ABSTRACT

DRAUT, AMANDA SCHELL. Changes in Bioactive Components of Muscadine Grapes During Microwave Processing and Storage. (Under the direction of Dr. Gabriel Keith Harris).

Muscadine grapes are native to the southeastern United States. Because of their short 6-8 week growing season, it is beneficial for growers to find a use for their produce that is an alternative to fresh sale. A whole muscadine grape puree was formulated to utilize waste products, muscadine skins and seeds in order to maximize its bioactive properties. While processing is required for safety and extended shelf life of such a product, it is desirable to do so by a method that would preserve the bioactive components present within the raw product. In order to investigate this, muscadine grapes were processed both in a model system at 95°C and in a continuous flow microwave system. Processed puree was stored at 37°C after both processes. Samples were analyzed for color on the L a b scale, total phenols by the Folin Ciocalteu assay (FC), total antioxidant capacity by the Oxygen Radical Absorbance assay (ORAC), total monomeric anthocyanins by the pH differential assay, and percent polymeric color by the index for color degradation and browning. Dielectric properties of the whole muscadine grape puree and of muscadine grape skins, pulp, and seeds were measured to assess appropriateness for a continuous flow microwave processing application.

In the model processing and storage system, grape purees were heated in a 95°C oil bath for 0, 3, 4, 5, and 6 minutes and then stored at 37°C for up to 8 weeks. After 6 minutes of processing, the Nesbitt and Triumph varieties exhibited 85.8% and 83.8% of their original (FC) values, and 106.1% and 105.1% of their ORAC values, respectively. After 8 weeks of storage at 37°C, Nesbitt and Triumph purees exhibited 88.6% and 110.6% of their original FC values and 83.3% and 77.5% of their original ORAC value, respectively. Monomeric
anthocyanin retention in Nesbitt grapes decreased with both processing (91.8%) and storage (18.3% from processed), while percent polymeric color retention decreased with processing (52.0%) and increased with storage (553.0%).

The dielectric constants generally decreased with increasing temperatures, whereas the dielectric loss factors increased with increasing temperature. Similarly, the loss tangent increased with increasing temperature and the depth of penetration decreased with increasing temperature. Results were similar among all varieties of whole muscadine grape puree. For the muscadine grape components, the seeds were different from the other parts, likely due to their lower water content. These results suggest that whole muscadine grape puree is compatible with microwave processing.

During the microwave processing study, both the Nesbitt and Frye varieties maintained high retention of total phenol content through grinding (115.9, 93.1%), microwave processing (140.7, 82.5%), and storage at 37°C for 4 weeks (100.4, 87.5%), respectively. These varieties also retained their antioxidant capacity through grinding (100.9, 90.7%), microwave processing (118.2, 100.4%), and storage (88.6, 61.1%). There were substantial decreases in total monomeric anthocyanins in the Nesbitt variety during grinding (68.8%), microwave processing (31.6%), and storage (38.4%). These results show that whole muscadine grape puree is a viable food matrix for microwave processing. This research suggests that whole muscadine grape puree has potential as a food product as processed by continuous flow microwave processing.
Changes in Bioactive Components of Muscadine Grapes During Microwave Processing and Storage

by
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DEDICATION

To Mom and Dad for your encouragement.

To Ryan for your constant support, love, and sanity.
BIOGRAPHY

Amanda Schell Draut was born on May 18, 1988 in Cincinnati, Ohio to Jim and Karen Draut. Throughout childhood, she dabbled in soccer, ballet, piano, swim team, flute, and choir before devoting herself to Arthurs Café for her last 2 years of high school. She graduated in 2005 from Walnut Hills High School. A Draut family culture that centered on food, a brief summer cooking class at Johnson and Wales University, and a chance encounter while taking the PSATs pointed her in the direction of food science as a possible future career. Her academic endeavors took her to Knoxville, Tennessee to follow her love of food and football at the University of Tennessee.

During her college career, she used her summers to travel and to gain experience whenever possible. These summers included teaching English at a summer camp at Tsinghua University in Beijing, China, traveling to learn about the agricultural practices and culture of Thailand, and an internship with Danisco Ingredients in New Century, Kansas, where she learned immeasurable amounts about the food industry and food matrices, including aiding in the development of a salt replacer that is currently in the Danisco product line. Throughout college, she worked in Marjorie Penfield’s food sensory lab. Amanda graduated in May of 2009 with a Bachelors of Science in Food Science with a Concentration in Science from the University of Tennessee and with a Certificate in Culinary Arts from the Culinary Institute at the University of Tennessee.

Ready for her next adventure, Amanda left immediately after graduation to complete an internship at ENITIAA in the Food Biochemistry lab in Nantes, France, studying the
conversion of lysine to furosine through Maillard Browning in bread. At the conclusion of
the internship, Amanda found herself in another new city, Raleigh, North Carolina, where
she started the crazy venture called graduate school. During this period, she acted as the
Program Coordinator for the Kannapolis Scholars program and as the Co-Chair of the Wine
and Cheese Committee and the Dairy Bar for the Food Science Club. Amanda hopes to work
in nutritionally-focused product development after graduation.
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CHAPTER 1 - Literature Review
1.1 Introduction

Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern United States. While the majority of muscadine grape production occurs in coastal states between Louisiana and North Carolina, there is over 1,000 acres of land devoted to the growth of these grapes in North Carolina alone. The North Carolina wine and grape industry contributes an annual economic impact of $1.28 billion. There are two types of muscadine grapes, “bronze” and “black”, that comprise over 300 varieties. In contrast to other grape species (*Vitis vinifera*), they do not grow in bunches, are much larger, have a thick, leathery skin, and hard, astringent seeds. These grapes can be attractive to growers and consumers alike for their disease resistance, anti-bacterial activity, potential health benefits, and unique flavor.

The disease resistance of muscadine grapes has made them uniquely fit for production in the southeastern United States in comparison to traditional bunch grapes. Muscadine grapes have a specific resistance to Pierce’s Disease, which is a common issue in *Vitis vinifera* grapes that are grown in the same region. Phenolic compounds, while thought to confer this disease resistance, also contribute to the anti-bacterial activity of muscadine grapes. Multiple studies have investigated the effect of muscadine grape components and extracts on pathogenicity. Many of these studies worked with gram negative microorganisms including *Eschericia coli* O157:H7, *Enterobacter sakazakii*, *Cronobacter sakazakii*, *Salmonella enteriditis*, and *Listeria monocytogenes*, pathogens that the food industry watches closely. The combination of disease resistance and anti-bacterial activity make muscadine grapes attractive because this results in potential for a higher yield in regions that...
are affected by issues such as Pierce’s Disease, but due to their low pH, there is no need for severe pasteurization procedures in order to yield a safe product.

