 254nm as an alternative treatment to thermal pasteurization of fresh juice products. At least a 5-log reduction on various test organisms has been shown with UV exposure of 400J/m^2 (Barbosa-Canovas, 2005). The limiting factor with this processing method is the surface penetration of UV through an opaque juice. No study has been done on antioxidant retention for UV light; however, due to the use of ambient temperatures during processing, it is predicted to have minimal effects.

Another alternative method is ohmic heating. This process involves the passage of alternating currents, generating internal heat as a result of electrical resistant. Temperatures can be between approximately 90°C and 150°C for one second operating times. This short processing time along with internal heat production can produce a high-quality product with minimal nutritional damage (Barbosa-Canovas, 2005). This process resulted in a 16% reduction of vitamin C in fresh orange juice, while thermal processing (90°C/90s) resulted in a slightly greater reduction of 22% (Leizerson and Shimoni, 2005).

3.2.1 Thermal pasteurization effects of antioxidants and phenolic compounds

Since thermal pasteurization is the current industry standard, the effects of various thermal processing conditions on antioxidant activity will be discussed. Temperature and time

both have an influence on the phenolics retention (Wagener, 1981). In addition to the antioxidant loss that is exhibited in processing filtration, thermal pasteurization has shown similar effects to these compounds. Even though the thermal processing's impact on ORAC in different fruit mediums has been thoroughly studied, results have been widespread and inconclusive. Each study described below used different temperatures, processing conditions, food mediums, and analytical methods; thus, linking the results of antioxidant activity retention to processing is difficult.

In whole fruits, anthocyanins can be heat sensitive. Grapes processed at 35°C resulted in most individual anthocyanins decreasing except malvidin 3-glucoside. This compound is highly methylated around the phenolic ring providing higher stability to heat. Anthocyanins without a high number of attachments decreased as a result of chemical and enzymatic degradation (Mori et al., 2007).

Phenolic compounds and antioxidants have been thoroughly studied in grape pomace as a result of drying. Drying red grape pomace peels at 60°C had no effect on the polyphenolic content, color, and antioxidant activity (Larrauri et al., 1997) while increasing the drying temperature from 37°C to 49°C to 60°C had a slight increase of total phenolics and antioxidant activity (Vaughan, 2003). At temperatures above 60°C, drying of winery pomace at 80°C and 100°C decreased the total phenol content by 10.3% and 15.7%, respectively (Lafka et al., 2007). Similarly, grape pomace dried at 100°C and 140°C reduced antioxidant activity by 28% and 50%, respectively, compared to 60°C drying (Larrauri et al., 1997). These four studies together show a consistent trend: drying temperatures up to 60°C result in an increase of phenolics and antioxidant activity while increasing temperatures after 60°C show decreases in phenolics

retentions. Therefore, after taking all four studies into consideration, 60°C is a critical threshold temperature for grape pomace phenolics and antioxidant activity.

The studies found below all pertain to the thermal processing effects on juice. Moro orange juice processed at 80°C for one minute resulted in anthocyanins increasing by 48%, while hydroxycinnamate, a non-flavonoid phenolic, increased by 20%. However, the scavenging activity decreased by 4%. The phenolic compound increase was probably caused by polymerization during processing and the inactivation of enzymes (Lo Scalzo et al., 2004). This processing condition used in this study is similar to flash pasteurization, the most commonly used pasteurization treatment in industry. Another study concluded that pasteurized blueberry juice (90°C/30s) resulted in higher antioxidant activity and phenolics values (Carlson, 2003). That study suggested that the addition of heat, without sustained boiling, has a positive impact on these two factors.

After pomegranate juice was processed at 100°C for 20 min, total phenolics content decreased by 7.1% (Alper et al., 2005). These temperature abusing processing conditions, which are much higher in temperature and processing times than industry conditions, were the most probable result in the phenolics degradation. In a different study, pomegranate juice was processed at 95°C for 30s and hot filled at 90°C (Perez-Vicente et al., 2002). The total anthocyanin concentration decreased by 14%, total phenols decreased by 2%, while free ellagic acid and antioxidant activity increased by 57% and 10%, respectively. The heat released hexahydroxydiphenoic acid which is then transformed to ellagic acid. Unlike the previous study, phenolic compounds showed greater stability due to the lower heat treatment time. The authors suggest the increase of antioxidant is contributed by the extraction of hydrolyzable tannins from juice particulates.

Strawberry juice was pasteurized at 85°C for 5 min in one liter bottles (Klopotek et al., 2005). The cooling method was not described. The phenolics decreased by 27% while the antioxidant activity decreased by 38%. It is assumed that the bottles were stored in ambient temperature after pasteurization. Juice was therefore stored for at least a few hours above ambient temperatures which could attribute to the compounds being reduced. These conditions are similar to the temperature abuse conditions seen in the first pomegranate study. Both treatments resulted in a high degradation of phenolics and antioxidant activity.

Various fruit juices were heated at 105°C/45s and 120°C/20 min, then cooled 20°C by immersion in an ice water bath for 10 min (Hernandez et al., 1997). These temperatures were even higher than the previous studies. The phenolics of chlorogenic acids decreased, although the weaker treatment had the least effect. Quercetin and phloetin derivatives in apple juice decreased equally with both thermal treatments. Furfural derivatives increased with heating time and temperature. The production of these derivatives is an effect of heat treatment as described by the following section. Additionally, there was an absence of flavan-3-ols which indicates the effect of a thermal treatment.

Muscadine grape juice processed at 75°C for 15s decreased anthocyanins (16%), phenolics (26%), and antioxidant capacity (10%). The heat treatment promoted the polyphenolic degradation to yield brown or polymerized pigments, negatively impacting the juice quality (Del Pozo-Insfran et al., 2006). In a different study, muscadine juice was processed (conditions not mentioned), and the total phenols increased by 43% (Auw, 1996). A similar observation was found supporting this increase in both total phenols and antioxidant activity values (Lee and Talcott, 2002).

Very few studies show the effects of different heat temperatures on antioxidant activity and total phenolics thresholds, especially in juice processing. However, the combined four studies, previously described, on pomace drying illustrate the overall effects on phenolics and antioxidant activity. The anthocyanins degradation kinetics study is most similar to the study of all four studies combined (Wang and Xu, 2007). The degradation of blackberry juice anthocyanins increased with higher temperatures (60°C to 90°C) and time. They also found that anthocyanins in sour cherries were more susceptible to higher temperatures. Yet the importance of determining specific threshold degradation temperatures, especially for juice, was not found in any study in literature.

3.2.2 Heat antioxidant mechanisms

The previous section illustrated the effects of various heat treatments on the phenolics and antioxidants in various fruits. This section will explain some overall mechanisms that contribute to these effects.

The antioxidant and phenolics increase effects could be explained by the following. During thermal treatments, natural antioxidants such as polyphenols and ascorbic acid are consumed as reactants in the Maillard reaction. As heating time is prolonged, possible antioxidant activity enhancement could occur due to the formation of antioxidant Maillard reaction products (MRPs) including furfural and 5-hydroxy-methylfurfural (HMF) (Nicoli et al., 1999). However, these products including the furfural derivatives are deterioration indicators of fruit juice that cause color and flavor change (Hernandez et al., 1997).

The basic mechanisms that explain the decrease of phenolics is the release of bound phenolic compounds, partial degradation of lignin releasing phenolic acid derivatives, and the beginning of thermal degradation of the phenolic compounds (Maillard and Berset, 1995). These

explanations could show the following transformations occurring in juice process. Peonidin and its glucoside form were the major phenolic components of grape juice before heat treatment. Malvidin and dimethoxy-flavylium became the most significant after pasteurization. Finally, piceatannol glucoside was a significant compound after the concentration step (Lee and Talcott, 2002). When compounds had methylated groups, they showed higher stability to oxidative and thermal conditions compared to compounds that exhibited glycosylation (Lee and Talcott, 2002).

A more in depth explanation shows how an anthocyanin system changes form when heated at 90°C. The anthocyanin color changed from red to pale red to colorless to yellow and finally, brown. Monomers significantly decreased from 98% to 14% of the total anthocyanins because of polymerization. Polymerization produces a new brown polymer with a larger molecular weight. The weak hydrophobic forces of anthocyanins also enhance the change of color. These compounds can also be condensed in the presence of flavylium salts which lead to colorless products (Tsai and Huang, 2004).

Color changes can also be associated with antioxidant changes. The yellow anthocyanin fraction is the main contributor for the increase in radical scavenging. The degradation of monomeric anthocyanins led to a reduction in the FRAP assay. Finally, polymerization did not affect the radical scavenging ability of the $ABTS^{+}$ radical (Tsai and Huang, 2004).

3.2.3 Thermal processing impact on sensory quality

Thermal processing has negatives effects on functional food properties (Pares et al., 2001). In orange juice, high temperatures may deteriorate quality and taste (Farnworth et al., 2001). Other pasteurization technologies such as high hydrostatic pressure show less degradation in quality. In an orange-lemon-carrot juice, high pressure processing (500MPa and 5 min) has a small and insignificant change on sensory quality (Fernández García et al., 2001).

The importance of any kind of processing is clear; juice that is unpasteurized has a much shorter shelf life because microbial load increases and flavor diminishes compared to processed juice (Butz and Tauscher, 2002).

High heat can affect the color, cloudiness, taste, and aroma of juice. These attributes all affect the overall's consumer acceptance of the product. Color degradation follows a first order degradation curve with temperature (Ávila and Silva, 1999). Pasteurization can prevent the cloud formation in juices, improving the quality (Farnworth et al., 2001). Prolong heating can cause gradual browning of juice, which shows the beginning of anthocyanin destruction (Ponting, 1960). Additionally, greater heating causes a decrease in blue and red color and an increase in lightness. Temperature, rather than total time-temperature input, has the greater influence on the change (Flora, 1976).

Taste and aroma in juices are mostly associated with the fruit's volatile compounds (Farnworth et al., 2001). Aroma compounds are more heat-sensitive than color compounds as heat more quickly degrades these compounds during pasteurization. Degradation of these compounds usually results in scorched, cooked, and burnt aromas as heating times and temperature increase (Flora, 1976). Heat can drive off these volatiles, decreasing the flavor. A certain amount of heat is required to decrease the flavor. For apple cider, heat treatment from 60°C to 80°C resulted in no differences in aromatic flavor (Rye and Mercer, 2003). 90°C treatment showed more than minimal aromatic degradation. Volatile compounds can be a number of compounds in fruits, but terpenes and ester are usually the most important volatiles contributing to flavor (Mikkelsen and Poll, 2002). Thermal treatments usually result in a decrease of terpenes. A process that uses very high temperatures and short times, such as HTST, results in less sensorial changes (Leino and Kallio, 1993; Viberg et al., 1997).

Anthocyanins are also sensitive to thermal treatment. Between 25-30% of the anthocyanins are lost during the overall juice processing flow diagram. The long heating periods had the most destructive effect on these compounds. HTST had a smaller reduction of anthocyanins than the heat pretreatment step, showing that flash pasteurization has a higher retention of anthocyanins and aroma contributing compounds. The only compound that did not decrease was the aroma compound of β-damascenone, which significantly increased. This compound produces a stewed fruit flavor. Thus, heat has both positive and negative effects on aroma compounds in fruit juices but a definite negative impact on anthocyanins (Mikkelsen and Poll, 2002).

3.3 Supercritical carbon dioxide process

Supercritical carbon dioxide is one of the alternative processing techniques mentioned previously. CO_2 is permitted in food applications because it is nontoxic, inflammable, inexpensive, and environmentally and physiologically safe. The critical temperature of CO_2 is 31.1°C, just above the ambient temperature which could minimize the problems of thermal degradation of delicate biological materials and natural products (Gui et al., 2007). Pressure also needs to be at least 1107MPa to be in the critical state.

During processing, highly pressurized CO_2 comes in contact with food for a certain amount of time in a batch, semibatch, or continuous way. When pressurized, carbon dioxide has the ability to diffuse through the food materials. Furthermore, carbon dioxide processing creates a medium where oxidization reactions are prevented or minimized. This could be of great interest to protect to the oxidative stability of the phenolic compounds (Beckman, 2004).

Supercritical carbon dioxide has been shown to effectively inactivate different microorganisms. Microbial inactivation can be controlled the pH lowering effect, inhibitory

effect of molecular carbon dioxide and bicarbonate ion, physical disruption of cells, and modification of cell membrane and extraction of cellular components (Daniels et al., 1985; Fraser, 1951; Ishikawa et al., 1995; Nakamura et al., 1994; Spilimbergo et al., 2002). The rate of cell inactivation is initially slow at first because carbon dioxide must penetration into the microbial cells. This penetration is a primary controlling factor in the inactivation and is determined by the amount of pressure. High pressure, a minimum of 1000atm, can result in cell damage. Once inside the cellular space, essential lipids and other vital components are extracted. The extraction is usually a slow process depending on the operation conditions, but the action is stimulated when the depressurization occurs. The expansion of fluid within the cells by the pressure release rapidly transfers the extracted intracellular materials out of the biological system to accelerate cell inactivation (Lin et al., 1992).

There is limited research on the effects of antioxidants due to supercritical or dense phase carbon dioxide processing. One study showed that dense phase carbon dioxide processing at 34.5MPa resulted in only a 2% reduction in both phenolics and antioxidant activity, much better than the reductions of at least 10% exhibited by thermal processing at 75°C/15s. No temperature was mentioned for the dense phase carbon dioxide processing study. In addition to high preservation of phenolic compounds, this 34.5MPa operating condition showed at least a five log reduction in yeast cells (Del Pozo-Insfran et al., 2006).

There are a limited number of published studies regarding the effects on the quality of foods. One study shows that dense phase carbon dioxide can improve physical and nutritional quality attributes such as cloud formation and stability, color, and ascorbic acid retention. In a treatment of 29MPa, 50°C, and 4hr, there was no significant difference in flavor, aroma, and

overall acceptability, while another study shows nutritional, color, and sensory quality improvements compared to untreated juice (Arreola et al., 1991; Kincal et al., 2006).

An in depth microbial inactivation study on yeasts show that higher temperatures have a greater inactivation effect than higher pressures. 2.63 logs cfu/ml, 4.68 logs cfu/ml, and 5.27 logs cfu/ml were the greatest log reductions at 35°, 50°C, and 60°C at pressures around 15MPa and times between 60 minutes to 140 minutes (Ferrentino, 2008).

3.3.1 Phenolic compound extraction with supercritical carbon dioxide

One of the factors affecting the phenolics retention is the possible extraction of these compounds during pressure release (Murga et al., 2000). As the CO_2 is vented from the juice, phenolics have the ability to be extracted. However, for the most part, CO_2 is a poor solvent for phenolics because of the difference in polarities. Most phenolic compounds are highly polar while CO_2 is completely non-polar. For projects that try to extract phenolic compounds from foods, usually an organic polar material is used (Dobbs et al., 1987). Experiment conditions, though, should be designed to minimize this extraction in juice processing. Temperature, pressure, contact duration time, and structural polarity of the juice compounds are all factors.

Higher pressures increase the effectiveness of extraction by CO_2 up to a certain threshold. After this threshold pressure, the higher CO_2 viscosity reduces the extraction or solvating ability. Around this critical pressure, higher temperatures may also decrease the extraction yield due to the reduction in density of the fluid. However, higher temperatures usually result in better extraction. Increasing the temperature to 60°C, grape anthocyanin extraction improves. However, the extraction resulted in low yields of phenols and anthocyanins even at the highest experimental conditions (Vatai et al., 2009). When there is a longer contact time between the CO_2 and juice, higher extractions result (Martinez, 2008).

4. Research Objectives

Literature suggests that phenolic compounds and antioxidant activity can be degraded with certain amounts of heat. Since no study has looked at thermal degradation threshold temperatures, this current project will use a variety of temperature and time conditions. The objective is to compare phenolics and antioxidant stability in pomegranate and muscadine juice at temperature conditions of 50°C to 80°C.