In addition to their disease resistance and antimicrobial activity, muscadine grapes have potential to positively affect a wide variety of health issues including Alzheimer’s disease, inflammation, cancer, and diabetes. For example, consumption of muscadine grape wine prevented oligomerization of amyloid beta peptides in mice, which are associated with cognitive decline related to Alzheimer’s disease\(^22\). Additionally, anti-inflammatory effects were reported in one study, conducted in mice, indicating that muscadine grape extracts from skins and seeds attenuated inflammation when topically administered. There was an additive effect when utilizing the whole grape extract, suggesting that multiple components in various parts of the muscadine grape can reduce inflammation\(^23\). Similarly, muscadine grape skin extract suppressed edema in rats, displaying anti-inflammatory properties with possible mechanisms including the inhibition of cytokine and superoxide release\(^24\). Anti-inflammatory effects can be relevant to many chronic diseases such as cancer and diabetes.

Muscadine pomace has displayed anti-carcinogenic activity \textit{in vitro}\(^17\). One study in prostate cancer cell lines showed that muscadine skin extract induced apoptosis and decreased cell proliferation through phosphatidylinositol 3-kinase-Akt and mitogen-activated protein kinase survival pathways\(^18\). Muscadine grape extracts also attenuated growth of Caco-2 colon carcinoma cells, cell death correlating with presence of ellagic acid glycosides\(^19\). In another study involving Caco-2 cells and HT-29 cells, administration of crude muscadine extracts, purified phenol-rich muscadine extracts, and purified anthocyanin-rich extracts resulted in cell apoptosis, with anthocyanin-rich extracts displaying the most
potent activity\textsuperscript{12}. While results from these \textit{in vitro} studies are promising, it is essential to have positive effects \textit{in vivo} to implicate anti-carcinogenic activities of these grapes.

Muscadine grapes have a high sugar content that lends their characteristic sweetness, but also have displayed anti-diabetic activity. While the brix content of these grapes is highly variable, depending mostly on the weather and the variety, most muscadine grapes have a total soluble sugar content between 9 and 17 °Brix\textsuperscript{25}. Most of the sugars that comprise this total soluble sugar content are glucose and fructose, with some sucrose\textsuperscript{26}. Despite these high levels of sugar, studies have suggested that muscadine grapes can have anti-diabetic activity. One study found that application of muscadine grape extracts inhibited \(\alpha\)-glucosidase and pancreatic lipase \textit{in vitro}, enzymes implicated in diabetes\textsuperscript{20}. Extracts of muscadine grape and seeds have also prevented formation of advanced glycation end products, \textit{in vitro}, which have been associated with complications involving diabetes\textsuperscript{16}. In humans, nightly supplementation of 150 ml of muscadine wine and dealcoholized wine both comparably moderated blood glucose, glycated hemoglobin, and insulin in participants with and without Type II diabetes\textsuperscript{14}. Further evidence involving \textit{in vivo} studies would be required to understand a possible linkage between muscadine grapes and anti-diabetic activity.

The suggested link between muscadine grapes and the suppression of chronic diseases is promising. However, in all of the aforementioned studies, the administered samples came from whole muscadine grape components, as opposed to a single compound. These components are rich in phenols, antioxidant capacity, anthocyanins, and possibly stilbenes including resveratrol. Separately, studies have investigated the positive effects of these compounds on chronic disease. Therefore, it is logical to try to preserve these bioactive
components, until they may be ingested by consumers. While pasteurization or sterilization of muscadine grape juice or puree is required before distribution to consumers, it is important to observe this process and to try to preserve these components. Alternative processing techniques such as microwave processing, copigmentation, dense phase CO\textsubscript{2} processing, and high pressure processing have previously been studied with a goal to attenuate the destruction of these important compounds. This review will strive to understand the health effects of these bioactive components, the presence of these components in muscadine grapes, and the effects of processing and storage on bioactive components.

1.2 Muscadine Grape Production and Consumption

Consumption of muscadine grapes is currently limited mostly to the southeastern United States due to the short, 4-6 week growing season. This limitation has also been reflected in how the grapes are consumed. In a survey conducted in 2001, researchers found that 93.8% of respondents consumed the grapes in their fresh fruit form. The remaining percentages were attributed to wine, jam or jelly, frozen fruit, and juice.\textsuperscript{27} Consumption of fresh grapes also tends to result in waste products including the thick, leathery skin and the hard, astringent seeds. By weight, these parts account for over 27% of the grape, traditionally thought of as waste\textsuperscript{28}. Importantly, the skin and the seeds of the muscadine grape have the highest concentrations of phenols, antioxidant capacity, and anthocyanins, the compounds that likely deliver the grapes’ disease resistance, anti-bacterial activity, and potential health benefits\textsuperscript{29}. Inclusion of these components into a finished product can result in a value-added product by utilizing components that are normally thought of as waste\textsuperscript{30}. 

\textsuperscript{5}
Extraction of the bioactive compounds from the skins and seeds has been investigated for use as a supplement, but a water-based extraction resulted in 48-69% losses of phenols and 48-64% losses of anthocyanins\textsuperscript{31}. Supplements are currently being developed, using the skins and the seeds of the grape, but it is appropriate to try to discover ways to incorporate these muscadine grape components into a normal diet in order to deliver the full bioactive potential of the muscadine grape\textsuperscript{32}.

1.3 Muscadine Grape Compounds of Interest

1.3.1 Phenols

Phenols are a class of secondary metabolite compounds that are produced by plants as a stress response. These compounds include phenolic acids, flavonoids, flavonols, flavones, flavanones, flavanols, anthocyanins, isoflavones, and chalcones. Phenols have at least one hydroxyl group attached to an aromatic ring and are generally water soluble. The chemical structure of phenolic acids causes them to have a variety of functions including antioxidant activity, chelating of metal ions, and the formation of hydrogen bonds that can cause precipitation or an astringent taste. For plants, these compounds have a biological function to protect them against pests, disease, oxidation, and ultraviolet rays and to serve as attractants for pollinating insects. Phenols can also be beneficial for human health and are interesting to the food industry for their use as antimicrobials and antioxidants that can aid in the preservation of a food product\textsuperscript{33}. 


In muscadine grapes, the health effects of phenol-rich fractions have been investigated. Phenolic acid fractions of muscadine grapes resulted in a 50% inhibition of growth in HT-29 and Caco-2 cell lines at a concentration of 0.5-3 mg/ml in vitro\textsuperscript{12}. Ellagic acid-rich fractions of black muscadine grape extracts induced apoptosis in Caco-2 colon carcinoma cells in vitro\textsuperscript{15}. While the ellagic acid-rich fractions were effective against these cells, there is evidence to suggest that ellagic acid alone would not have the effect that a combination of phenolic acids would have. Another study investigated effects of ellagic acid, quercetin, and resveratrol upon human leukemia cells (MOLT-4) and found that while the separate compounds were effective in inhibiting cell growth, a synergistic effect occurred when the compounds were combined, displaying more than an additive effect\textsuperscript{34}.

The total phenol content of the component parts of the muscadine grape are reported in Table 1.1. The seeds have the highest total phenol content, followed by the skins, whole grape, and pulp. Muscadine grapes are rich in a variety of phenols including (-)-epicatechin, (+)-catechin, ellagic acid, myricetin, quercetin, gallic acid, and kaempferol (Table 1.2). While the whole muscadine puree includes all of these compounds, (-)-epicatechin, (+)-catechin, and gallic acid tend to be located in the seed, while ellagic acid, myricetin, quercetin, and kaempferol are located in the skins. Ellagic acid is an acid hydrolytic product of ellagitannin that was first identified in muscadine grapes after precipitation in clarified muscadine juice and wine\textsuperscript{35}. Other ellagic acid derivatives present in muscadine grapes include ellagitannin, epigallocatechin and digalloylglucose\textsuperscript{36}. 
**Table 1.1.** Total Phenol Content of Muscadine Grape Components. All values were determined using the Folin Ciocalteu Assay. GAE= gallic acid equivalents, FW= fresh weight muscadine, DW= dry weight muscadine

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Grape</td>
<td>1.69-4.25 mg GAE/g FW</td>
</tr>
<tr>
<td>Skin</td>
<td>2.62-5.46 mg GAE/g FW</td>
</tr>
<tr>
<td>Seed</td>
<td>15.36-32.59 mg GAE/g FW</td>
</tr>
<tr>
<td>Seed Powder</td>
<td>32.13 mg GAE/g DW</td>
</tr>
<tr>
<td>Pulp</td>
<td>0.12-0.33 mg GAE/g FW</td>
</tr>
<tr>
<td>Juice</td>
<td>0.33-1.28 mg GAE/ml</td>
</tr>
<tr>
<td>Wine</td>
<td>0.25-1.86 mg GAE/ml</td>
</tr>
</tbody>
</table>

**Table 1.2.** Phenol Content of Muscadine Grape Components. All values were determined using the HPLC. GAE= gallic acid equivalents, FW= fresh weight muscadine, DW= dry weight muscadine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-epicatechin</td>
<td>Whole</td>
<td>0.07-0.72 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>4.50-18.98 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed Powder</td>
<td>0.96 mg/g DW</td>
</tr>
<tr>
<td>(+)-catechin</td>
<td>Whole</td>
<td>0.05-0.86 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>3.20-14.25 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed Powder</td>
<td>0.12 mg/g DW</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>Whole</td>
<td>0.03-0.10 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>0.06-0.22 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Juice</td>
<td>&lt;0.01-0.10 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>&lt;0.01-0.01 mg/ml</td>
</tr>
<tr>
<td>Myricetin</td>
<td>Whole</td>
<td>&lt;0.01-0.01 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>0.02-0.20 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Juice</td>
<td>&lt;0.01-0.05 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>&lt;0.01-0.10 mg/ml</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Whole</td>
<td>&lt;0.01-0.02 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>0.01-0.04 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Juice</td>
<td>&lt;0.01-0.02 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>&lt;0.01-0.04 mg/ml</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Whole</td>
<td>&lt;0.01-0.01 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>&lt;0.01-0.03 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Juice</td>
<td>&lt;0.01-0.03 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>&lt;0.01-0.03 mg/ml</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Whole</td>
<td>&lt;0.01-0.01 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.02-0.12 mg/g FW</td>
</tr>
<tr>
<td></td>
<td>Seed Powder</td>
<td>0.99 mg/g DW</td>
</tr>
</tbody>
</table>
In previous research, studies have shown that phenols tend to be relatively stable to processing and storage. One study found a decrease in total soluble phenolics after 60 days of storage at 20°C and 37°C in muscadine juice and wine. Samples that were stored at 37°C generally had lower levels of phenols than the samples that were stored at 20°C, by the end of the 60 day storage period. In green tea, catechins including (-)-epigallocatechin gallate and (-)-epicatechin gallate followed a first order degradation pattern. There is evidence to suggest that the presence of lactic acid bacteria can degrade some phenolics, such as tannic acid wherein high molecular weight tannins can be depolymerized and lower molecular weight tannins can be decreased, resulting in an increased gallic acid concentration. In cell culture, application of lactic acid bacteria has resulted in gallic acid degradation. Because of the wide variety of phenols, more research would be required to fully understand any mechanisms of phenol degradation during processing or storage.

The bioavailability of phenols has been studied. In one *in vitro* study, codigestion of raspberry phenol extracts with ice cream and breakfast cereal resulted in a lower uptake, while codigestion with bread and minced beef did not, suggesting that food matrix can be very important for the bioavailability of a compound. Due to the wide variety of phenols that exist, it would be beneficial for more research to be completed regarding these specific groups of compounds.

1.3.2 Antioxidant Capacity

Phenol content is highly correlated with antioxidant capacity. Antioxidant capacity is a measure of the ability to prevent oxygen radical formation and to quench pre-formed
oxygen radicals. Oxygen radicals are molecules that are in an unstable state and can transfer
an electron from itself to other molecules. This transfer of electrons can result in a chain
reaction that leads to off flavors in foods, cell death, and inactivation of certain molecules.
Antioxidants serve as reducing agents that can accept the free electron, but in a more stable
state than would occur in other molecules. It is common in the food industry for synthetic
antioxidants (such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and
tert-Butylhydroquinone (TBHQ)) and natural antioxidants (such as ascorbic acid and
tocopherols) to be used in order to prevent oxidation that can lead to off-flavors\textsuperscript{43}. In
addition to the benefits of flavor preservation, antioxidants have been used in the food
industry to appeal to a consumer’s desire to purchase healthy foods\textsuperscript{44}.

In muscadine grapes, there is an average antioxidant capacity of 17.6±7.1 µM trolox
equivalents /g fresh weight muscadine. Similar to phenols, the highest concentration of
antioxidant capacity is in the seeds, followed by the skin, whole fruit, and then the pulp\textsuperscript{29}.
The predominant compounds that lend antioxidant capacity to muscadine grapes include
phenolic compounds, especially anthocyanins for the black varieties. Because of this,
antioxidant capacity tends to be positively correlated with both total phenol content and total
monomeric anthocyanin content.

In order to understand antioxidant capacity of a finished food product, it is essential
to investigate its degradation patterns. Studies have generally found that antioxidants follow
a pseudo first-order degradation pattern in applications such as dried apples and vitamin C in
citrus juice\textsuperscript{45,46}. The antioxidant degradation in dried apples was also dependent upon water
activity, with apples with higher water activity undergoing faster antioxidant degradation. In
one study, muscadine wine had a higher antioxidant capacity with storage time than muscadine juice. The antioxidant capacity also tended to be more dependent upon storage time than upon storage temperature (37°C and 20°C) after 60 days, but all of the 37°C samples decreased, while only one of the 20°C decreased after the given time period\textsuperscript{37}. Antioxidant capacity has been a trendy topic in food marketing in previous years, but phenols and more specifically anthocyanins have received growing attention recently.

1.3.3 Anthocyanins

Anthocyanins are natural compounds that are found in black varieties of muscadine grapes and are useful in terms of health and food quality. Anthocyanins are natural flavonoid pigments that are found in plants as part of the class of secondary metabolites and are considered to be phenolic compounds. The main structure of an anthocyanin is made up of an A ring that is attached to a pyrilium ring, which is then linked to a B phenolic ring (Figure 1.1). The biological purpose of anthocyanins is as a pigment in the range of red to blue, which can lend color in the grape and can also act as a shield against ultraviolet radiation as a benefit to the plant.