Most industrial juicing processes filter the pomace particulates out of the juice before the juice is pasteurized. Some of literature studies that were previously discussed show that heating releases a proportion of the phenolic compounds from the pomace into the juice. In addition, the filtering studies show that removing the pomace results in significant antioxidant and phenolic losses. Thus, it might be beneficial for the juice industry not to remove the pomace as much or at all from the juice before pasteurization. This current project will compare filtering before and after thermal processing on antioxidant activity and phenolics.

Emerging processing technologies can be possible alternatives to thermal processing in the future. Further research is needed with better equipment design and studies showing microbial inactivation and antioxidant retentions. In this project, the alternative technology of supercritical carbon dioxide will be compared with thermal processing for a range of processing conditions. Antioxidant and phenolic retention along with a microbial inactivation will be analyzed.

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CHAPTER 2

THERMAL PROCESSING AND FILTRATION

1. Introduction

Previous literature has shown that phenolic compounds that contribute to antioxidant activity in juices are susceptible to processing and heat. In one study, the clarification processing step in açaí, a highly antioxidant rich fruit, resulted in a 27% loss in total polyphenolics, 20% reduction in both total anthocyanins and antioxidant activity, and 15% loss in non-anthocyanin polyphenols (Pacheco-Palencia et al., 2007). Two other studies found that pomegranate juice thermally processed at 95°C/30s and 100°C/20 min showed a 2% and 7% reduction in total phenolics (Alper et al., 2005; Perez-Vicente et al., 2002). These studies only showed the effects of antioxidants and phenolics to these specific time and temperature conditions. The overall conclusion from literature studies is that heat has a detrimental effect on antioxidant retention. In addition, heat can have a negative impact on sensory quality, specifically at temperatures greater than 80°C. Volatiles can be driven off and the flavor can diminish (Rye and Mercer, 2003).

Juice processors in industry should therefore become more interested in determining optimal process conditions to retain health contributing antioxidants as antioxidants and functional foods are one of the top ten trends in the food industry (Sloan, 2009). Safety is also extremely important for juice processors because they must meet microbiological inactivation requirements that are set by FDA HACCP regulations. The FDA recommends several temperature and time combinations that effectively achieve a five log reduction in targeted

pathogens (Mazzotta, 2001). In general, juice processors use thermal processing as a means of pasteurization to achieve this microbial reduction. .

This thermal processing study will analyze the effects of multiple thermal processing conditions on phenolics and antioxidant retention because this data is missing from literature. Only one study that could be found explored a range of processing temperatures on juice antioxidants. This study found that anthocyanin degradation rates in blackberry juice increased with as temperatures increased from 60°C to 90°C (Wang and Xu, 2007). However, this study did not show specific temperatures when antioxidant compounds started to degrade in the juice. Another area missing in juice processing literature is the effect of filtration on antioxidants. No study has compared antioxidant retention with juices filtered both before and after heat treatment.

Due to these gaps in the literature of fruit juice processing, there were several objectives of this project. The first objective was to determine the effects of several temperature and time combinations on the phenolics and antioxidants in filtered pomegranate and muscadine juices. Both fruits were grouped together in this study because they are Georgia commodities that show promising economic impacts in the future. The second objective was to analyze antioxidant and phenolics retention for muscadine juices filtered both before and after heat treatment. The filtration was effectively achieved by a centrifugal process. Thus when filtration is mentioned, a centrifugal process was used. Filtering after thermal treatment may allow for the pomace particulate antioxidants to be extracted into the juice due to heat. Finally, the last objective was to determine the microbial inactivation of various thermal processing conditions on *Zygosacchromyces bailii*, a common juice spoilage yeast organism which has a higher resistance to heat than most pathogenic species (Combina et al., 2008).

2. Materials and Methods

2.1 Thermal Processing

Thermal processing was performed using a batch system. Fifteen ml of juice in a centrifuge vial was moved to 4°C storage from -10°C storage twelve hours before thermal treatment. Immediately before treatment, the juice was added to a Pyrex test tube. A thermocouple and shaker, which were designed to fit inside the test tube for temperature measurement and constant heating during processing, were placed in the test tube. The test tube containing the juice was placed in boiling water and mixed with a manual shaker until the juice reached the experimental temperature. Immediately, the test tube was placed in a water bath to maintain the temperature. After holding the temperature constant (±1°C), the processed juice was cooled with ice until room temperature. Juice was then added to 2ml centrifuge vials and stored at -10°C prior to analysis.

Temperatures and residence times were chosen between conditions used in low temperature-long time (LTLT) processes and high temperature-short time (HTST) processes. LTLT processes use approximately 50°C/30 min conditions while HTST processes use approximately 80°C/1min conditions. **Table 2.1** shows the processing conditions used for this project. Each processing condition was conducted twice.

Table 2.1. Thermal processing conditions used for total phenolics and antioxidant analysis.

Temperature (°C)	Time (min)	Time (min)	Time (min)	Time (min)
50	1	10	20	30
60	1	10	20	30
70	1	10	20	30
80	1	10	20	30

2.2 Juice Preparation

Pomegranate Juice

Pomegranates (Ponder Farm, Ty Ty, GA) of the North TR (2nd harvest) variety were used. Initially, thirteen varieties of pomegrantes were analyzed. This variety was chosen because it had one of the highest antioxidant values and a many fruits were available. Juice was prepared by separating the arils from the pith and blending with a home blender. The pomegranate mixture was then passed through fine cheese cloth to eliminate large particulates. The filtered juice was then transferred to 15 ml centrifuge vials and stored at -10°C for no greater than one week.

Muscadine Juice

Purple muscadine juice was received from Paulk Vineyards (Wray, GA). In the first part of this project, muscadine juice was left unfiltered prior to pasteurization. For the second part, the juice was centrifuged prior to pasteurization. The centrifuge (Sorvall RC-6 Plus, Waltman, MA) conditions were 3000xg for 3 min in 500 ml bottles containing 400 ml of juice. After centrifugation, the filtered juice was added to one main collection vessel and the filtrate was discarded. Once all of the juice was centrifuged and collected, it was mixed while transferred to 15 ml centrifuged vials. This ensured all of the juice samples to have identical properties. The juice was then frozen at -10°C. For the third part, muscadine juice was filtered after processing. The thermally treated juice, stored in 2 ml centrifuge vials, was centrifuged at 3000xg for 3min.

2.3 Total Phenolics

The total phenolics contents were measured using an adapted Folin-Ciocalteu's phenol reagent assay (Naczk and Shahidi, 1989). A gallic acid stock solution was first prepared to an approximate concentration of 0.32mg/ml in 5% methanol-95% water solution. Also a blank (5%

methanol-95% water) was prepared. A standard curve was conducted using different concentrations of this stock solution. 0.25 ml of filtered juice samples and standard curve solutions were added to 4.0 ml of deionized water. After vortexing, 0.25 ml of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO) was added. After vortexing and waiting for 5 min, 0.5ml of saturated sodium carbonate (Fisher Scientific, Pittsburg, PA) was added. Then, the absorbance of the samples was measured at 750nm by an Agilent 8453 diode-array spectrophotometer (Agilent Technologies, Wilmington, DE) after a one hour incubation period to allow for color development. Absorbance to gallic acid concentration conversion was performed using the standard curve of known gallic acid concentrations that were prepared. Each processing sample was analyzed in duplicate, thus giving quadruplicate readings at each processing condition. The difference in values from the unprocessed value is calculated by the following formula:

$$\%\,\text{Change}_i = \frac{\text{Phenolics or ORAC}_i}{\text{Phenolics or ORAC}_{\text{unprocessed}}} \text{ x } 100$$

2.4 ORAC

Oxygen radical absorbance capacities (ORAC) of juice samples were measured using the method (Prior et al., 2003) modified for use with a FLUOstar Omega microplate reader (BMG Labtech, Durham, NC), using fluorescein (3,6-dihydroxy-spiro[isobenzofuran-1[3*H*],9[9*H*]-xanthen]-3-one) disodium salt (Sigma-Aldrich, St. Louis, MO) as the fluorescent probe. Juices were diluted appropriately with phosphate buffer (75mM, pH 7.4) made of mono- and di-basic potassium phosphate (VWR Internal, Suwanee, GA). The assay was carried out in a Costar 96well (Costar #3631) opaque clear bottom micro-well assay plate (Fisher Scientific, Suwanee, GA). Initially 20µL of diluted juice samples and Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, St. Louis, MO) standards (6.25, 12.50,

25, 50, 100μM) along with 40μL of blank solution (75mM, pH 7.4 phosphate buffer) were added to each well using an automatic pipet. The FLUOstar Omega microplate reader, equipped with two automated injectors, was then programmed to add 400 μL of fluorescein (0.11μM) (Sigma-Aldrich, St. Louis, MO), followed by 150μL of azobis (2-amidino-propane) dihydrochloride (AAPH) (Sigma- Aldrich, St. Louis, MO) (31.6mM) to each well. Fluorescence readings (excitation 485nm, emission 520nm) were recorded after the addition of fluorescein and AAPH (2,2-azobis [2-amidinopropane] dihydrochloride) (Sigma-Aldrich, St. Louis, MO), and every 192s thereafter until a 95% loss of fluorescence was reached. Final fluorescence measurements were expressed relative to the initial reading. Results were calculated based upon difference in areas under the fluorescein decay curve between the blank, samples, and standards. The standard curve of each analysis was obtained by plotting the five concentrations of TE against the net area under the curve (AUC) of each standard. Final ORAC values were calculated using the regression equation between the TE concentration and the AUC and are expressed as micromoles (μmol) of TE equivalents per L of juice.

2.5 Microbiological Inactivation Study

Zygosacchromyces bailii (ATCC 42476) (Difco, Franklin Lakes, NJ), a yeast spoilage organism, was inoculated at a concentration between 10^6 and 10^7cfu/ml in muscadine grape juice immediately before thermal processing. These organisms were grown in a yeast and malt (YM) broth (Sigma- Aldrich, St. Louis, MO) test tube for no more than 48hr. The cells were centrifuged (Beckman Coulter-Allegra X-22R, Fullerton, CA) at 3000xg for 4 min and then washed twice with sterile 0.1% peptone (Difco, Franklin Lakes, NJ). After washing, the cells were transferred to 15ml of muscadine grape juice.

After the inoculated juice was processed, plating occurred on YM agar (Sigma- Aldrich, St. Louis, MO) plates acidified with 10% tartaric acid (Sigma- Aldrich, St. Louis, MO) to a pH between 3 and 4. Plates were spiral plated using a Spiral Autoplater 20.7 (Spiral BioTech Inc., Bethesda, MD) and stored at 30°C for 4 days. Processing conditions for the microbiological inactivation study are shown in **Table 2.2**.

Table 2.2. Thermal processing conditions used for microbiological inactivation study.

Temperature (°C)	Time (min)	Time (min)	Time (min)	Time (min)
50	1	5	10	20
60	1	5	10	20
70	1	3	5	10
80	0.5	1	4	7

2.6 Statistical Analysis

Analysis of Variance (ANOVA) was conducted to determine significant difference between treatments for each of the assays performed. An alpha level $p< 0.05$ was used in the analysis. Treatment differences were determined using Tukey's HSD Test. Statistical analysis was conducted using JMP Software (SAS Institute, Cary, NC).

Statistical analysis was performed on quadruplicates. Two samples were processed at each condition and duplicates were analyzed for each sample, giving four readings.

3. Results and Discussion

3.1 Pomegranate Juice

3.1.1 Phenolics

Phenolics were analyzed for all thermal processing conditions of filtered pomegranate juice to determine if either temperature or time significantly affected the phenolics retention. Phenolic values at each processing condition can be found in **Table 2.3**. Temperature was a

significant factor ($p<0.05$) affecting phenolics while residence time was not. The interaction of temperature and time also did not significantly affect the phenolics. This statistics information is found in **Appendix A.1**. The processing temperatures of 70°C and 60°C had significantly better phenolics retention than 80°C, while juice treated at 50°C did not have significantly different retentions than the other temperatures. These results can be found in the statistics output in **Appendix A.2**. **Figure 2.1** shows total phenolics are the highest at the processing temperature of 70°C**. Table 2.3** shows that 70°C and 1min was the only temperature and time combination which was significantly different from any other combination (80°C and 20 min). This condition of 70°C and 1min had 2.55% higher phenolics than the unprocessed sample, while the 80°C and 20 min condition had 2.11% lower phenolics. All other conditions did not deviate more than ±2% from the unprocessed juice's total phenolics.

Table 2.3. Pomegranate juice thermal processing data.

Temp (°C)	Time (min)	Total Phenolics (mg GAE/10ml)	% Change	ORAC (μmoles Trolox equiv/ml)	% Change
Unprocessed		15.25 ± 0.103 ^{ab}	N/A	15.21 ± 0.086 ^{bc}	N/A
50	1	15.10 ± 0.197 ^{ab}	-0.93	15.01 ± 0.144 ^{d}	-1.37
50	10	15.13 ± 0.378 ^{ab}	-0.79	15.02 ± 0.084 ^{d}	-1.31
50	20	15.22 ± 0.093 ^{ab}	-0.24	15.03 ± 0.103 ^{d}	-1.24
50	30	15.27 ± 0.219 ^{ab}	0.14	15.02 ± 0.113 ^{d}	-1.30
60	1	15.34 ± 0.111 ^{ab}	0.61	15.28 ± 0.099 ^{bc}	0.43
60	10	15.29 ± 0.427 ^{ab}	0.27	15.42 ± 0.117 ^{ab}	1.35
60	20	15.30 ± 0.116 ^{ab}	0.33	15.41 ± 0.078 ^{ab}	1.25
60	30	15.44 ± 0.389 ^{ab}	1.21	15.39 ± 0.100 ^{ab}	1.15
70	1	15.64 ± 0.141^{a}	2.55	15.42 ± 0.098 ^{ab}	1.34
70	10	15.35 ± 0.076 ^{ab}	0.63	15.51 ± 0.098 ^{a}	1.95
70	20	15.37 ± 0.469 ^{ab}	0.76	15.48 ± 0.071 ^{ab}	1.70
70	30	15.41 ± 0.127 ^{ab}	1.04	15.55 ± 0.087 ^{d}	2.18
80	1	15.09 ± 0.225 ^{ab}	-1.03	15.03 ± 0.059 ^{d}	-1.20
80	10	14.99 ± 0.284 ^{ab}	-1.69	15.06 ± 0.120 ^{cd}	-1.03
80	20	14.93 ± 0.352 ^{b}	-2.11	14.97 ± 0.068 ^{d}	-1.61
80	30	14.99 ± 0.349 ^{ab}	-1.73	15.02 ± 0.056 ^{d}	-1.29

Average values are determined from two processing runs and duplicate analyses for each run.
Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

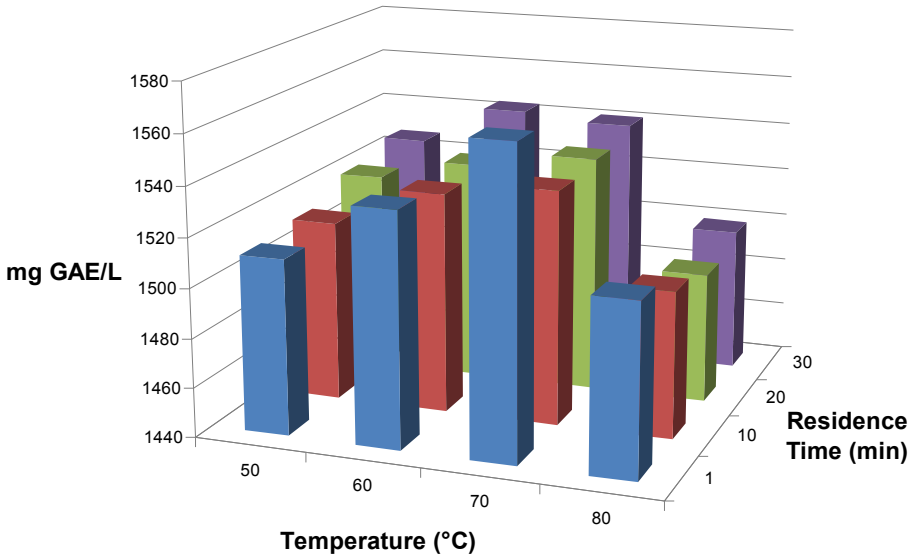

Figure 2.1. Pomegranate juice thermal processing total phenolics graph.

3.1.2 ORAC

ORAC was used to determine the antioxidant activity of these thermally processed conditions. ORAC values can also be found in **Table 2.3.** Similar results were found for ORAC values. Temperature was found to be a significant factor ($p<0.05$) affecting ORAC values. Time was also found to be barely significant when taking into account both temperature and time in the model. However, the significant difference in time was that the 10 min residence time showed significant higher antioxidant retentions than 1min. Processing at 70°C displayed significantly better antioxidant retention values than 60°C, which had significantly better antioxidant values than 80°C and 50°C. These statistics are found in **Appendix A.3** and **A.4**.

ORAC values and total phenolics result show that values reach a peak at 70°C. These phenolics and antioxidant values of juice processed at 70°C were significantly higher than the values for the unprocessed juice. The decrease in both ORAC and phenolics values at 80°C

show that compounds and ORAC values may begin to show instability and activity after 70°C. At 70°C and below, higher temperatures resulted in the increase of phenolics and ORAC values.

3.2 Muscadine Juice

3.2.1 Preliminary Study

This preliminary study shows the overall basic effects of filtration (centrifugation) and thermal processing on phenolics and ORAC values in muscadine juice. However, the filtration effects will be focused in more detail. Paulk Vineyard's final muscadine juice product, which was filtered and pasteurized, was compared with the unprocessed and unfiltered juice for total phenolics and ORAC values. Additionally, the unfiltered and unprocessed muscadine juice was filtered in both 2ml and 500ml containers. Therefore, juice that is completely unprocessed, only filtered, and both filtered and pasteurized was compared. **Table 2.4** shows the antioxidant and phenolics values of each juice processing state.

Table 2.4 Muscadine juice preliminary study data.

	TP (mg GAE/10ml)	% TP Lost	ORAC (μmoles Trolox equiv/ml)	%ORAC lost	Brix (%)
Unprocessed and Unfiltered	13.27 ± 0.310		22.14 ± 0.218		13.7
Unprocessed and filtered in 2ml centrifuge vials	9.431 ± 0.107	29.0	15.47 ± 0.080	30.1	13.6
Unprocessed and filtered in 500ml bottles	8.552 ± 0.155	39.5	14.62 ± 0.104	33.9	10.7
Paulk Vineyard's final muscadine product	7.461 ± 0.075	43.8	12.55 ± 0.064	43.3	13.5

The completely unprocessed juice had the highest total phenolics and antioxidant values compared to the three processed samples. The two filtered-only samples had higher values than

the juice that had been both filtered and pasteurized. These results show that both the thermal treatment and filtration had a decreasing effect on the total phenolics and ORAC values.

The decreasing antioxidant activity of filtration was attributed to the removal of particulates from the juice, as particulates have a significant amount of phenolic compounds (Pacheco-Palencia et al., 2007). During both filtering processes with 2ml and 500ml of juice, approximately 15% (v/v) of particulates from the juice was removed. Yet, between 29.0 and 39.5% of the total phenolics and ORAC values was lost. Since the particulates and phenolics values do not match, the pomace particulates must have a higher overall phenolics and antioxidant contribution by volume than the juice. These particulates come from the pulp, seeds, or skin of the muscadine grape. Literature verifies that a high proportion of phenolic compounds exist in these fruit parts (Schieber et al., 2001; Vine, 2002; Yildirim et al., 2005).

The two filtration methods yielded different phenolics and ORAC values, even though the same centrifugal conditions were used. The difference in the filtration methods was the batch size. The larger filtration batch size (500ml) resulted in a greater decrease in total phenolics and antioxidant compared to the smaller batch size (2ml). The difference in values can either be explained by the following two hypotheses: the amount of particulates removed or the amount of soluble solids was different. Therefore, the Brix of all four juice samples was measured and reported in **Table 2.4**.

The 500ml filtration resulted in the lowest Brix value (10.7%), much lower than the 13.5-13.7% values observed for the other three samples. Therefore, the larger batch size filtration removed more soluble solids than the smaller batch size. Thus, the amount of soluble solids could be another contributing factor to the antioxidant activity decrease in addition to the amount of particulates removed during filtration. No literature could be found to support this hypothesis.

3.2.2 Unfiltered Phenolics and ORAC

The data for juice that has been heat treated but left unfiltered before and after processing can be found in **Table 2.5.** Neither temperature nor time had any significance ($p<0.05$) on the level of total phenolics after thermal processing in unfiltered juice. The order of temperature conditions displaying the highest phenolics retention was 50°C > 70°C > 60°C > 80°C, while time showed the following trend: 1min > 10 min > 20 min > 30 min.

ORAC values showed temperature had a significant ($p<0.05$) effect on the antioxidant retention. The processing temperature of 80°C was also found to have significantly lower antioxidant retention values than all other conditions.

Table 2.5. Muscadine juice unfiltered thermal processing data.

Temperature (°C)	Time (min)	Total Phenolics (mg GAE/10ml)	% Change	ORAC (μmoles Trolox equiv/ml)	% Change
Unprocessed	N/A	13.27 ± 0.31 [a]	N/A	22.14 ± 0.218 [a]	N/A
50	1	13.30 ± 0.25 [a]	0.16	22.32 ± 0.226 [a]	0.84
50	10	13.27 ± 0.23 [a]	0.00	22.39 ± 0.219 [a]	1.12
50	20	13.30 ± 0.32 [a]	0.2	22.356 ± 0.235 [a]	1.00
50	30	13.14 ± 0.30 [a]	-1.00	22.13 ± 0.227 [a]	-0.03
60	1	13.14 ± 0.32 [a]	-1.00	22.39 ± 0.205 [a]	1.14
60	10	13.07 ± 0.40 [a]	-1.56	22.04 ± 0.225 [a]	-0.44
60	20	13.09 ± 0.45 [a]	-1.41	22.23 ± 0.215 [a]	0.42
60	30	13.24 ± 0.44 [a]	-0.25	22.28 ± 0.230 [a]	0.65
70	1	13.19 ± 0.39 [a]	-0.66	22.19 ± 0.203 [a]	0.26
70	10	13.30 ± 0.24 [a]	0.20	22.41 ± 0.227 [a]	1.22
70	20	13.17 ± 0.28 [a]	-0.77	22.21 ± 0.242 [a]	0.32
70	30	13.06 ± 0.12 [a]	-1.6	22.35 ± 0.220 [a]	0.94
80	1	13.17 ± 0.39 [a]	-0.77	21.94 ± 0.234 [a]	-0.88
80	10	13.03 ± 0.36 [a]	-1.82	21.89 ± 0.204 [a]	-1.10
80	20	12.99 ± 0.18 [a]	-2.12	22.18 ± 0.196 [a]	0.19
80	30	13.02 ± 0.29 [a]	-1.9	22.03 ± 0.222 [a]	-0.49

Average values are determined from two processing runs and duplicate analyses for each run. Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

Three main conclusions can be made from the data. Total phenolics and ORAC values had slightly different statistical significances and trends as temperature was a significant parameter for ORAC values. The standard deviations for each unfiltered sample were higher

than the three filtered samples in the preliminary study. Finally, all samples were not statistically different from one another after thermal treatment.

Particulates in the unfiltered juice could possibly be the reason for these conclusions. When performing the spectrophotmetric analyses, the particulates interfered and bounced the light flow. This interference created a higher analysis standard deviation than what occurred for the filtered juice. Since these particulates must be removed before analysis to obtain great precision, the results in this section are not useful to determine the effects of thermal treatment on phenolics and ORAC values. Therefore, all other studies will use filtered juice for analysis of phenolics and antioxidant activities.

3.2.3 Filtering Before Thermal Processing Phenolics

This section uses filtered juice before thermal treatment as in the pomegranate juice study. **Table 2.6** shows the data.

Table 2.6. Muscadine juice filtering before thermal processing data.

Temperature (°C)	Time (min)	Total Phenolics (mg GAE/10ml)	% Change	ORAC (μmoles Trolox equiv/ml)	%Change
Unprocessed		8.552 ± 0.155 ^{bcde}	N/A	14.62 ± 0.104 ^{def}	N/A
50	1	8.509 ± 0.044 ^{cde}	-0.51	14.73 ± 0.102 ^{cde}	0.74
50	10	8.624 ± 0.078 ^{abcd}	0.84	14.84 ± 0.101 ^{bcd}	1.46
50	20	8.570 ± 0.017 ^{bcde}	0.20	14.74 ± 0.103 ^{cde}	0.81
50	30	8.490 ± 0.013 ^{cde}	-0.72	14.25 ± 0.107 ^{g}	-2.57
60	1	8.824 ± 0.014 ^{ab}	3.18	14.94 ± 0.097 ^{bc}	2.12
60	10	8.689 ± 0.012 ^{abc}	1.60	14.82 ± 0.094 ^{bcd}	1.36
60	20	8.856 ± 0.054 ^{a}	3.55	15.01 ± 0.098 ^{ab}	2.64
60	30	8.885 ± 0.021 ^{a}	3.89	15.20 ± 0.083 ^{a}	3.96
70	1	8.499 ± 0.037 ^{cde}	-0.63	14.63 ± 0.094 ^{de}	0.05
70	10	8.679 ± 0.070 ^{abc}	1.48	14.73 ± 0.0104 ^{cde}	0.74
70	20	8.473 ± 0.039 ^{cdef}	-0.93	14.53 ± 0.0105 ^{ef}	-0.66
70	30	8.526 ± 0.063 ^{cde}	-0.31	14.63 ± 0.089 ^{de}	0.05
80	1	8.382 ±0.104 ^{def}	-1.99	14.92 ± 0.104 ^{bc}	2.03
80	10	8.195 ± 0.104 ^{ef}	-4.18	14.20 ± 0.110 ^{g}	-2.88
80	20	8.348 ± 0.166 ^{def}	-2.39	14.28 ± 0.107 ^{fg}	-2.37
80	30	8.329 ± 0.208 ^{ef}	-2.61	14.52 ± 0.103 ^{ef}	-0.69

Average values are determined from two processing runs and duplicate analyses for each run.
Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

Temperature was the only significant individual factor affecting total phenolics ($p<0.05$). The temperature and time interaction term was also found to be significant. 60°C showed significantly higher total phenolics values than 50°C and 70°C, which had significantly higher values than 80°C. At the processing temperature of 50°C for all time conditions, average values for total phenolics were not significantly different than the unprocessed sample. In all temperature and time combinations, 60°C had the highest levels of phenolics compared to all other processing conditions. These trends can be observed in **Figure 2.2** while the statistics can be seen in **Appendix A.5 and A.6**.

The condition of 60°C and 30 min had the greatest phenolics retention of all conditions, having 3.89% greater phenolics than the unprocessed sample. The juice processed at 80°C and 10 min had the lowest phenolics value which was 4.18% lower than the unprocessed sample.

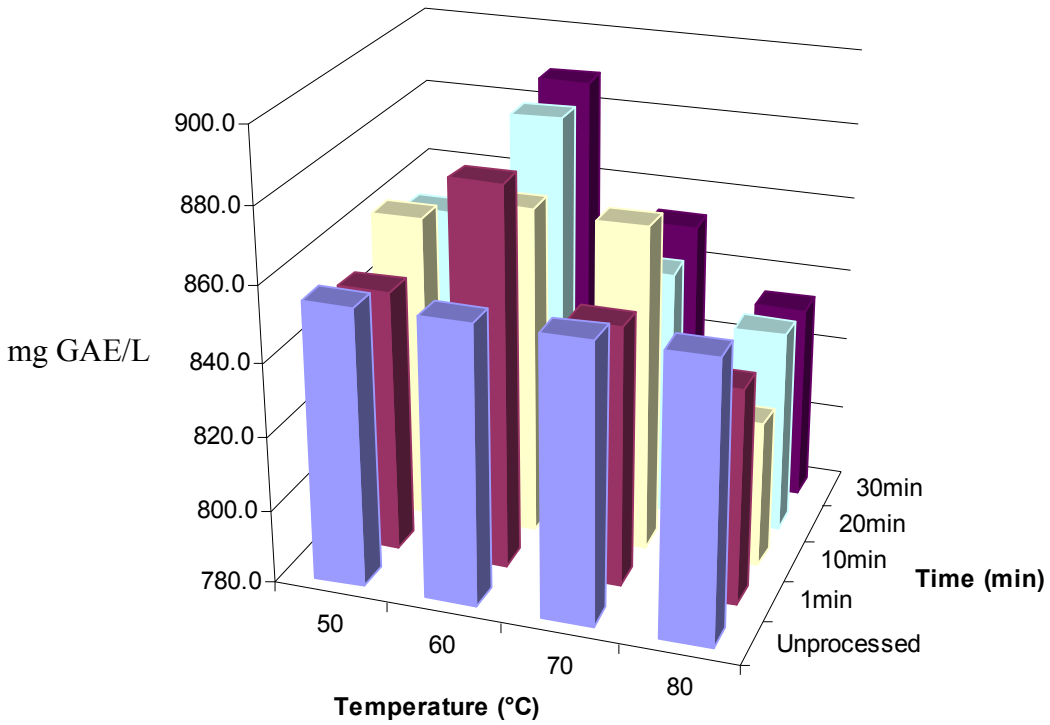

Figure 2.2 Muscadine juice filtering before thermal processing phenolics graph.

3.2.4 Filtering Before Thermal Processing ORAC

ORAC results show a very close agreement with the total phenolics for juice that has been filtered before thermal processing. **Table 2.6** also shows antioxidant values after the thermal treatment. Temperature was the only significant individual factor ($p<0.05$) affecting antioxidant retention. Temperature and time interaction also had a significant effect on the antioxidant retention. 60°C was found to have higher ORAC retention than 50°C and 70°C, which had significantly higher values than 80°C. All samples processed at 60°C showed a significant increase in ORAC values than the unprocessed sample. 70°C processing showed no significant differences in antioxidant values from the unprocessed sample. For the 80°C juice samples, two processing times of 10 min and 20 min showed a significant decrease in ORAC values compared to the unprocessed sample. Like the total phenolics, 60°C/30min and 80°C/10 min had the highest and lowest antioxidant activities, respectively, of all processing conditions. The 60°C and 30 min condition had a 3.96% higher ORAC value than the unprocessed sample while the 80°C and 10 min condition had a 2.88% decrease.

ORAC and total phenolics result show that values reach a peak at 60°C in muscadine juice. The decrease in both ORAC and phenolics values at 70°C show that compounds and ORAC values may begin to show instability and activity after 60°C. At 60°C and below, higher temperatures resulted in the increase of phenolics and ORAC values. These degradation temperature values for muscadine juice are approximately 10°C lower than what was found in pomegranate juice.

The difference in phenolic compounds could be the reason for the differences in juice antioxidant threshold degradation temperatures. Literature shows that phenolic compounds with varying degrees of glycosylation, methylation, number of hydroxyl groups, and other ring

substitution have different antioxidant activities (Lee and Talcott, 2002). No specific literature was found comparing these phenolic compound attributes in muscadine juice, pomegranate juice or similar systems.