The structure of anthocyanins allows for a pH-dependent color change (Figure 1.2). The red color occurs due to the presence of conjugated double bonds when the structure is in its flavium cation form at a pH of <2 and the blue color occurs in the quinonoidal base form at a pH >6. Due to recent concerns about a possible connection between artificial colorants and hyperactivity in children, the food industry has been increasingly interested in the use of
natural colorants, such as anthocyanins\textsuperscript{47}. In addition to their practical purpose as colorants for the food industry, anthocyanins have been correlated with health promoting properties relating to cancer, heart disease and diabetes. While positive health effects of anthocyanins have been reported \textit{in vitro} and in animal models, there is limited evidence in humans to understand any possible connections, especially with respect to dosage\textsuperscript{48}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{anthocyanin_structure.png}
\caption{Structure of an Anthocyanin\textsuperscript{50}}
\end{figure}
Anthocyanins are the main contributors to the purple color in the black varieties of muscadine grapes and are predominantly located in the skin. Previously reported values for total anthocyanin content of muscadine grapes include 4.1 mg/g dry weight in the Jumbo variety, with the vast majority occurring in the skins\textsuperscript{51}. The major anthocyanins that have been identified in the muscadine grape include delphinidin-3,5-diglucoside (1.6 mg/g dry weight), cyanidin-3,5-diglucoside (0.3 mg/g dry weight), petunidin-3,5-diglucoside (0.6 mg/g...
dry weight), peonidin-3,5-diglucoside (0.1 mg/g dry weight), and malvidin-3,5-diglucoside (0.2 mg/g dry weight) in the Jumbo variety\textsuperscript{51}. The juice and wine of the Noble variety contains 10.6-606 mg/L delphinidin, 20.2-441 mg/L cyanidin, 9.9-797 mg/L petunidin, 12.0-48.4 mg/L pelargonidin, 32.1-518 mg/L peonidin, and 24.2-526 mg/L malvidin.

In order to preserve these compounds, it is important to understand their degradation patterns. Anthocyanins undergo first order degradation, reported in applications such as blood oranges, black current, strawberry paste, blackberry juice, mangosteen peel, blueberry juice, purple potato, and grape juice, with results depending on °Brix in some cases\textsuperscript{53-60}. Heat-treated blackberry juice (95°C for 3 minutes, hot filled) decreased in total monomeric anthocyanins (up to 65%) and increased in percent polymeric color (7%)\textsuperscript{61}. Anthocyanin degradation increases exponentially at temperatures above 125°C, wherein they are broken down into their aglycone forms and then further degraded\textsuperscript{50}.

During storage anthocyanins degrade, negatively correlating with storage time, as reported in a variety of fruit juices\textsuperscript{62}. Stability of anthocyanins in muscadine grape juice and wine has been studied. Anthocyanin content of decreased in both the juices and wines after 60 days of storage at 20°C and 37°C, a higher temperature generally causing more anthocyanin degradation. The greatest anthocyanin losses occurred in delphinidin-3,5-diglucoside and petunidin-3,5-diglucoside\textsuperscript{37}. Another study showed the negative effect of heating upon anthocyanins, most notably in delphinidin-3,5-diglucoside and petunidin-3,5-diglucoside\textsuperscript{63}. The total anthocyanin content of muscadine grape juice decreased logarithmically when heated at 21, 46, 71, 96, and 121°C for 0-360 min. The temperature had a greater influence upon the anthocyanin content than the process time (Figure 1.3)\textsuperscript{64}. 

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After storage of blackberry juice at 25°C for 6 months, total monomeric anthocyanins decreased by 69-75% and percent polymeric color increased from 14.4-36.2%\(^1\).

**Figure 1.3.** Anthocyanin Degradation vs. Time at Various Temperatures\(^6^4\)

Changes to anthocyanins of muscadine grapes during processing and storage can occur in two main forms: the polymerization of monomeric anthocyanin molecules with other anthocyanin or catechin molecules and the degradation of the anthocyanin into its constituent components\(^6^5\). The polymerization of anthocyanins can lead to a brown color, while the degradation of anthocyanins can lead to a lightening in color. These changes are dependent upon a myriad of conditions including sugar content, water activity, pH, and temperature.

Changes in anthocyanins during heating and storage can be highly dependent upon pH\(^6^6\).\(^6^7\). In separate studies using strawberry, elderberry, and black carrot anthocyanin-rich concentrates, at pH 1.0 and pH 3.5, anthocyanin degradation was traced throughout heating
for 6 hours at 95°C. Most notably, the half lives of the anthocyanins at a pH of 1.0 were much longer than those at a pH of 3.5. At a pH of 1.0, anthocyanin glycosides were first deglycosylated, before the A and the B rings were split into their phenolic acid and aldehyde constituents (Figure 1.4). In contrast, samples that were heated at a pH of 3.5 first developed into their chalcone glycoside form, with the opening of the pyrillium ring before deglycosylation and degradation into its phenolic acid and aldehyde constituents (Figure 1.5). The chalcone form of the anthocyanin is highly unstable, readily breaking into its constituents that have an overall lower antioxidant value. Because pH 3.5 is within the pH range of most juices, including muscadine grapes, this is likely a predominant mechanism for the degradation of anthocyanins during processing.

Figure 1.4. Anthocyanin Degradation at pH 1.0
While it is extremely important to understand how anthocyanins are degraded in order to try to better preserve them, it is also important to understand how anthocyanins might work in the human body, especially through their bioavailability. Foods that are eaten with a certain compound of interest have the ability to affect its absorption, but digestion of anthocyanin extracts from raspberries was not affected \textit{in vitro} by codigestion\textsuperscript{41}. The main location of anthocyanin absorption is in the small intestine, with aglycones more easily diffusing due to their smaller size\textsuperscript{68}. Unabsorbed anthocyanins may then travel to the colon where they are fermented by colonic microflora and their phenolic acid metabolites may be absorbed\textsuperscript{48}. More studies need to be conducted in order to fully understand how different anthocyanins and anthocyanin metabolites may be absorbed and how that could be affected by outside traits.
1.3.4 Resveratrol

While the presence and concentration of resveratrol in muscadine grapes has been a point of some contention, background regarding resveratrol and studies relating to resveratrol will be discussed. Resveratrol (3, 4, 5-trihydroxy-trans-stilbene) is a common component of several plants (Figure 1.6). Resveratrol is a type of agylcone stilbene, which is formed as a secondary product in plants and trees, having anti-fungal properties which result in a phytoalexin quality. In their natural forms, stilbenes are often difficult to recover from their plant sources to make a pure and high quality extract. It is important to note that resveratrol, in its natural form, will vary not only in food source, but also in the quality of that specific source from plant to plant. Factors such as cultivar, growing conditions, growing season, disease prevalence, and time of exposure to stress will all affect the amount of resveratrol in a plant.

![Figure 1.6. Structure of trans-resveratrol](image)

Both resveratrol on its own and muscadine grape extracts have been shown to induce apoptosis in carcinogenic cells, but this activity occurred through separate mechanisms. One study found that muscadine grape skin extract targeted the phosphatidylinositol 3-kinase-Akt
and mitogen-activated protein kinase survival pathways while resveratrol stopped cells at the G1-S phase transition and decreased expression of cyclin D1 and cyclin-dependent kinase 4 proteins in prostate cancer cell lines in vitro. This suggests that there is something beyond the possible stilbene content of muscadine grapes that may lead to its anti-carcinogenic activity.