3.2.5 Filtering After Thermal Processing Phenolics

This section shows the effect of not removing particulates until thermal processing has occurred. Filtering after thermal treatment may allow for the pomace particulate antioxidants to be extracted into the juice due to heat. Currently, most juice processors filter out most particulates prior to heat treatment.

The unprocessed sample, which was also filtered, had a total phenolics value of 9.431mg GA equiv/10ml. All of the process samples had values greater than 10.06 mg GA equiv/10ml, which is a minimum average increase of 6.6%. **Table 2.7** shows all of the data for all thermal treatments.

Table 2.7. Muscadine juice filtering after thermal processing data.

Temperature (°C)	Time (min)	Total Phenolics (mg GAE/10ml)	% Change	ORAC (μmoles Trolox equiv/ml)	%Change
Unprocessed	N/A	9.431 ± 0.107 ^{c}	N/A	15.47 ± 0.079 ^{g}	N/A
50	1	10.16 ± 0.082 ^{ab}	7.73	16.98 ± 0.093 ^{cde}	9.76
50	10	10.23 ± 0.075 ^{ab}	8.43	16.84 ± 0.094 ^{ef}	8.86
50	20	10.24 ± 0.028 ^{ab}	8.60	17.02 ± 0.091 ^{cde}	10.01
50	30	10.51 ± 0.100 ^{a}	11.42	17.35 ± 0.101 ^{a}	12.14
60	1	10.11 ± 0.086 ^{ab}	7.16	16.85 ± 0.100 ^{ef}	8.92
60	10	10.11 ± 0.226 ^{ab}	7.16	17.05 ± 0.096 ^{bcde}	10.20
60	20	10.15 ± 0.180 ^{ab}	7.62	16.93 ± 0.092 ^{def}	9.43
60	30	10.10 ± 0.068 ^{ab}	7.04	16.98 ± 0.097 ^{cde}	9.76
70	1	10.24 ± 0.033 ^{ab}	8.54	17.00 ± 0.097 ^{cde}	9.89
70	10	10.06 ± 0.185 ^{b}	6.64	16.93 ± 0.092 ^{def}	9.43
70	20	10.21 ± 0.082 ^{ab}	8.31	17.18 ± 0.106 ^{abcd}	11.06
70	30	10.21 ± 0.100 ^{ab}	8.31	17.20 ± 0.099 ^{abc}	11.15
80	1	10.13 ± 0.082 ^{ab}	7.39	16.72 ± 0.105 ^{f}	8.08
80	10	10.30 ± 0.184 ^{ab}	9.17	17.35 ± 0.103 ^{a}	12.10
80	20	10.28 ± 0.180 ^{ab}	9.00	17.28 ± 0.103 ^{ab}	11.68
80	30	10.18 ± 0.123 ^{ab}	7.96	17.13 ± 0.110 ^{abcd}	10.71

Average values are determined from two processing runs and duplicate analyses for each run. Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

Neither temperature nor time had any significant effect on phenolics retention ($p<0.05$). Though, the statistics show that all processing conditions had significantly higher phenolics retentions than the unprocessed sample ($p<0.05$).

This result supports the hypothesis that phenolics could be extracted out of the particulates and into the juice during heat treatment. This study also illustrates that the different temperature and time combinations do not have an effect on this extraction process. It seems that the particulates only require a small amount of heat to produce extraction results.

3.8 Muscadine Juice Filtering After ORAC

The ORAC values also show a close agreement with what was found for the total phenolics. All processed samples had significantly higher antioxidant retentions compared to the unprocessed muscadine juice ($p<0.05$). The processed condition with the lowest antioxidant retention had an 8.1% higher value compared to the unprocessed sample. **Table 2.7** also shows antioxidant values for the thermal treatments. Temperature did not have any significant effect on the ORAC ($p<0.05$). Time, on the other hand, was found to have a significant effect ($p<0.05$). 30 min and 20 min residence times had significantly higher antioxidant retentions than the 1 min residence time. These results show that particulates encountering prolonged heat can result in higher ORAC value juices. By filtering after processing, juice processors can obtain approximately a 10% increase in ORAC values in their juice, while still producing a filtered, clear beverage.

3.9 Total Phenolics Content and ORAC Correlation

Previous literature has shown several different total phenolics and ORAC relationships ranging from 0.73 to 0.99 between in various fruits and fruit juices (Lee and Talcott, 2004;

Pacheco-Palencia et al., 2007). However, no papers illustrated the relationship after these fruits have undergone heat treatment. The pomegranate study had a R^2 of 0.637 as seen in **Appendix A.9**. The muscadine filtering before processing study produced a R^2 of 0.649 as seen in **Appendix A.10**. The R^2 of the filtering after processing study was 0.497 as seen in **Appendix A.11**. These correlation values show that after thermal processing, the relationship between ORAC and total phenolics slightly decreases in juice.

3.10 Microbiological Inactivation Study

Filtered muscadine juice was used. Of the processing conditions used in **Table 2.2**, only juice processed at 50°C had yeast colony growth. All other samples did not have enough colonies to count. The possible reasoning behind this is the great degree of heat transfer in a test tube heated to the experimental temperature in boiling water. Heat can much more rapidly penetrate the entire juice medium than if processed under a much larger scale. Data is shown in the **Table 2.8**.

Table 2.8. Microbial inactivation with thermal processing

Temperature (°C)/ Time (min)	Log (cfu/ml)
Unprocessed	6.547
50/1	5.675
50/5	4.456
50/10	3.786
50/20	2.235
60/1	ND
60/5	ND
60/10	ND
60/20	ND
70/1	ND
70/3	ND
70/5	ND
70/10	ND
80/0.5	ND
80/1	ND
80/4	ND
80/7	ND

ND = Not detectable

At 50°C, the D value for *Zygosacchromyces bailii* in muscadine juice was determined to be 5.04 min (R^2 was 0.9368). This determination is shown by the **Appendix A.4** graph. A D value at 50°C for *Zygosacchromyces bailii* in grape juice was found to be 4.18 min by literature (Raso et al., 1998). These two values are highly similar and the possible difference is the high transfer occurring in the small batch size of this study. This study also suggests that *Zygosacchromyces bailii* cannot survive in temperatures at 60°C or greater with an effective heat transfer method.

4. Conclusions

This study illustrates the effects of various thermal processing treatments on total phenolics and antioxidants. Filtered pomegranate juice thermally processed at 70°C showed the highest total phenolics antioxidant retention even compared with unprocessed juice. Phenolics degradation was visible at temperatures greater than 70°C. Therefore, juice processors are recommended to use temperatures at or below 70°C to preserve antioxidant retention during thermal pasteurization of pomegranate juice. At these temperatures, juice processors need to determine adequate residence time that provide a 5-log pathogen inactivation for their specific process. An exact residence time, that meets inactivation requirements, is not proposed by the FDA.

The studies conducted on muscadine juice showed that both filtration and thermal processing are detrimental to the total phenolics and ORAC retention. By only filtering the juice, approximately 30% of phenolics and ORAC values were lost, yet only 10-15% of pomace particulates were removed. This result suggests that pomace particulates have a greater overall effect to ORAC values than the juice. Muscadine juice that was filtered prior to processing showed that 60°C was the temperature that produced the highest total phenolics and antioxidant

retention even compared with the unprocessed juice. Phenolics degradation was visible at processing temperatures greater than 60°C in muscadine juice. Therefore, muscadine juice exhibited phenolics degradation at a temperature 10°C lower than in pomegranate juice. Muscadine juice that was filtered after thermal processing showed that the thermal conditions did not have an effect on the antioxidant retention. Since the particulates were left in the juice before thermal processing, all conditions showed antioxidant and total phenolic retentions at least 8% higher than the unprocessed sample. The prolonged heat extracted some of the phenolics and antioxidant activity out of the particulates and into the juice, providing higher values. This result suggests that juice processors should filter after thermal treatment in order to maintain high ORAC values that are lost during filtration.

Total phenolics and ORAC correlations were found to be between 0.497-0.649 after thermal treatment for pomegranate and muscadine juice. These values were slightly below the 0.73-0.99 values of unprocessed juice found in literature. This result shows that thermal treatment decreases the relationship between phenolics and ORAC.

The microbiological inactivation study shows that the high heat transfer occurring in the test tube processing was highly successful at killing *Zygosacchromyces bailii*, a yeast spoilage organism. Temperatures greater than 50°C killed all organisms. At 50°C, the D value was calculated to be 5.04 minutes, slightly greater than the 4.18 minute D value found in literature.

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CHAPTER 3

SUPERCRITICAL CARBON DIOXIDE PROCESSING

1. Introduction

Previous literature has shown that phenolic compounds that contribute to ORAC are susceptible to processing and heat. Two studies found that pomegranate juice processed at 95°C/30s and 100°C/20 min showed a 2% and 7% reduction in total phenolics (Alper et al., 2005; Perez-Vicente et al., 2002). Other studies using different juices have also found a similar reduction in both phenolics and antioxidants (Klopotek et al., 2005; Larrauri et al., 1997).

Consumption of natural antioxidants, naturally present in juices, is becoming a common trend for consumers. Elizabeth Sloan in April 2009 edition of Food Technology Magazine states, "Interest in naturally functional foods and whole food nutrition is likely to be one of the strongest health trends for the next 10 years." This includes "foods naturally high in phytochemicals and nutrients (Sloan, 2009)." As the demand and desire for higher antioxidant juices becomes greater, there is a possible need for an alternative pasteurization technology to be developed.

Alternative technologies have been referred to as nonthermal processing because the temperature of the juice is held below the temperatures necessary for current thermal pasteurization. Therefore, phenolic compounds are expected to undergo minimal degradation during nonthermal processing (Barbosa-Canovas, 2005). One alternative technology that exists is supercritical carbon dioxide.

Supercritical and dense phase carbon dioxide has not been extensively studied to show how various processing conditions affect the microbial inactivation and antioxidant levels in various food matrices. One such study showed that dense phase carbon dioxide processing at 34.5MPa showed only a 2% reduction in both phenolics and ORAC values, much better than the reductions of at least 10% exhibited by thermal processing (75°C and 15s). In addition to high preservation of phenolic compounds, this operating condition showed at least a five log reduction in yeast cells (Del Pozo-Insfran et al., 2006). Only one processing condition was used in this study so the optimum condition to preserve the antioxidant retention cannot be concluded.

Therefore, the purpose of this study is to evaluate how different supercritical carbon dioxide processing conditions affects antioxidant retentions in juices. This will be done by using several temperature, pressure, and residence time combinations and measuring the total phenolics and ORAC retentions in muscadine and pomegranate juice. In addition, microbial inactivation will be studied for *Zygosacchromyces bailii* for the various processing conditions.

2. Materials and Methods

2.1 Processing

The experimental apparatus was a batch reactor. **Figure 3.1** shows a 99.9% deep tube carbon dioxide feed tank (Toccoa Welders, Toccoa, GA (1) which was regulated (2) to reach 5.52MPa before being fed into a pump (Haskel Gas Booster AGT-6215, Huntington Beach, CA) (3) operating at a 41.4MPa capacity. The pump was regulated by an air regulator valve (4). The high pressure carbon dioxide was then fed into a stainless steel, high pressure vessel (Autoclave Engineers, Erie, PA) (5). Carbon dioxide was vented at the bottom of the vessel. This high pressure vessel was insulated in an incubator chamber (Instron, Normwood, MA) (6) that was temperature controlled by a PID controller (Instron 3119-005, Normwood, MA) (7). At the start

of each experiment, 50ml juice taken 4°C storage was heated to the experimental processing temperature using a hot/stir plate (Fisher Scientific, Pittsburg, PA) under constant mixing conditions with a stir bar. The juice was immediately injected into the top of the pre-heated vessel. The top and bottom of the vessel was then securely attached to the processing lines. When the system reached the specified experimental conditions of pressure, the treatment time was recorded. After processing, the heat supply to the chamber was shut off and the chamber was opened. Additionally, carbon dioxide was purged from the vessel and a heater (Master Flow Heat Blower Model AH-501, Racine, WI) (8) was used to prevent the venting valve (9) from freezing. The processed juice was then poured into a 50ml centrifuge tube and placed in ice to chill and then stored at -10°C for less than one week until total phenolics and ORAC values were analyzed.

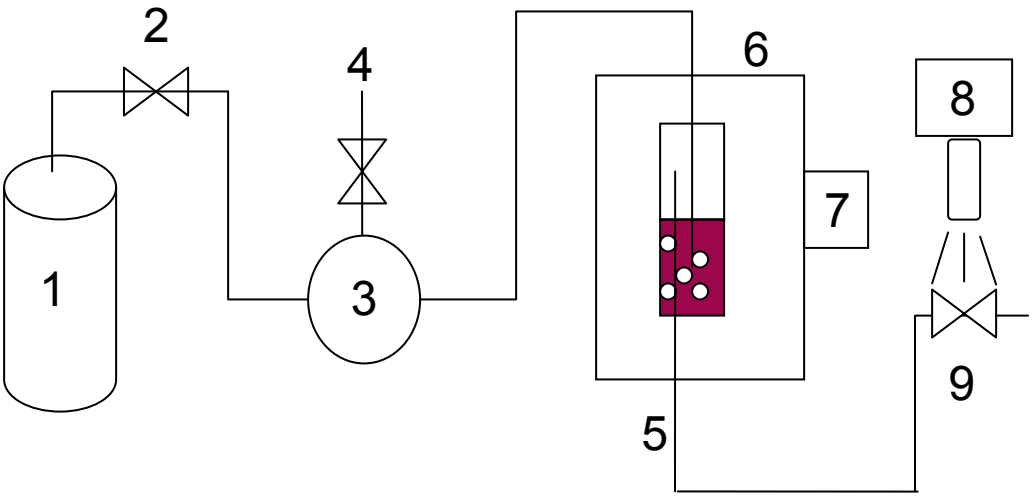

Figure 3.1. Supercritical carbon dioxide processing system. 1 is the dip tube carbon dioxide tank. 2 is the tank regulator. 3 is the high pressure pump (41.4MPa max). 4 is the air supply regulator. 5 is the venting carbon dioxide tube. 6 is the temperature controlled incubator. 7 is the PID controller. 8 is the heater. 9 is the venting valve.

Temperature, pressure, and residence time were the varying factors during processing. Three conditions of each factor were used giving 27 overall conditions. The conditions were

chosen because temperatures in literature ranged from 25°C-60°C, pressure ranged from 6.89MPa-48.3MPa, and residence times from 10 min-100 min (Gunes et al., 2005). Based on these wide ranges of conditions, a preliminary study was performed to determine the exact conditions to use.

The preliminary study was conducted at 27.6MPa to see if the total phenolics or ORAC values changed due to the preheating step, processing temperature, or residence time in the supercritical carbon dioxide vessel. Initially, a concern was that the juice heat pretreatment occurring on the hot plate could affect the antioxidant quality. The study verified that this processing step did not have an effect on the phenolics content. Different holding times of 15 and 30 min had similar antioxidant contents at the different temperatures. The results also showed that of the 35°C, 45°C, 55°C processing temperatures, 45°C showed the highest antioxidant (ORAC) retentions. From this study and the findings in literature, the following experimental conditions were then chosen as seen in **Table 3.1**. Each processing condition was duplicated.

Table 3.1. Supercritical carbon dioxide processing conditions for total phenolics and antioxidant analysis.

Variables	Conditions
Temperature (°C)	35°C 45°C 55°C
Pressure (MPa)	20.7MPa 27.6MPa 34.5MPa
Residence Time (min)	10 min 20 min 30 min

2.2 Juice Preparation

2.2.1 Muscadine Juice

Raw muscadine juice was received from Paulk Vineyards (Gray, GA) in an unprocessed and unfiltered state. It was filtered using a centrifugal process (Sorvall RC-6 Plus, Waltman, MA) of 3000xg for 4 min. Approximately, 400ml of unprocessed and unfiltered juice were added to 500ml centrifugal bottles and centrifuged using the stated conditions. After centrifugation, the filtered juice was added to one main collection vessel while the filtrate was discarded. Once all of the juice was filtered and collected, it was mixed while transferred to 50ml centrifuged vials. This ensured all of the juice samples to have identical properties. The juice was then frozen at -10°C for no more than 14 days prior to processing.