Literature reporting on the content of resveratrol and its derivatives, including its glucoside form, piceid, in muscadine grapes varies greatly, with some researchers reporting copious amounts of resveratrol and others reporting none. One study found resveratrol in levels between 4.8 and 26.4 mg/g berry in the whole fruit, 2.7-23.5 mg/g berry in the fruit without the seeds, and 27.4-62.2 mg/g seed in the seeds. Values reported for the black varieties were higher than those of the bronze varieties. In other studies, researchers have found trans-resveratrol to be only at detection limits or not detected completely. Stilbene content of muscadine juice, including resveratrol, piceid, and their isomers were found to be present in the Noble and Carlos varieties, with extraction best with a hot press method or with cold press from frozen and then thawed berries. A possible explanation for the wide range of reported values is that resveratrol is produced as a stress-response compound, meaning that the external conditions and/or weather need to cause the plant stress in order for these compounds to be produced.

When making a product such as muscadine juice or wine, there are several processing options that could be used to separate the liquid phase from the solid phase. These processing parameters have been shown to affect the trans-resveratrol content of muscadine juice, with a hot press method creating detection limits of the compound and the cold press
methods not having any detectable trans-resveratrol\textsuperscript{72}. One study found that grape extracts rich in resveratrol and piceid were stable for 4 years at 40°C without exposure to light\textsuperscript{74}. However, in the presence of light, trans-resveratrol is converted to cis-resveratrol, which is an unstable form and has a different effect on the body\textsuperscript{75}. Absorption of resveratrol has been reported at about 70% of oral dosage (25mg), but most of the dosage was excreted through the urine\textsuperscript{76}. More research is required to understand if there are, indeed, consistent stilbene levels in muscadine grapes and how these stilbenes might affect health.

1.4 Processing of Muscadine Grape Puree

With occurrence of remaining product after saturation of the market during the short growing season, there is an opportunity for products made from frozen muscadine grapes. One such product that could be made to capitalize on the potential of the muscadine grape is a whole muscadine grape puree, designed to utilize the leftover yield, and the potential waste products that can result in a higher total phenol content, antioxidant capacity, and total anthocyanin content. This product would have the potential to be marketed to people across the United States, or even the world. In addition, the sweetness and the distinctive flavor of the muscadine grape would mask the astringency that is contributed by the seeds.

In comparison to a comparable juice product, whole muscadine grapes contain more total phenols, antioxidant capacity, and total anthocyanins\textsuperscript{77}. The component parts of the muscadine grape also have unique bioactive compounds. The seeds account for 87.1% of phenolic compounds, including hydrolyzable tannins, flavan-3-ols and condensed tannins, ellagic acid derivatives, and quercetin rhamnoside. The skin of the muscadine grape
accounts for 11.3% of phenolic compounds including hydrolyzable tannins, flavonoids, including anthocyanin 3,5-diglucosides, quercetin, myricetin, and kaempferol glycosides. The combination of the component muscadine parts would create a cocktail of these bioactive compounds that could benefit health and food quality. The whole muscadine grape puree can be used as an ingredient to be added to final products including smoothies, ice cream, and popsicles.

While a whole muscadine grape puree can best utilize the bioactive potential of the muscadine grape, there is an FDA mandated requirement of pasteurization in order to confer a 5-log reduction of pathogenic bacteria in juice products and purees. Further, a sterilization process is a harsher processing treatment, but could be desirable in order to create a shelf stable product. It is therefore important to understand what changes might occur to the bioactive components of the muscadine grapes during processing and to try to preserve these compounds until they may reach the consumer. While the above pasteurization procedure is appropriate for a refrigerated product, a sterilization procedure is more appropriate for the muscadine grape puree. Sterilization allows for the product to be shelf stable, which aids in transportation and storage costs, facilitating addition of this raw ingredient to finished food products. While the most important characteristic of a food product is its safety, it is still essential to deliver the bioactive components, which are a main intrigue of this value-added product.
1.5 Alternative Processing for Bioactive Component Retention

Alternative forms of processing have been investigated for their potential to preserve flavor and nutrients that are characteristic of the raw material, while still resulting in the necessary microbial death. Several processing techniques have been utilized for this purpose including high pressure processing, dense phase CO₂ processing, copigmentation, and continuous flow microwave processing.

1.5.1 High Pressure Processing

High pressure processing has been employed to improve bioactive compound stability in muscadine grapes. This form of processing uses intense pressure in order to extend shelf life through the destruction of pathogens and spoilage microorganisms. With high hydrostatic pressure processing in muscadine grapes, greater anthocyanin and antioxidant activity losses occurred at 400 MPa (70% loss) when compared to treatment at 550 MPa (46% loss). The drawback to this form of processing was that it failed to inactivate polyphenol oxidase, suggesting that a blanching step could be required\(^8^0\). In contrast to these findings, a study conducted in strawberries indicated that high pressure processing between 400 and 600 MPa can inactivate polyphenol oxidase and preserve phenol, anthocyanins, and color\(^8^1\). It is also essential for the matrix of the product to be such that it would not be negatively affected by use of elevated levels of pressure.
1.5.2 Dense Phase CO₂ Processing

Another alternative to traditional processing, dense phase CO₂ processing, has also been applied in muscadine grapes. This form of processing uses pressure and CO₂ to inactivate pathogens and spoilage microorganisms. In comparison to traditional thermal pasteurization, dense phase CO₂ processing better preserved phenols, antioxidant capacity, anthocyanins, and ascorbic acid through processing and storage. A higher level of CO₂ (16%) was more effective in preventing bioactive degradation than the samples that were treated with the lower level of CO₂ (8%). Dense phase CO₂ processing incurs a comparable flavor to unprocessed samples, but samples processed by traditional thermal pasteurization had lower hedonic scores during sensory testing²²-²⁴.

1.5.3 Copigmentation

Copigmentation involves the addition of colorless flavonoids in order to complex with anthocyanins to aid in their preservation. This technique has been studied using rosemary and thyme polyphenolic extracts in muscadine grapes, reducing phytochemical losses during high hydrostatic pressure processing and storage²⁰. Copigmentation in samples processed through traditional thermal pasteurization and dense phase CO₂ processing also better preserved the bioactive components of the muscadine grape juice, while resulting in a product with better organoleptic scores²². The addition of red clover extract at a 1:8 ratio was also effective in color and anthocyanin stability over storage²⁵. Addition of ascorbic acid, however, was found to decrease monomeric anthocyanin stability²⁶. This occurrence
could be attributed to the oxidation of ascorbic acid into $\text{H}_2\text{O}_2$ constituents that then react with anthocyanins to decrease their stability\textsuperscript{87}.