2.2.2 Pomegranate Juice

Pomegranates (Ponder Farm, Ty Ty, GA) of the North TR (2^{nd} harvest) variety were used. Juice was prepared by separating the arils from the pith and blending with a home blender. The pomegranate mixture was then passed through fine cheese cloth to eliminate large particulates. The filtered juice was then transferred to 50ml centrifuge vials and stored at -10°C.

Prior to processing, the frozen juice samples were placed in a 4°C cooler overnight. Immediately after taking the sample out of the cooler, it was heated on a stir plate to the experimental processing temperature.

2.3 Total Phenolics

The total phenolics contents were measured using an adapted Folin-Ciocalteu's phenol reagent assay (Naczk and Shahidi, 1989). A gallic acid stock solution was first prepared to an approximate concentration of 0.32mg/ml in 5% methanol-95% water solution. Also a blank (5% methanol-95% water) was prepared. A standard curve was conducted using different

concentrations of this stock solution. 0.25 ml of filtered juice samples and standard curve solutions were added to 4.0 ml of deionized water. After vortexing, 0.25 ml of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO) was added. After vortexing and waiting for 5 min, 0.5ml of saturated sodium carbonate (Fisher Scientific, Pittsburg, PA) was added. Then, the absorbance of the samples was measured at 750nm by an Agilent 8453 diode-array spectrophotometer (Agilent Technologies, Wilmington, DE) after a one hour incubation period to allow for color development. Absorbance to gallic acid concentration conversion was performed using the standard curve of known gallic acid concentrations that were prepared. Each processing sample was analyzed in duplicate, thus giving quadruplicate readings at each processing condition. The difference in values from the unprocessed value is calculated by the following formula:

$$\%\ \text{Change}_i = \frac{\text{Phenolics or ORAC}_i}{\text{Phenolics or ORAC}_{\text{unprocessed}}} \text{ x } 100$$

2.4 ORAC

Oxygen radical absorbance capacities (ORAC) of juice samples were measured using the method (Prior et al., 2003) modified for use with a FLUOstar Omega microplate reader (BMG Labtech, Durham, NC), using fluorescein (3,6-dihydroxy-spiro[isobenzofuran-1[3*H*],9[9*H*]-xanthen]-3-one) disodium salt (Sigma-Aldrich, St. Louis, MO) as the fluorescent probe. Juices were diluted appropriately with phosphate buffer (75mM, pH 7.4) made of mono- and di-basic potassium phosphate (VWR Internal, Suwanee, GA). The assay was carried out in a Costar 96well (Costar #3631) opaque clear bottom micro-well assay plate (Fisher Scientific, Suwanee, GA). Initially 20µL of diluted juice samples and Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, St. Louis, MO) standards (6.25, 12.50, 25, 50, 100µM) along with 40µL of blank solution (75mM, pH 7.4 phosphate buffer) were added

to each well using an automatic pipet. The FLUOstar Omega microplate reader, equipped with two automated injectors, was then programmed to add 400 µL of fluorescein (0.11µM) (Sigma-Aldrich, St. Louis, MO), followed by 150µL of azobis (2-amidino-propane) dihydrochloride (AAPH) (Sigma- Aldrich, St. Louis, MO) (31.6mM) to each well. Fluorescence readings (excitation 485nm, emission 520nm) were recorded after the addition of fluorescein and AAPH (2,2-azobis [2-amidinopropane] dihydrochloride) (Sigma-Aldrich, St. Louis, MO), and every 192s thereafter until a 95% loss of fluorescence was reached. Final fluorescence measurements were expressed relative to the initial reading. Results were calculated based upon difference in areas under the fluorescein decay curve between the blank, samples, and standards. The standard curve of each analysis was obtained by plotting the five concentrations of TE against the net area under the curve (AUC) of each standard. Final ORAC values were calculated using the regression equation between the TE concentration and the AUC and are expressed as micromoles (µmol) of TE equivalents per L of juice.

2.5 Microbiological Inactivation Study

Zygosacchromyces bailii (ATCC 42476) (Difco, Franklin Lakes, NJ), a yeast spoilage organism, was inoculated at a concentration between 10^6 and 10^7cfu/ml in muscadine grape juice immediately before supercritical carbon dioxide processing. These organisms were grown in a yeast and malt (YM) broth (Sigma- Aldrich, St. Louis, MO) test tube for no more than 48hr. The cells were centrifuged (Beckman Coulter-Allegra X-22R, Fullerton, CA) at 3000xg for 4 min and then washed twice with sterile 0.1% peptone (Difco, Franklin Lakes, NJ). After washing, the cells were transferred to 50ml of muscadine grape juice.

After the inoculated juice was processed, plating occurred on YM agar (Sigma- Aldrich, St. Louis, MO) plates acidified with 10% tartaric acid (Sigma- Aldrich, St. Louis, MO) to a pH

between 3 and 4. Plates were spiral plated using a Spiral Autoplater 20.7 (Spiral BioTech Inc., Bethesda, MD) and stored at 30°C for 4 days. The processing conditions for the microbial inactivation study are shown in **Table 3.2**.

Table 3.2. Supercritical carbon dioxide processing conditions for microbiological inactivation study.

Variables	Conditions
Temperature (°C)	35°C 45°C 55°C
Pressure (MPa)	20.7MPa 27.6MPa 34.5MPa
Residence Time (min)	10 min 30 min

2.6 Statistical Analysis

Analysis of Variance (ANOVA) was conducted to determine significant difference between treatments for each of the assays performed. An alpha level $p < 0.05$ was used in the analysis. Treatment differences were determined using Tukey's HSD Test. Statistical analysis was conducted using JMP Software (SAS Institute, Cary, NC).

Statistical analysis was performed on quadruplicates. Two samples were processed at each condition and duplicates were analyzed for each sample, giving four readings.

3. Results and Discussion

3.1 Muscadine Juice

3.1.1 Phenolics

Phenolics were analyzed for all processing conditions to determine if temperature, pressure, or residence time had any significant effects on the phenolics retention. Phenolic values at each processing condition can be found in **Table 3.3**. Temperature and pressure were

individually significant factors ($p < 0.05$) affecting phenolics while the combination of temperature and time also was found to be significant. Temperature ($p<0.05$) had a more significant contribution to the antioxidant retention than pressure ($p<0.05$). Taking into account all variables, the 45°C processing temperature had significantly higher phenolics retentions than 35°C which had significantly higher retentions than 55°C. 27.6MPa had significantly higher phenolic retentions than 20.7MPa and 34.5MPa. Individually, residence time was not critical in the phenolics retention of the supercritical carbon dioxide process ($p>0.05$). However, the interaction of time and temperature showed significant effects on the retention ($p<0.05$). The condition with the highest phenolics retention was 45°C, 27.6MPa, and 20 min, which had 2.12% higher phenolics than the unprocessed juice. The condition of 55°C, 34.5MPa, and 30 min had the lowest phenolics retention, 1.52% lower than the unprocessed juice.

At 20.7MPa, phenolics increased with residence time at 35°C and 45°C with a higher rate of increase found at 45°C. At this same pressure, phenolics remained constant with residence time at 55°C. 27.6MPa shows similar trends at the various temperatures, except phenolics slightly decreased at 55°C processing times. At 55°C and 27.6MPa, values increased with the shortest residence time then decreased quickly. Additionally, at 10 min, phenolics increased from 35°C to 55°C at 27.6MPa. At 34.5MPa, 45°C shows increasing values with increasing residence time while 55°C shows decreasing values with increasing residence time. Overall, the middle experimental conditions of temperature, pressure, and residence time (45°C, 27.6MPa, 20 min) showed greater phenolics values than the other conditions used.

Table 3.3. Muscadine juice supercritical processing data.

Temperature (°C)	Pressure (MPa)	Residence Time (min)	Total Phenolics (mg GAE/10ml)	% Change	ORAC (μmoles Trolox equiv/ml)	% Change
Unprocessed	0	0	8.562 ± 0.135 ^{bc}	N/A	14.69 ± 0.112 ^{bcd}	N/A
35	20.7	10	8.524 ± 0.164 ^{abc}	-0.44	14.64 ± 0.121 ^{bcd}	-0.36
35	27.6	10	8.665 ± 0.052 ^{bc}	1.20	14.73 ± 0.099 ^{abcd}	0.29
35	34.5	10	8.537 ±0.095 ^{bc}	-0.29	14.64 ± 0.112 ^{bcd}	-0.35
35	20.7	20	8.614 ± 0.100 ^{bc}	0.61	14.80 ± 0.112 ^{abc}	0.77
35	27.6	20	8.706 ± 0.046 ^{a}	1.68	14.85 ± 0.104 ^{abc}	1.12
35	34.5	20	8.567 ± 0.109 ^{bc}	0.06	14.77 ± 0.125 ^{abcd}	0.54
35	20.7	30	8.659 ± 0.124 ^{bc}	1.13	14.85 ± 0.100 ^{abc}	1.09
35	27.6	30	8.711 ± 0.045 ^{a}	1.73	14.85 ± 0.109 ^{abc}	1.11
35	34.5	30	8.548 ± 0.148 ^{bc}	-0.17	14.67 ± 0.099 ^{abcd}	-0.15
45	20.7	10	8.620 ± 0.072 ^{bc}	0.67	14.80 ± 0.096 ^{abc}	0.76
45	27.6	10	8.699 ± 0.080 ^{ab}	1.60	14.82 ± 0.108 ^{abc}	0.90
45	34.5	10	8.565 ± 0.079 ^{bc}	0.03	14.84 ± 0.118 ^{abc}	1.04
45	20.7	20	8.677 ± 0.047 ^{ab}	1.33	14.83 ± 0.124 ^{abc}	0.97
45	27.6	20	8.744 ± 0.120 ^{a}	2.12	14.90 ± 0.105 ^{ab}	1.45
45	34.5	20	8.680 ± 0.045 ^{ab}	1.37	14.85 ± 0.111 ^{abc}	1.12
45	20.7	30	8.671 ± 0.068 ^{bc}	1.26	14.89 ± 0.102 ^{ab}	1.37
45	27.6	30	8.704 ± 0.063 ^{a}	1.65	14.95 ± 0.122 ^{a}	1.78
45	34.5	30	8.680 ± 0.025 ^{ab}	1.37	14.90 ± 0.114 ^{abc}	1.42
55	20.7	10	8.602 ± 0.032 ^{bc}	0.47	14.75 ± 0.108 ^{abcd}	0.38
55	27.6	10	8.678 ± 0.090 ^{ab}	1.35	14.90 ± 0.112 ^{ab}	1.40
55	34.5	10	8.670 ± 0.154 ^{bc}	1.26	14.89 ± 0.100 ^{abc}	1.35
55	20.7	20	8.562 ± 0.098 ^{bc}	-0.01	14.73 ± 0.109 ^{abcd}	0.29
55	27.6	20	8.553 ± 0.072 ^{bc}	-0.11	14.79 ± 0.103 ^{abcd}	0.65
55	34.5	20	8.539 ± 0.047 ^{bc}	-0.27	14.63 ± 0.098 ^{bcd}	-0.41
55	20.7	30	8.512 ± 0.071 ^{bc}	-0.58	14.59 ± 0.111^{cd}	-0.68
55	27.6	30	8.457 ± 0.068 ^{bc}	-1.23	14.59 ± 0.104 ^{cd}	-0.68
55	34.5	30	8.432 ± 0.101^{c}	-1.52	14.50 ± 0.104 ^{d}	-1.27

Average values are determined from two processing runs and duplicate analyses for each run. Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

3.1.2 ORAC

ORAC was used to determine the ORAC values of these processed conditions. ORAC values at each processing condition can be found in **Table 3.3.** Temperature and pressure were found to be individually significant factors ($p <0.05$) affecting ORAC values while the combination of temperature and time also was a significant factor. The condition with the highest antioxidant retention was 45°C, 27.6MPa, and 30 min, which had 1.78% higher ORAC

values than the unprocessed juice. The condition of 55°C, 34.5MPa, and 30 min had the lowest antioxidant retention, 1.27% lower than the unprocessed juice.

The antioxidant retention had similar trends as the phenolics retention for each of the pressures, temperatures and residence times, and the same factors had significant effects. The correlation relationship will be explained in Section *3.3*.

3.2 Pomegranate Juice

3.2.1 Phenolics

The pomegranate juice study showed similar results to the muscadine juice study as described below. Values can be found in **Table 3.4**. Temperature and pressure were individually significant factors ($p < 0.05$) affecting the phenolics retention while the combination of temperature and time also was found to be significant. Temperature ($p<0.05$) was found have a greater impact on the phenolics retention than pressure ($p<0.05$). A temperature of 45°C was found to produce significantly higher phenolics retentions than 35°C which was found to have higher retentions than 55°C. Processing at 27.6MPa had significantly higher phenolics retentions than 20.7MPa and 34.5MPa. Thus, the two intermediate processing conditions for both temperature and time were optimal conditions in the experiment for phenolics retention.

The highest temperature and time (55°C and 30 min) condition had significantly lower phenolics retentions than all other temperature-time combinations. At the processing temperature of 55°C, phenolics values decreased with increasing residence times. This study shows that processing with supercritical carbon dioxide at temperatures above 50°C with increasing residence times has a detrimental effect on the phenolics retention. The condition with the highest phenolics retention was 35°C, 27.6MPa, and 10 min, which had 1.87% higher

phenolics than the unprocessed juice. The condition of 55°C, 34.5MPa, and 30 min had the lowest phenolics retention, 1.56% lower than the unprocessed juice.

Table 3.4. Pomegranate juice supercritical processing data.