### 1.5.4 Continuous Flow Microwave Processing

Microwave processing is a promising technique that has not yet been employed in muscadine grapes. All microwave technology utilizes the same concept, whether it is in a home-use microwave or on an industrial scale. Microwave processing is a method of heating, whereby microwaves are sent through a given sample, exciting the polar molecules within the sample. These polar molecules then rotate, resulting in friction that creates heat. The major difference between the two types of microwave systems is the frequency used, with in-home microwaves utilizing a microwave frequency of about 2450 MHz, while industrial microwaves tend to be run at a frequency of 915 MHz, allowing for more even heating.

The heat created by the microwaves serves to process the samples, but is a very efficient form of energy transfer that requires a much shorter ‘come up’ time (the time it takes to reach the preferred processing temperature) than traditional processing. In contrast to traditional heat processing, which heats from the outside of a sample towards the inside, microwave processing can generate heat wherever polar molecules exist within a matrix. This results in a much more efficient process that can minimize overcooking of a sample, while still resulting in the same level of safety. This technology has the potential of better nutrient and flavor preservation than traditional forms of processing.
Microwave processing on an industrial scale can be done in either a batch system or a continuous flow system. Both of these types of systems are comprised of a microwave source, a waveguide, and an applicator. In an industrial food production system, the microwave source is usually a magnetron. The magnetron would then be attached to a metal wave guide that would direct the microwaves to go towards the applicator, where the microwaves would reach a sample (Figure 1.7). In order to understand if a given sample would be appropriate for use in an industrial microwave, it is essential to understand its dielectric properties, which characterize that particular material’s ability to reflect, transmit, or absorb the microwave energy.\textsuperscript{89}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{continuous_flow_microwave_system.png}
\caption{Simplified Representation of Continuous Flow Microwave Processing System.\textsuperscript{88}}
\end{figure}
Continuous flow microwave processing has been successfully used in a variety of systems including apple juice and sweet potatoes, but efficient microwave processing can be highly dependent upon a given food matrix, parameters that can partially be quantified by the measurement of dielectric properties\textsuperscript{90-92}. These properties can be broken into two categories: dielectric constant ($\varepsilon'$), being efficiency of energy storage, and dielectric loss factor, being the ability to transfer electromagnetic energy into heat energy and the energy that is dissipated in the system ($\varepsilon''$). Interactions between the dielectric properties and a sample can be described using the loss tangent, utilizing both the dielectric constant and the dielectric loss factor (Equation 1). In addition, depth of penetration, the distance (m) required to dissipate 63\% of the microwave energy, can be calculated using these measures (Equation 2). This calculation can be used to understand the potential to achieve uniformity in processing a sample\textsuperscript{93}.

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'}$$ \hspace{1cm} [1]

$$d_p = \frac{c}{2\pi f \sqrt{2 \varepsilon' \left[ 1 + \sqrt{\left(\frac{\varepsilon''}{\varepsilon'}\right)^2 - 1} \right]}}$$ \hspace{1cm} [2]

The dielectric properties of grape juice and wine (Vitis vinifera) have been studied at a temperature of 20°C between 200MHz and 3Gz, finding that at a given frequency, wine might have a higher dielectric constant than juice, while at another frequency there may be an opposite effect\textsuperscript{94}. The frequency is essential for understanding dielectric properties, with smaller frequencies having a higher dielectric constant and a lower dielectric loss factor than larger frequencies\textsuperscript{95}. Temperature of the sample has a similar effect, with higher temperatures resulting in a lower dielectric constant and higher dielectric loss factor than
lower temperatures. The industry standard frequency of 915 MHz has been chosen in order to improve the coupling of microwave energy dependent on these dielectric properties, resulting in a safe product with superior food quality.

Microwave processing results in a microbialy safe product, while yielding higher levels of bioactive components and better flavor preservation than occurs with traditional processing. In a study conducted using apple juice, continuous flow microwave pasteurization resulted in a more efficient microbial destruction than traditional heat pasteurization\(^9\). Microwave processing can be effective in inactivating polyphenol oxidase in strawberry puree, while only minutely modifying anthocyanin concentrations\(^9\). This indicates that it is possible for microwave processing to attenuate the action of polyphenol oxidase, effectively reducing one threat to stability of anthocyanins and other phenols. In red cauliflower, microwave processed samples had a much higher retention of anthocyanins (95.4\%) than the traditionally heat treated samples (19.8\%)\(^9\). Microwave processing also resulted in preservation of quality parameters in purple sweet potato puree including an increase in total phenol content by 5.9\%, a decrease in monomeric anthocyanin content by 14.5\% and relative stability of antioxidant capacity\(^9\). Continuous flow microwave processing has been shown to have the potential for the preservation of quality parameters, but has not yet been tested in a muscadine grape application.

1.6 Conclusion

Muscadine grape puree has potential as a shelf-stable value-added product that can be used as an ingredient in producing finished products such as smoothies, ice cream, and
popsicles. By utilizing the whole muscadine puree, bioactive components that are naturally occurring within the muscadine grape including phenols, antioxidant capacity, and anthocyanins, may be incorporated to benefit the health of the consumer and product quality in terms of spoilage and safety, flavor, and color. In order to make the most of these labile bioactive components, it is appropriate to utilize alternative processing techniques to avoid overheating and phytochemical degradation. Continuous flow microwave processing is a promising technique that can minimize bioactive compound degradation due to its decreased come up time and efficiency in transferring energy through processing. Research is required to understand the potential for continuous flow microwave processing in the application of muscadine grape puree and its effects upon phenols, antioxidant capacity, and anthocyanin content through processing and storage.

1.7 Literature Cited


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CHAPTER 2- Effects of Processing and Storage on Bioactive Components of Muscadine Grapes (*Vitis rotundifolia*)
2.1 Abstract

Whole muscadine grape purees (including skin, seed, and pulp) from Nesbitt (a “black” variety and Triumph (a “bronze” variety) were evaluated for color and bioactive component stability during thermal processing and in an accelerated storage model system. Total phenol content was measured by the Folin-Ciocalteau (FC) assay. Total antioxidant activity was measured by the Oxygen Radical Absorbance Capacity (ORAC) assay. For the Nesbitt grapes, monomeric anthocyanin content was also measured by a pH differential method and percent polymeric color was measured by the index for color degradation and browning. Grape purees were heated in a 95°C oil bath for 0, 3, 4, 5, and 6 minutes and then stored at 37°C for up to 8 weeks. After 6 minutes of processing, the Nesbitt and Triumph varieties exhibited 85.8% and 83.8% of their original (FC) values, and 106.1% and 105.1% of their ORAC values, respectively. After 8 weeks of storage at 37°C, Nesbitt and Triumph purees exhibited 88.6% and 110.6% of their original FC values and 83.3% and 77.5% of their original ORAC value, respectively. Monomeric anthocyanin retention in Nesbitt grapes decreased with both processing (91.8%) and storage (18.3% from processed), while percent polymeric color retention decreased with processing (52.0%) and increased with storage (553.0%). Based on the data, muscadine grape puree was robust throughout processing and storage in terms of bioactive component and color stability.