Temperature (°C)	Pressure (MPa)	Residence Time (min)	Total Phenolics (mgGAE/10ml)	% Change	ORAC (μmoles Trolox Equiv/ml)	% Change
Unprocessed	0	0	15.26 ± 0.153 ^{abcde}	N/A	15.24 ± 0.116 ^{abcde}	N/A
35	20.7	10	15.23 ± 0.232 ^{abcde}	-0.22	15.21 ± 0.111 ^{abcde}	-0.18
35	20.7	20	15.37 ± 0.128 ^{abcde}	0.87	15.37± 0.116 ^{abcd}	1.01
35	20.7	30	15.36 ± 0.186 ^{abcde}	-0.05	15.38 ± 0.108 ^{abcd}	0.13
45	20.7	10	15.27 ± 0.132 ^{abcde}	-0.57	15.38 ± 0.100 ^{abcd}	-0.02
45	20.7	20	15.42 ± 0.111 ^{abcd}	0.96	15.37 ± 0.117 ^{abcd}	-0.05
45	20.7	30	15.48 ± 0.079 ^{abcd}	0.37	15.44 ± 0.098 ^{abc}	0.47
55	20.7	10	15.26 ± 0.108 ^{abcde}	-1.41	15.29 ± 0.107 ^{abcde}	-1.01
55	20.7	20	15.29 ± 0.142 ^{abcde}	0.18	15.28 ± 0.090 ^{abcde}	-0.06
55	20.7	30	15.11± 0.062 ^{de}	-1.17	15.15 ± 0.117 ^{cde}	-0.88
35	27.6	10	15.39 ± 0.078 ^{abcd}	1.87	15.28 ± 0.078 ^{abcde}	0.86
35	27.6	20	15.44 ± 0.088 ^{abcd}	0.31	15.40 ± 0.094 ^{abcd}	0.79
35	27.6	30	15.45 ± 0.083 ^{abc}	0.07	15.40 ± 0.105 ^{abcd}	0.01
45	27.6	10	15.42 ± 0.094 ^{abcd}	-0.20	15.44 ± 0.214 ^{abcd}	0.24
45	27.6	20	15.55 ± 0.163 ^{a}	0.86	15.45 ± 0.098 ^{abc}	0.10
45	27.6	30	15.48 ± 0.090 ^{abc}	-0.47	15.50 ± 0.120 ^{a}	0.31
55	27.6	10	15.44 ± 0.089 ^{abcd}	-0.23	15.44 ± 0.104 ^{abcd}	-0.38
55	27.6	20	15.28 ± 0.093 ^{abcde}	-1.07	15.33 ± 0.105 ^{abcde}	-0.71
55	27.6	30	15.18 ± 0.085 ^{abcde}	-0.66	15.14 ± 0.105 ^{de}	-1.29
35	34.5	10	15.21± 0.126 ^{abcde}	0.21	15.16 ± 0.115 ^{bcde}	0.12
35	34.5	20	15.31 ± 0.153 ^{abcde}	0.65	15.31± 0.139 ^{abcde}	1.04
35	34.5	30	15.21± 0.185 ^{abcde}	-0.70	15.23 ± 0.120 ^{abcde}	-0.58
45	34.5	10	15.29 ± 0.127 ^{abcde}	0.56	15.38 ± 0.120 ^{abcd}	1.03
45	34.5	20	15.44 ± 0.082 ^{abcd}	1.00	15.41± 0.106 ^{abcd}	0.17
45	34.5	30	15.42 ± 0.042 ^{abcd}	-0.12	15.44 ± 0.085 ^{abcd}	0.18
55	34.5	10	15.52 ± 0.171 ^{ab}	0.62	15.46 ± 0.124 ^{ab}	0.14
55	34.5	20	15.29 ± 0.072 ^{abcde}	-1.50	15.18 ± 0.085 ^{bcde}	-1.81
55	34.5	30	15.05 ± 0.142 ^{abcd}	-1.56	15.05 ± 0.108 ^{e}	-0.84

Average values are determined from two processing runs and duplicate analyses for each run. Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

3.4 ORAC

ORAC analysis had similar significant parameters as total phenolic content. **Table 3.2** also shows the ORAC values for each processing condition. Temperature and pressure were found to be individually significant factors ($p<0.05$) affecting antioxidant retention while the

combination of temperature and time also was found to be significant ($p<0.05$). The significant factors for both phenolics and antioxidant retentions were the same. The processing temperature of 45°C had significantly higher antioxidant retentions than 35°C and 55°C. The processing pressure of 27.6MPa had significantly higher antioxidant retentions than 34.5MPa.

The condition with the highest antioxidant retention was 35°C, 34.5MPa, and 20 min, which had 1.04 % higher ORAC values than the unprocessed juice. The condition of 55°C, 34.5MPa, and 20 min had the lowest antioxidant retention, 1.81% lower than the unprocessed juice.

3.5 Total Phenolics and ORAC Correlation Study

Due to the close agreement in the total phenolics and ORAC retentions, a correlation was performed. Previous literature has shown several different total phenolics and ORAC relationships ranging from 0.73 to 0.99 between in various fruits and fruit juices (Lee et al., 2002 and Pacheco-Palencia et. al., 2007). However, no papers illustrated the relationship after these fruits have undergone processing.

In the muscadine juice, the average values of the total phenolics and ORAC from the 27 processing conditions showed a correlation value of 0.801, as shown in **Appendix A.5**. The pomegranate juice correlation shown in **Appendix A.5** was 0.814. These correlation values show that after supercritical carbon dioxide processing, the relationship between ORAC values and total phenolics is similar to what was found in literature. Additionally, the processing had a similar effect to the relationship in both pomegranate and muscadine juice.

3.6 Processing Effect on Total Phenolics and ORAC Discussion

As discussed, the highest temperature (55°C) and pressure (34.5MPa) showed the lowest values in both ORAC value and phenolics retentions. Additionally, the highest temperature and

time combinations also had the lowest retentions compared to lower temperature and time combinations. One possible reason for the loss of phenolics during higher temperature and longest residence times is extraction. After processing, the depressurization step caused some juice to exit with the carbon dioxide. This juice loss could either caused by carbon dioxide becoming soluble in the juice or the carbon dioxide extracting the juice. No literature study supports either hypothesis. At the highest temperature, pressure, and residence time, the carbon dioxide is possibly becoming more soluble in the juice or extracting some of these compounds during the depressurization step (Murga et al., 2000). Heat effects can also be another reason for the observed results. Phenolic compounds in the muscadine juice were found to decrease at 60°C in the thermal processing chapter. The instability of the compounds during the supercritical carbon dioxide processing at 55°C could be therefore caused by the heating or from the extraction loss from the carbon dioxide.

3.7 Microbiological Inactivation Study

Filtered muscadine juice was used in this analysis. This study had 18 different processing conditions. Results can be found in **Figure 3.2.** Temperature and time had both significant contributions ($p<0.05$) on the log reduction of *Zygosacchromyces bailii*, while pressure was not significant. 55°C was found to have a significantly higher inactivation than 35°C and 30 min had significantly higher inactivation than 10 min. This result shows that when designing a supercritical process to inactivate organisms, pressure would not have a significant effect on microbial inactivation, as temperature had, with similar equipments and settings used in this study. The treatment with the highest temperature, pressure, and residence time (55°C, 34.5 MPa and 30 min), had a 3.85 log reduction, the greatest inactivation of all conditions. Of all the

conditions used in the microbial study, the 45°C, 27.6MPa, and 30 min observed the highest retention of ORAC values in the previous study. This condition produced a 2.54 log reduction.

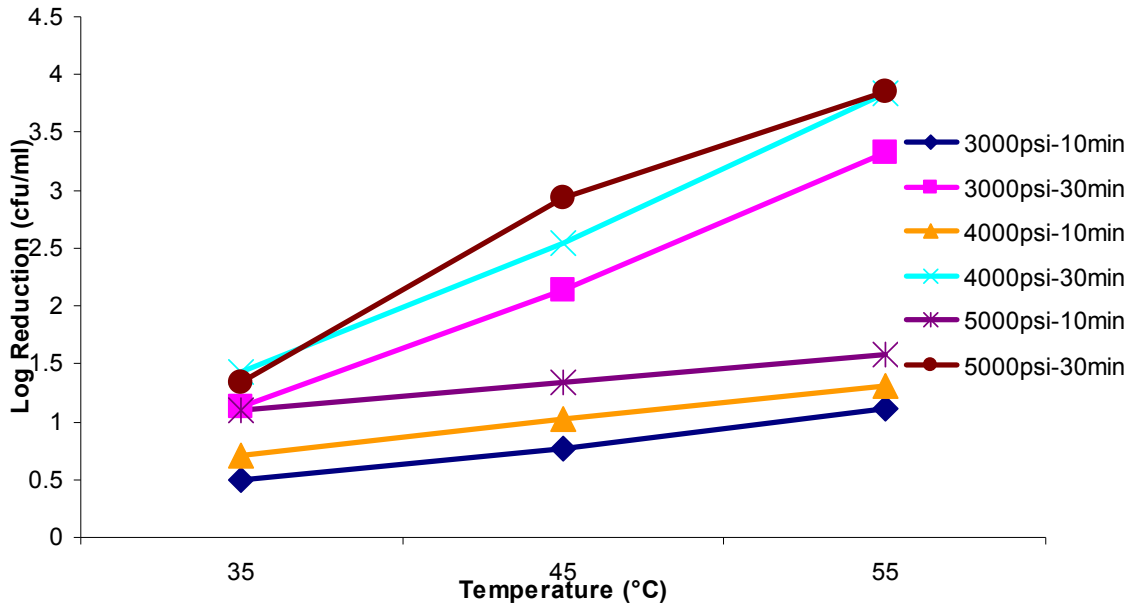

Figure 3.2. Microbiological inactivation with *Zygosacchromyces bailii* using supercritical processing.

4. Conclusions

This paper illustrates the effects of various supercritical carbon dioxide processing treatments on total phenolics and antioxidants. For both the muscadine and pomegranate juice studies, temperature and pressure were both found to be individually significant to the phenolics and antioxidant values during processing. 45°C and 27.6MPa were the two best operating conditions to retain antioxidants. Additionally, the interaction of time and temperature was also found to be significant. 45°C and times of 10 min and 20 min had the two highest antioxidant values after processing. These conditions in the study were both optimal for antioxidant and phenolics retention for muscadine and pomegranate juice.

The correlation study shows that phenolics and ORAC correlation were 0.801 and 0.814 for muscadine and pomegranate juices, respectively. These values were within the 0.77-0.93

correlation range found in literature for unprocessed juice. Therefore, supercritical processing does not have an effect on the structure to function relationship of phenolics.

The microbial inactivation study shows only temperature and time were individually significant. 55°C and 30 min had the greatest microbial inactivation of *Zygosacchromyces bailii*. These conditions produced a 3.85 log reduction. The condition of 45°C, 34.5MPa, and 30 min showed the overall best ORAC value result in pomegranate juice, having a 2.54 log reduction.

Overall, this supercritical carbon dioxide system was effective at ORAC value retention and microbial inactivation. The system used in this study was a small batch reactor. Continuous systems which can be more advantageous because of greater throughput could possibly display different behavior when processed at different temperatures, pressures, and residence times. The need to do similar research such as this one on larger and continuous systems is critical before industry sees this is as a viable technology for juice processing. Yet, the initial studies show promising results for this technology.

5. References

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CHAPTER 4

DISCUSSION AND CONCLUSIONS

1. Introduction

The antioxidant and phenolic retention results from the thermal processing and supercritical processing chapters are compared and analyzed in this discussion and conclusions section. The thermal processing chapter found that temperature and the interaction of time and temperature were significant factors affecting the phenolics and antioxidant retentions. In the supercritical processing chapter, also temperature and the interaction of time and temperature significantly affected the phenolics and antioxidant retention. Since both studies illustrated that temperature and time are both significant factors in the retention of compounds, this conclusion study grouped both supercritical and thermal processes by temperature and time. Then, all groupings were compared for phenolics and ORAC value retentions to see which processes were optimal at compound retention. Analysis of variance and means separations by Tukey's HSD ($p<0.05$) were conducted to determine significant difference between treatments using JMP Software (SAS Institute, Cary, NC).

In industrial practices, the most common condition for thermal processing is 80-85°C for a one minute holding time (Mazzotta, 2001). Since this is the standard process, all supercritical conditions and other thermal conditions with respect to phenolics and antioxidant retentions will be compared. Supercritical processing is an emerging nonthermal process that uses much lower temperatures than thermal treatments. It has been reported that lower temperatures used for nonthermal processes could lead to less phenolics and ORAC value degradation than thermal

treatments (Barbosa-Canovas, 1998). This overall comparison was performed to see which process and their conditions have the best overall retention of compounds. Filtered muscadine and pomegranate juice were used in the study.

2. Discussion

2.1 Muscadine Juice

2.1.1 Phenolics

Table 4.1 shows the phenolics values for muscadine juice, grouped by the temperature and time conditions of the processing treatments. The unprocessed sample had a phenolics value of 8.552 mg gallic acid equivalents/10ml. The treatment of 80°C and 1min, the current industry standard for thermal pasteurization, had a lower phenolics value than the unprocessed sample at 8.382mg gallic acid equivalents/10ml. This treatment resulted in a 2.0% reduction from the raw juice's phenolics. The supercritical processing conditions that had the highest phenolics retentions were the conditions of 45°C and 20 min/30 min. These values were 8.700 and 8.685 mg gallic acid equivalents/10ml, which were 1.7% higher than the unprocessed juice. Therefore, the supercritical processing had an increasing effect on the phenolics from the unprocessed sample while the thermal treatment had a reduction. Phenolics values of these supercritical and thermal treatments significantly differed ($p<0.05$) by greater than 5%.

A couple of studies found in literature showed the effects of thermal treatments on muscadine juice phenolics. Muscadine grape juice processed at 75°C for 15s decreased in phenolics by 26% (Del Pozo-Insfran et al., 2006). Other studies showed that thermal treatment increased phenolics, by 43% in one case (Auw et al., 1996; Lee and Talcott, 2002). Processing conditions were not mentioned. Thus, literature suggests inconclusive effects of heat on the

phenolics in muscadine juice. This study processed at 80°C and 1min showed minimal changes in muscadine juice phenolics.

Phenolic and antioxidant increases as a result of heat processing can be explained by the following. During thermal treatments, natural antioxidants such as polyphenols and ascorbic acid are consumed as reactants in the Maillard reaction. As heating time is prolonged, possible ORAC value enhancement could occur due to the formation of antioxidant Maillard reaction products (MRPs) including furfural and 5-hydroxy-methylfurfural (HMF) (Nicoli et al., 1999). The decrease in phenolics as a result of thermal processing can be explained by the following mechanism. Heat can lead to the degradation of lignins, releasing phenolic acids. This is the beginning steps to degradation of phenolic compounds (Maillard and Berset, 1995). Anthocyanin state changes can also produce higher or lower phenolics values depending on the states. For example, when anthocyanins change to a yellow fraction form as a result of heat, phenolics values increase (Tsai and Huang, 2004).

In **Figure 4.1**, the density, which is the distribution of muscadine phenolics values over the range of temperature and time combinations for both thermal and supercritical processing, is shown. This figure also shows the average phenolics values to be higher for supercritical processing than thermal processing, taking into account all conditions. Thermal processing showed a wider distribution of values, meaning higher and lower phenolics values were produced compared to supercritical processing. Therefore, the effect of specific processing conditions on phenolics did not differ as much for supercritical processing as for thermal processing. Additionally, thermal processing conditions at 60°C had the highest phenolics values compared to all the supercritical conditions, while the thermal processing conditions at

80°C had the lowest phenolics values compared to all other conditions. All discussed conditions are shown in bold and italicized.

Table 4.1. Muscadine juice thermal and supercritical phenolics

Treatment	Temperature (°C)	Time (min)	Phenolics (mg GAE/10ml)
Thermal	60	30	8.885 [a]
Thermal	60	20	8.856 [ab]
Thermal	60	1	8.823 [abc]
Supercritical	***45***	***20***	***8.700*** [***abcd***]
Thermal	60	10	8.689 [abcde]
Supercritical	***45***	***30***	***8.685*** [***abcde***]
Thermal	70	10	8.679 [abcdef]
Supercritical	50	10	8.650 [bcde]
Supercritical	35	30	8.639 [cde]
Supercritical	35	20	8.629 [cde]
Supercritical	45	10	8.628 [cde]
Thermal	35	10	8.624 [abcdefg]
Supercritical	35	10	8.575 [defg]
Thermal	50	20	8.570 [cdefghi]
Unprocessed			***8.552*** [***cdefghi***]
Supercritical	55	20	8.551 [defgh]
Thermal	70	30	8.526 [defghi]
Thermal	50	1	8.509 [defghi]
Thermal	70	1	8.499 [defghi]
Thermal	50	30	8.490 [defghi]
Thermal	70	20	8.473 [efghi]
Supercritical	55	30	8.467 [fghi]
Thermal	***80***	***1***	***8.382*** [***ghij***]
Thermal	80	20	8.348 [hij]
Thermal	80	30	8.329 [ij]
Thermal	80	10	8.195 [j]

Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

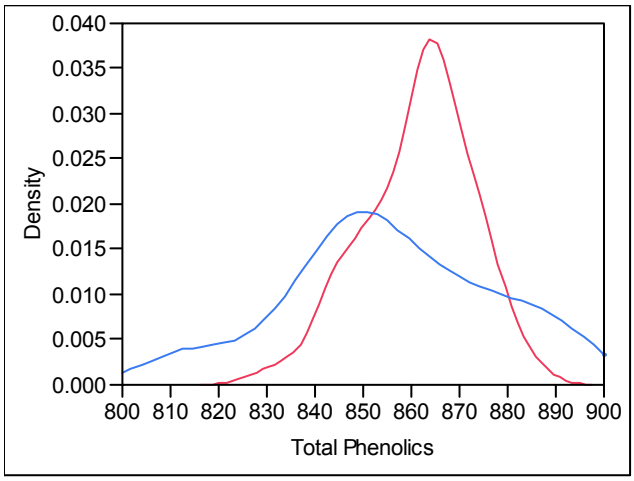

Figure 4.1. Distribution of muscadine phenolics values over range of temperature and time combinations. Red is supercritical processing and blue is thermal processing. Values are in mg GAE/L.

2.1.2 ORAC

ORAC had slightly different results than the phenolics. These results are shown in **Table 4.2**. The unprocessed sample had an ORAC value of 14.62µmoles Trolox equivalents/ml. The industry's thermal processing condition had a higher value of 14.92µmoles Trolox equivalents/ml. The supercritical conditions with the highest antioxidant retentions (45°C and 20/30 min) had values of 14.91 and 14.86µmoles Trolox equivalents/ml. These values show that this thermal processing condition increased the raw juice's ORAC values by 2.0%, while the supercritical processing conditions increased activity by 1.9%. Statistical analysis shows that no significant differences exist between these supercritical and the thermal conditions for antioxidant retention ($p<0.05$). Additionally, this thermal processing result had significantly higher antioxidant retention than the unprocessed sample, while the supercritical conditions were not statistically different.

Only one study found in literature showed the effects of heat treatment on antioxidants in muscadine juice. This study showed that processing at 75°C for 15s decreased ORAC values by

10% (Del Pozo-Insfran et al., 2006). This result is much different than the 2.0% increase found for the thermal treatment of 80°C and 1 minute in this study.

The thermal processing conditions at 60°C had the highest antioxidants while the thermal processing conditions at 80°C had the lowest. All supercritical antioxidant retentions were between these two thermal processing temperatures. According to **Figure 4.2**, thermal processing showed a wider distribution of values, meaning higher and lower phenolics values were produced compared to supercritical processing. Therefore, the effect of specific processing conditions on ORAC values did not differ as much for supercritical processing as for thermal processing. All discussed conditions are shown in bold and italicized.

Table 4.2. Muscadine juice thermal and supercritical ORAC

Treatment	Temperature (°C)	Time (min)	ORAC (µmoles Trolox equiv/ml)
Thermal	60	30	15.20 [a]
Thermal	60	20	15.01 [ab]
Thermal	60	1	14.94 [abc]
Thermal	***80***	***1***	***14.92*** [b]
Supercritical	***45***	***30***	***14.91*** [bcd]
Supercritical	***45***	***20***	***14.86*** [bcd]
Supercritical	55	10	14.84 [bcde]
Thermal	50	10	14.84 [bcde]
Thermal	60	10	14.82 [bcde]
Supercritical	45	10	14.82 [bcde]
Supercritical	35	20	14.81 [bcde]
Supercritical	35	30	14.79 [bcde]
Thermal	50	20	14.74 [bcdef]
Thermal	70	10	14.73 [bcdef]
Thermal	50	1	14.73 [cdef]
Supercritical	55	20	14.72 [cdef]
Supercritical	35	10	14.67 [ef]
Thermal	70	1	14.63 [def]
Thermal	70	30	14.63 [def]
Unprocessed			***14.62*** [def]
Supercritical	55	30	14.56 [f]
Thermal	70	20	14.53 [fg]
Thermal	80	30	14.52 [fg]
Thermal	80	20	14.28 [gh]
Thermal	50	30	14.25 [gh]
Thermal	80	10	14.20 [h]

Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

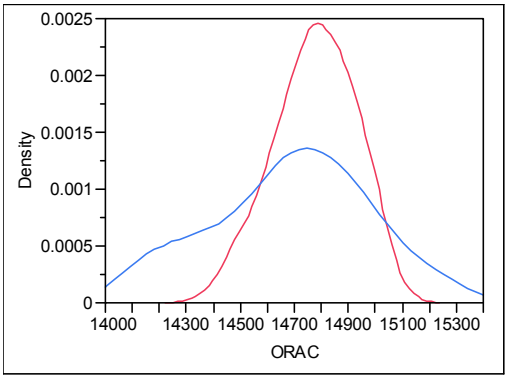

Figure 4.2. Distribution of muscadine antioxidant values over range of temperature and time combinations. Red is supercritical processing and blue is thermal processing. Values are in µmoles Trolox equiv/L

2.2 Pomegranate Juice

2.2.1 Phenolics

The unprocessed pomegranate juice had a phenolics value of 15.25 mg gallic acid equivalents/10ml. Similar to the muscadine juice study, the supercritical processing conditions with the highest phenolics retentions were 45°C and 20/30 min. These values were 1547.0 and 15.50 mg gallic acid equivalents/10ml. The industry thermal processing condition had a value of 15.09 mg gallic acid equivalents/10ml. All phenolics values are shown in **Table 4.3.** These values show that this thermal processing condition decreased the raw juice total phenolics by 1.0%, while the supercritical processing condition increased phenolics by 1.4%. Unlike the muscadine juice study, the higher phenolics retentions exhibited by the supercritical processing were not significantly greater than the thermal processing condition ($p<0.05$).

Studies in literature have found similar findings. Pomegranate juice that was processed at 95°C for 30s and hot filled at 90°C had a 2% decrease in phenolics (Perez-Vicente et al., 2002). In another study, pomegranate juice that was processed at 100°C for 20 minutes showed a 7.1% decrease in phenolics (Alper et al., 2005). These two literature studies show that higher processing temperature above industry's standard temperature of 85°C result in greater phenolics degradation. Therefore, the 1.0% loss in phenolics for the 80°C and 1min processing condition coincides with the findings in literature for thermal processing.

For pomegranate juice, the thermal processing condition that had the highest phenolics retentions were 70°C, while the lowest retentions occurred at 80°C. These were the highest and lowest retentions of all conditions including supercritical processing. According to **Figure 4.3**, thermal processing showed a wider distribution of values, meaning higher and lower phenolics values were produced compared to supercritical processing. Therefore, the effect of specific

processing conditions on phenolics did not differ as much for supercritical processing as for thermal processing. All discussed conditions are shown in bold and italicized.

Table 4.3 Pomegranate juice thermal and supercritical phenolics

Treatment	Temperature (°C)	Time (min)	Phenolics (mg GAE/10ml)
Thermal	70	1	15.64 [a]
Supercritical	***45***	***20***	***15.47*** [***ab***]
Supercritical	***45***	***30***	***15.46*** [***ab***]
Thermal	60	30	15.44 [abcde]
Thermal	70	30	15.41 [abcde]
Supercritical	55	10	15.41 [abc]
Supercritical	35	20	15.37 [abcd]
Thermal	70	20	15.37 [abcde]
Thermal	70	10	15.36 [abcde]
Thermal	60	1	15.34 [abcde]
Supercritical	35	30	15.34 [abcde]
Supercritical	45	10	15.33 [abcde]
Thermal	60	20	15.30 [abcde]
Thermal	60	10	15.29 [abcde]
Supercritical	55	20	15.29 [abcde]
Supercritical	35	10	15.28 [abcde]
Thermal	50	30	15.27 [abcde]
Unprocessed			***15.25*** [***abcde***]
Thermal	50	20	15.22 [abcde]
Thermal	50	10	15.13 [bcde]
Supercritical	55	30	15.11 [cde]
Thermal	50	1	15.11 [bcde]
Thermal	***80***	***1***	***15.09*** [***bcde***]
Thermal	80	10	15.00 [cde]
Thermal	80	30	14.99 [de]
Thermal	80	20	14.93 [e]

Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

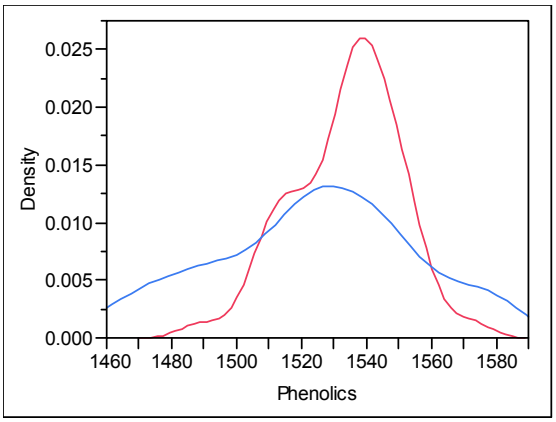

Figure 4.3. Distribution of pomegranate phenolics values over range of temperature and time combinations. Red is supercritical processing and blue is total thermal processing. Values are in mg GAE/L.

2.2.2 ORAC

The unprocessed pomegranate juice sample had an ORAC value of 15217.4µmoles Trolox equivalents/ml. The highest antioxidant retention with supercritical processing was found with the conditions of 45°C and 20 min. The ORAC value for this supercritical processed juice was 15395.2µmoles Trolox equivalents/ml. The industry's condition of thermal processing resulted in an ORAC value of 15050.5µmoles Trolox equivalents/ml. These values show that this thermal processing condition decreased the raw juice ORAC values by 1.2%, while this supercritical processing condition increased ORAC values by 1.2%. All pomegranate juice antioxidant data can be found in **Table 4.4**. This supercritical condition had a significantly higher antioxidant retention than the thermally processed sample ($p<0.05$) with a 2.2% higher value. Neither processing treatment was significantly different from the unprocessed sample.

One study that processed pomegranate juice thermally did not show similar findings for the ORAC retention. After pomegranate juice was processed at 95°C for 30s and hot filled at 90°C, antioxidant increased by 10% (Perez-Vicente et al., 2002). This is a much higher antioxidant retention result than the 1.2% decrease.

The thermally processed samples at 70°C had the highest antioxidant retentions, while the 50°C and 80°C had the lowest retentions of all treatments including supercritical carbon dioxide. According to **Figure 4.4**, thermal processing showed a wider distribution of values, meaning higher and lower phenolics values were produced compared to supercritical processing. Therefore, the effect of specific processing conditions on antioxidants did not differ as much for supercritical processing as for thermal processing. All discussed conditions are shown in bold and italicized.

Table 4.4. Pomegranate juice thermal and supercritical ORAC

Treatment	Temperature (°C)	Time (min)	ORAC (μmoles Trolox equiv/ml)
Thermal	70	30	15.54 [a]
Thermal	70	10	15.50 [ab]
Thermal	70	20	15.46 [ab]
Thermal	60	10	15.45 [ab]
Thermal	60	20	15.42 [abc]
Thermal	60	30	15.42 [abc]
Thermal	70	1	15.40 [abcd]
Supercritical	***45***	***20***	***15.40*** [***ab***]
Supercritical	45	10	15.39 [ab]
Supercritical	45	30	15.3460 [abc]
Supercritical	35	20	15.3402 [abc]
Supercritical	55	10	15.3320 [abcd]
Supercritical	35	30	15.3256 [abcde]
Supercritical	55	20	15.3015 [abcdef]
Supercritical	35	10	15.2890 [abcdefg]
Thermal	60	1	15.2686 [abcdefgh]
Supercritical	55	30	15.2381 [bcdefgh]
Unprocessed			***15.2174*** [***bcdefgh***]
Thermal	80	10	15.0806 [cdefgh]
Thermal	***80***	***1***	***15.0505*** [***defgh***]
Thermal	80	30	15.0403 [efgh]
Thermal	50	20	15.0354 [fgh]
Thermal	50	10	15.0077 [gh]
Thermal	50	1	15.0055 [gh]
Thermal	80	20	14.9893 [h]
Thermal	50	30	14.9854 [h]

Means with the same superscript letter are not significantly different at $p<0.05$ within a column.

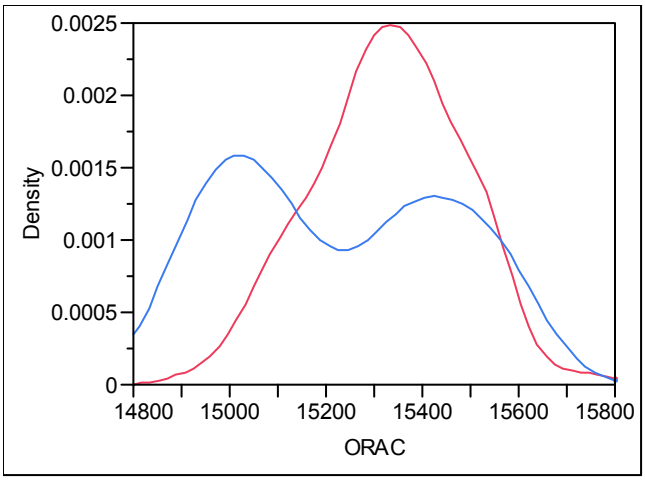

Figure 4.4. Distribution of pomegranate antioxidant values over range of temperature and time combinations. Red is supercritical processing and blue is total thermal processing.

2.3 Microbiological Inactivation

A comparison was made between the microbiological inactivation of *Zygosacchromyces bailii*, a yeast spoilage organism using both supercritical and thermal processing conditions. Both studies used filtered muscadine juice.

The thermal processing study showed that processing temperatures at 60°C and above resulted in complete inactivation of the organism. At 50°C, the D value for this organism was 5.04 minutes.

The supercritical conditions of 55°C, 34.5MPa, and 30 min had the greatest microbial inactivation of *Zygosacchromyces bailii*. These conditions produced a 3.85 log reduction. The condition of 45°C, 34.5MPa, and 30 min, which had the overall best ORAC value retention in pomegranate juice, resulted in a 2.54 log reduction. Overall, the supercritical processing did not have as much inactivation as the thermal processing conditions.

Since a 5 log reduction is required to pasteurize juices, this supercritical process must be combined with another process to approximately achieve the last 2 logs of inactivation.

2.4 Combining treatments and future work

As discussed in the phenolics and antioxidant studies of pomegranate and muscadine juice, supercritical processing had a positive impact on the retention of both the phenolics and ORAC values. Therefore, this process can be a viable process for industries to use to preserve nutrients while pasteurizing juices. However, this study shows that the complete log reduction cannot be achieved with only a supercritical process. Therefore, a combination treatment of supercritical processing with thermal processing could be a viable process combination for industrial pasteurization of juices.

No studies have shown the effects of combining supercritical processes and thermal processing for any type of processing. To determine the optimal process combination, anther study will be needed. However, if the individual effects of antioxidant and phenolics retention of the treatments are the same for a combined process than this combination could be useful. The supercritical condition of 45°C, 34.5MPa, and 30 min had increased phenolics retention of 1.4-1.6% in pomegranate juice and muscadine juice after processing. These conditions had a 1.1-1.9% increase in antioxidant retention in muscadine juice and pomegranate.

To have the best overall effect on the phenolics and antioxidant retention with the thermal processing part of the treatment, different conditions should be used on pomegranate and muscadine juice. For muscadine juice, a process of 60°C and 30 min will have the best overall effect on the compounds. In this study, these thermal conditions resulted in a 3.9-4.0% increase in muscadine phenolics and antioxidants after processing. However, a 30 minute residence time for juice processors lowers their throughput and increases their energy usage. Therefore, it may be recommended to use a process of 60°C and 1 minute. These conditions have lower phenolics retentions (2.1-3.1% increases) but the juice throughput would be higher and the energy usage would be lower.

For muscadine juice, 70°C and 30 minutes had the best overall effect on phenolics and antioxidants of all the thermal processing conditions. Yet, the energy consumption and the low throughput would not be beneficial for a juice company. Similarly, 70°C and 1 minute will still have a beneficial impact on the phenolics and antioxidants.