KEYWORDS: muscadine, anthocyanins, polyphenols, antioxidants processing, color
2.2 Introduction

Muscadine grapes (Vitis rotundifolia) are native to the southeastern United States and are non-bunch grapes that are unique due to their large size, characteristic flavor, thick skin, and numerous seeds. These grapes contain high levels of phenols, antioxidant capacity, and anthocyanins, which can result in the grapes’ disease resistance, antibacterial activity, and positive health effects. In addition, phenols can serve as natural antimicrobials, antioxidants as natural flavor preservers, and anthocyanins as natural colorants that can contribute to quality in a food product. More than 93% of muscadine grapes are consumed as fresh grapes and the leathery skin and the hard seed are often discarded. While the skins and the seeds of the muscadine grape are often considered to be waste products, they comprise over 27% by weight of the grape and are the most valuable grape components in terms of bioactive compounds. Therefore, a whole muscadine grape puree may be an ideal way to utilize these valuable waste products and to increase health parameters and food quality.

In order to understand the type and levels of bioactive components that might be ingested by the consumer of such a product it is important to investigate the chemical changes that occur during processing and storage. Previous studies have reported changes in bioactive components including phenols, antioxidant capacity, ascorbic acid, and anthocyanins in multiple food matrices throughout processing and storage. While there is not extensive research on the subject, there have been studies that suggested a pseudo-first order degradation pattern in phenols and antioxidant capacity. Multiple articles have reported
that anthocyanins follow a first order degradation pattern, but that this degradation pattern may be affected by the individual food matrix, especially relating to the sugar content of the sample\textsuperscript{25-32}.

While there have been studies that have investigated the effects of processing and storage on muscadine grape juice\textsuperscript{34-37} and muscadine pomace extract\textsuperscript{38}, there is currently no published data regarding the effects of processing and storage on the bioactive components and color of muscadine grape puree. The purpose of this study, therefore, was to investigate changes of bioactive components in this unique food matrix. We examined the effects of heat processing and elevated temperature storage on two varieties of whole muscadine grape puree, one black and one bronze, by evaluating changes in color, total phenol content, total antioxidant capacity, total monomeric anthocyanin content, and percent polymeric color.

2.3 Materials and Methods

2.3.1 Materials

Nesbitt (a purple, or “black” variety) and Triumph (a green, or “bronze” variety) muscadine grapes from the 2010 harvest were donated by Cottle Farms (Faison, NC, USA). These grapes were received frozen and then stored at -80°C (Kelvinator Series 100 Manitowac, WI, USA). Sodium acetate, sodium phosphate, and sodium carbonate were obtained from Fisher Scientific (Waltham, MA, USA). All other chemicals and solvents were purchased from Sigma Aldrich (St. Louis, MO, USA). All of these chemicals and solvents were analytical grade.
2.3.2 Sample Preparation

Thawed muscadine grapes were blended in a Blixer 3 (Jackson, MS, USA) for 2 min until homogenized. After blending, 40±2 g samples of muscadine puree were immediately weighed into plastic-lined foil pouches. The pouches were then closed and heat sealed both above and below the closure seam (American International Electric Impulse Sealer, Industry, CA, USA) (Figure 2.1).

![Figure 2.1. Process Flow Diagram.](image)

2.3.3 Processing Conditions

Pureed, packaged samples were heated in a mineral oil bath at 95°C with constant agitation (Barnant Co., Barrington, IL, USA). The come up time (the time needed to get from the original puree temperature to 95°C) was 3 min. Processing time points were taken at 0 min, 3 min (come up time) and after an additional 1, 2, and 3 min. These samples will
from here forth be denoted as 0 min, 3 min, 3+1 min, 3+2 min, and 3+3 min. Samples were placed in an ice water bath after processing. All samples were processed in duplicate (Figure 2.1). Before chemical analysis, the foil pouches were cut open and then mixed thoroughly to achieve homogeneity.

2.3.4 Storage Conditions

Samples that had been processed at 95°C for 3+3 min were placed in an incubator (Isotemp Oven 630G Fisher Scientific) at 37°C. These samples were taken out of the incubator at 0, 2, 4, 6, and 8 weeks for further analysis. The samples were stored in duplicate.

2.3.5 Colorimeter Measurement

Samples were measured on a Hunter L a b colorimeter after calibration (Hunter DP-9000; Reston, VA) directly after the pouches were opened. The ΔE value was calculated as follows (Equation 1) with all samples compared to the 0 min time point and with the understanding that a difference of greater than 1 signifies a difference that is detectable to the naked eye.

\[
\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}
\]  

[1]
2.3.6 Extraction Parameters

In order to extract the muscadine grape bioactive components, 10 g of each blended sample was weighed into a conical centrifuge tube and combined with 30 ml of 0.5% acetic acid in methanol. The filled centrifuge tubes were vortexed and allowed to rest for 10 min before centrifugation at 4200 G for 20 min (IEC CL31 Multispeed Centrifuge, Thermo Electron Corporation). The supernatant was then poured into an Erlenmeyer flask and 20 ml of acidified methanol was added, vortexed, and allowed to sit for 20 min. This last step was repeated an additional 2 times. The extracted supernatant was evaporated in a TurboVap II (Upssala, Sweden) under a nitrogen stream of 7-12 psi and a water bath temperature of 45°C until the volume was less than 25 ml. This was then brought to a 25 ml volume with acidified methanol. Extractions were done in triplicate.

2.3.7 Total Phenol Content

Total phenol content was measured by the Folin Ciocalteu assay. A high-throughput method that was designed for a 96-well plate was utilized\textsuperscript{30}, including a gallic acid standard curve. Samples were diluted using deionized water. Folin Ciocalteu reagent (1M) was then added to the wells and mixed in a plate reader (EnSpire 2300 Multilabel Reader, Perkin Elmer, Waltham, MA, USA). After standing for 2 min, 20% sodium carbonate solution was added and again the plate was mixed in the plate reader. After 10 min, the samples were read in the plate reader at 690 nm. This analysis was conducted in triplicate at room temperature and reported as mg gallic acid equivalents/g fresh weight muscadine (mg GAE/g FW).
2.3.8 Total Antioxidant Capacity

Total antioxidant capacity was measured by the Hydrophilic- Oxygen Radical Absorbance Capacity assay (H-ORAC) using a trolox standard. Samples and standards were diluted using 0.075 M phosphate buffer. After addition of the samples and standard curve to a 96-well plate, 70 nM fluorescein was added. After equilibration of the pipetted plate to 37°C for 15 min, 153 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added. An excitation wavelength of 483 nm and an emission wavelength of 525 nm were used to tabulate oxygen radical absorbance capacity over a period of 90 min at 37°C in a Tecan Safire plate reader (Tecan Group Ltd., Männedorf, Switzerland). Samples were tested in triplicate and reported as µmol trolox equivalents/g fresh weight muscadine (µmol trolox equiv/g FW).