3. References

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APPENDIX

A.1 Pomegranate thermal processing phenolics ANOVA

Source	**P value**
Temperature	0.0003
Time	0.6364
Temperature*Time	0.9394

A.2 Pomegranate thermal processing phenolics mean comparison for temperature

Temp (°C)	Average value (mg GAE/10ml)
70	15.44 [a]
60	15.34.[ab]
50	15.18 [bc]
80	15.00 [c]

A.3 Pomegranate thermal processing ORAC ANOVA

Source	**p value**
Temperature	<.0001
Time	0.0471
Temperature*Time	0.1677

A.4 Pomegranate thermal processing ORAC mean comparison for temperature

Temp (°C)	Average value (μmoles Trolox equiv/ml)
70	15.47 [a]
60	15.38.[ab]
80	15.04 [bc]
50	15.00 [c]

A.5 Muscadine filtering before thermal processing phenolics ANOVA

Source	**p value**
Temperature	<.0001
Time	0.9838
Temperature*Time	0.0084

A.6 Muscadine filtering before thermal processing total phenolics mean comparison for temperature

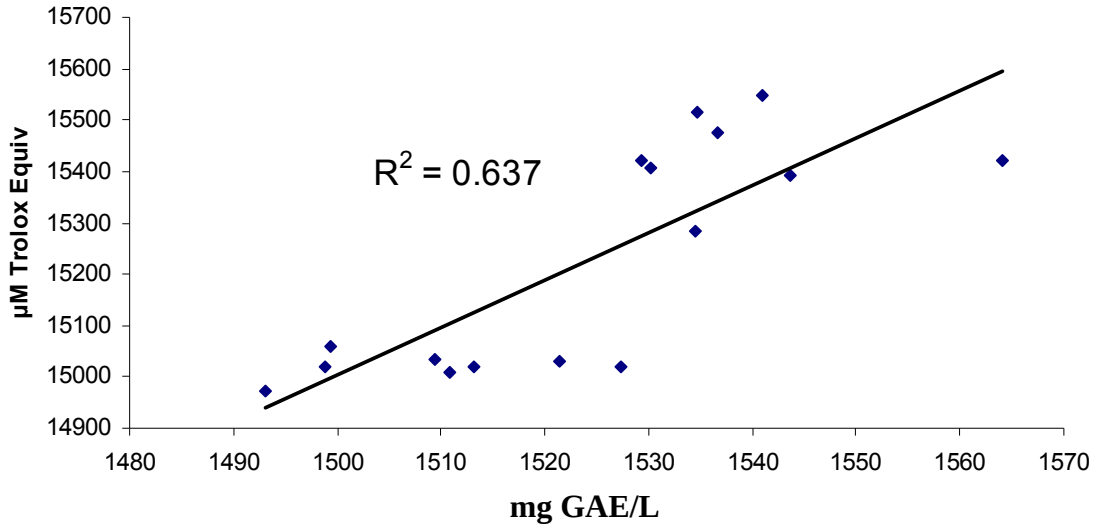
Temp (°C)	Average value (mg GAE/10ml)
60	8.814 [a]
50	8.548 [b]
70	8.544 [b]
80	8.314 [c]

A.7 Muscadine filtering before thermal processing ORAC ANOVA

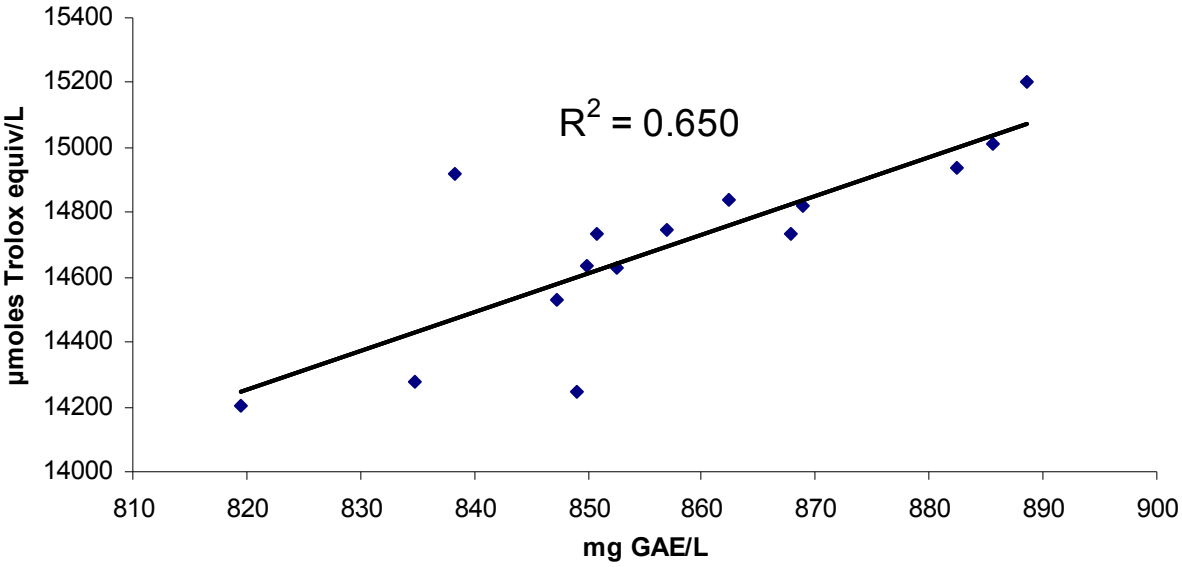
Source	**p value**
Temperature	<.0001
Time	0.9979
Temperature*Time	0.0078

A.8 Muscadine filtering before thermal processing ORAC mean comparison for temperature

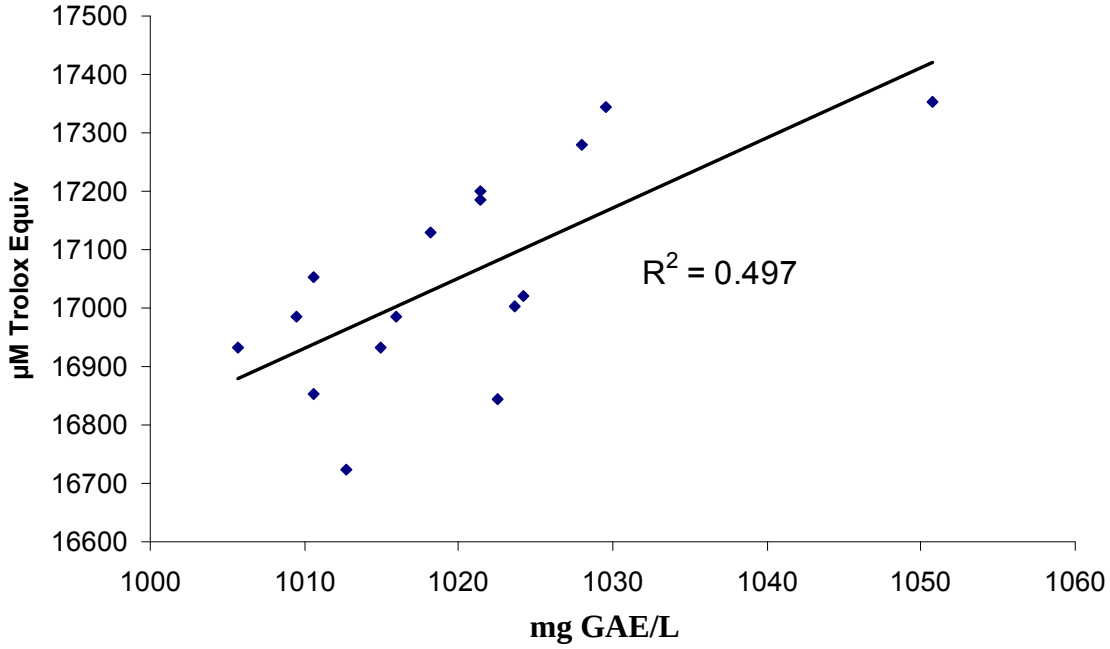
Temp (°C)	Average value (μmoles Trolox equiv/ml)
60	14.99 [a]
50	14.64 [b]
70	14.63 [b]
80	14.48 [c]



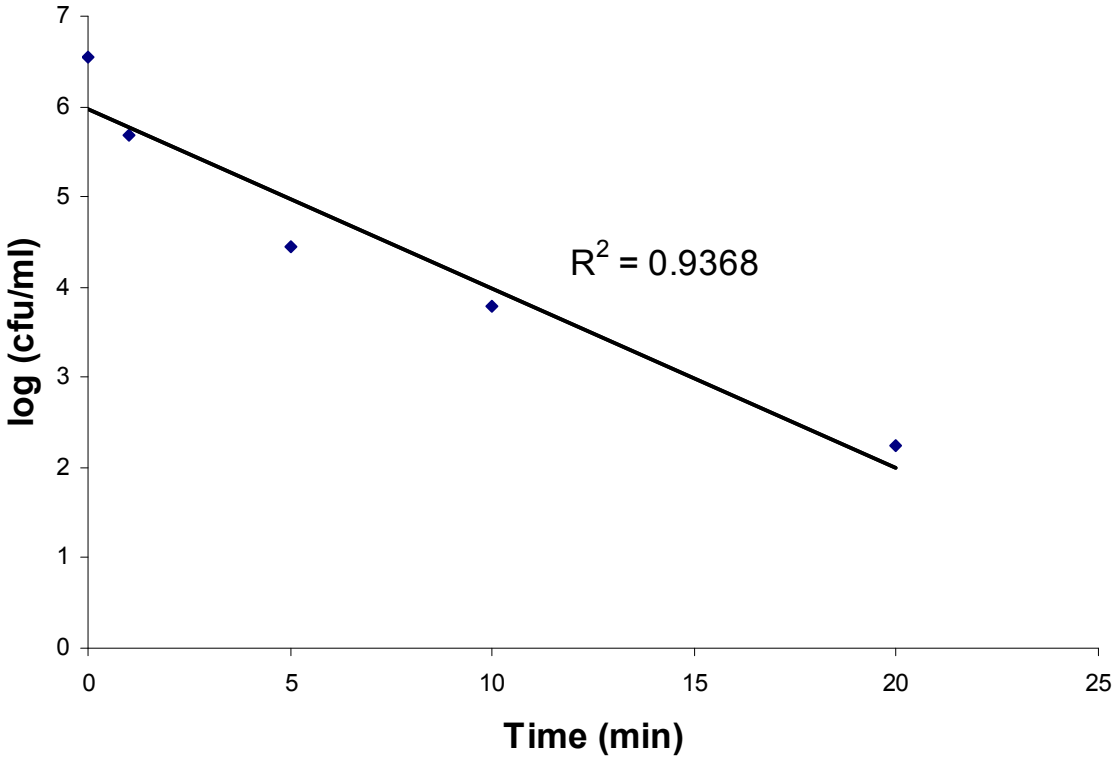
A.9 Pomegranate juice total phenolics and ORAC correlation after thermal processing. R^2 was 0.637.



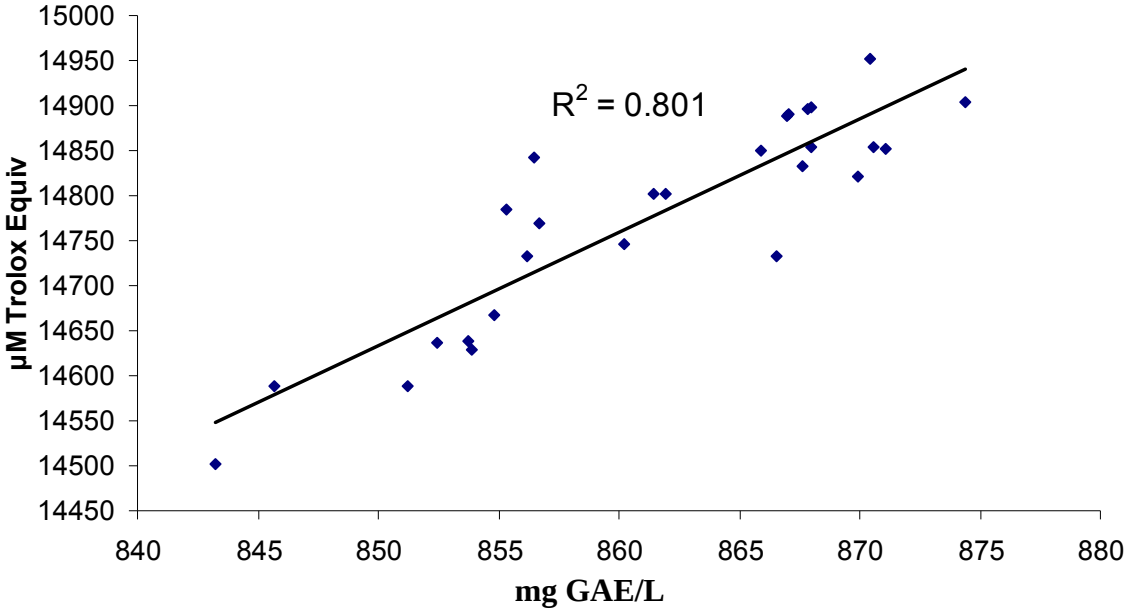
A.10 Muscadine juice filtering before thermal processing total phenolics and ORAC correlation.



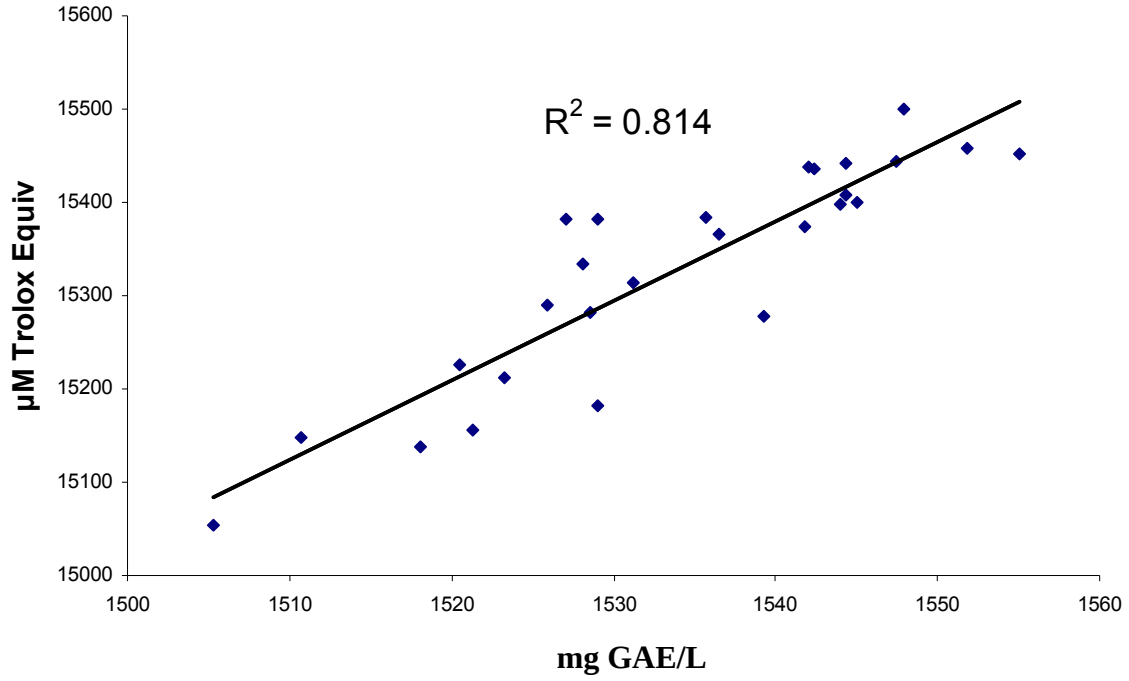
A.11 Muscadine juice filtering after thermal processing total phenolics and ORAC correlation.



A.12 Microbiological thermal inactivation study at 50°C. D value was calculated to be 5.04 min.



A.13 Muscadine juice total phenolics and ORAC correlation after supercritical processing



A.14 Pomegranate juice total phenolics and ORAC correlation after supercritical processing