2.3.9 Total Monomeric Anthocyanin Content

Total monomeric anthocyanin content was analyzed using the pH Differential assay\(^{40}\). Samples were diluted at 1:10 using 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5), separately, and allowed to sit for 15 min before reading. The spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA) was zeroed using deionized water and then samples were read at both 520 nm and 700 nm. Absorbance (A) of the diluted samples was calculated using Equation 2.

\[
A = (A_{\lambda, 520nm} - A_{700nm})_{pH 1.0} - (A_{\lambda, 520nm} - A_{700nm})_{pH 4.5} \quad [2]
\]

The diluted absorbance was used to find final anthocyanin concentrations (mg/g fresh weight muscadine) expressed as cyanidin-3-glucoside equivalents according to Equation 3.
Monomeric anthocyanin pigment (mg/liter) = \( \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \) [3]

Where MW= molecular weight, DF= dilution factor, and \( \varepsilon \)= molar absorptivity

2.3.10 Percent Polymeric Color

Percent polymeric color was determined by the Index for Pigment Degradation, Polymeric Color, and Browning\(^4\). The samples were diluted to 1:10 with deionized water in duplicate. Additional deionized water was added to one of these samples as a control and an equal amount of 0.2 g/ml potassium metabisulfite solution was added to the other. The samples were allowed to equilibrate for 15 min at room temperature before reading. The spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA) was blanked with deionized water and then samples were each read at 420, 520, and 700 nm. Percent polymeric color was calculated at follows (Equations 4, 5, 6).

\[
\text{Polymeric Color (bisulfite-treated samples)} = [A_{420\text{nm}} - A_{700\text{nm}}] + [A_{520\text{nm}} - A_{700\text{nm}}] \times DF
\]

Where DF = dilution factor

\[
\text{Color Density (water-treated samples)} = [A_{420\text{nm}} - A_{700\text{nm}}] + [A_{520\text{nm}} - A_{700\text{nm}}] \times DF
\]

Percent Polymeric Color = (polymeric color / color density) \times 100

2.3.11 Statistical Analysis

Statistical analysis was done using the Tukey test (P<0.05) on SAS software (Cary, NC, USA). Values were reported as mean ± standard error of the mean. All samples were processed in duplicate and analyses were conducted in triplicate.
2.4 Results and Discussion

The effect of processing and storage upon color, total phenol content, total antioxidant capacity, total monomeric anthocyanin content, and percent polymeric color was studied in whole muscadine grape puree. One black variety, Nesbitt, and one bronze variety, Triumph, were utilized in order to understand differences that occur between the two types of muscadine grapes. While there were significant differences in all of the studied parameters including color, total phenol content, and total antioxidant capacity, the muscadine grape puree proved to be a robust matrix throughout processing and storage. The total monomeric anthocyanin content and the percent polymeric color in the black, Nesbitt variety had the most notable differences during processing and storage, suggesting that anthocyanins are the most labile or the most likely to polymerize in this scenario. In addition, the processed samples became lighter, while the stored samples became browner. The total monomeric anthocyanin content decreased in the Nesbitt variety for both processed and stored samples, while the percent polymeric color decreased in processed samples and increased in stored samples. These results were reinforced by the color data.

2.4.1 Processing Study

In the Nesbitt variety, the samples were lighter, redder, and yellower with processing time. The ΔE values suggested that the unprocessed samples were significantly different than all of the processed samples, that the 3 min samples were different from the other processed samples, and that the 3+1 and 3+2 min samples were different from the 3+3 min samples (Table 2.1). These results reflected a visible lightening of the samples. The
Triumph variety followed a similar trend as the Nesbitt variety during processing. The samples became significantly lighter and redder with processing \((P>0.05)\) (Table 2.2). In this case, the \(\Delta E\) values for the samples showed that there was a visible difference between the processed and the unprocessed samples, but not between the processed samples.

Table 2.1. Nesbitt Muscadine Grape Puree L a b Color with Processing Time at 95°C.  
\(^a\)Values with the same letter within columns are not significantly different (Tukey, \(P>0.05)\).  
\(^b\)\(\Delta E\) Values are all compared to the 0m time point.

<table>
<thead>
<tr>
<th>Process Time</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>(\Delta E)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>16.7±0.1 c(^a)</td>
<td>14.8±0.2 c</td>
<td>-1.1±0.1 d</td>
<td></td>
</tr>
<tr>
<td>3m</td>
<td>19.5±0.5 b</td>
<td>17.6±0.3 b</td>
<td>-0.3±0.3 c</td>
<td>4.0</td>
</tr>
<tr>
<td>3m+1m</td>
<td>20.5±0.2 ab</td>
<td>18.4±0.1 a</td>
<td>0.5±0.1 b</td>
<td>5.4</td>
</tr>
<tr>
<td>3m+2m</td>
<td>21.1±0.1 a</td>
<td>18.5±0.1 a</td>
<td>0.8±0.1 ab</td>
<td>6.0</td>
</tr>
<tr>
<td>3m+3m</td>
<td>21.3±0.1 a</td>
<td>18.5±0.1 a</td>
<td>1.4±0.1 a</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Table 2.2. Triumph Muscadine Grape Puree L a b Color with Processing Time at 95°C.  
\(^a\)Values with the same letter within columns are not significantly different (Tukey, \(P>0.05)\).  
\(^b\)\(\Delta E\) Values are all compared to the 0m time point.

<table>
<thead>
<tr>
<th>Process Time</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>(\Delta E)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>41.3±0.1 c(^a)</td>
<td>5.0±0.1 b</td>
<td>17.7±0.1 a</td>
<td></td>
</tr>
<tr>
<td>3m</td>
<td>44.2±0.2 ab</td>
<td>4.6±0.1 b</td>
<td>17.5±0.1 a</td>
<td>2.9</td>
</tr>
<tr>
<td>3m+1m</td>
<td>44.3±0.1 b</td>
<td>4.9±0.1 b</td>
<td>17.5±0.1 a</td>
<td>3.0</td>
</tr>
<tr>
<td>3m+2m</td>
<td>44.7±0.1 a</td>
<td>5.7±0.2 a</td>
<td>17.6±0.1 a</td>
<td>3.5</td>
</tr>
<tr>
<td>3m+3m</td>
<td>44.7±0.1 a</td>
<td>6.0±0.1 a</td>
<td>17.5±0.1 a</td>
<td>3.5</td>
</tr>
</tbody>
</table>

After extraction, samples were then analyzed for bioactive component content. The total phenol content of both the Nesbitt and the Triumph samples decreased significantly after processing (Figure 2.2). There was a significant difference in the Nesbitt variety between the 0 min, 3 min, and 3+1 min time points and the 3+2 min time point, and then again at the 3+3 min time point (Table 2.3). This significant difference accounted for 95.0%
total phenol retention between the unprocessed and the 3+2 min time point and 85.8% retention between the unprocessed and 3+3 min time point (Figure 2.4). In the Triumph variety, there was a significant difference between the 0 min, 3 min time points and the 3+1, 3+2, and 3+3 min time points (Table 2.4). This accounted for 88.8% phenol retention between the unprocessed and the 3+1 min time point (Figure 2.5).

Figure 2.2. Total Phenol Content vs. Processing Time at 95°C for Nesbitt and Triumph Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

Table 2.3. Changes in Bioactive Components of Nesbitt Muscadine Grape Puree During Processing at 95°C. ^Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Process Time</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
<th>Monomeric Anthocyanin Content (mg equiv/g FW)</th>
<th>Percent Polymeric Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>2.376±0.017 a</td>
<td>35.084±0.390 c</td>
<td>0.489±0.008 a</td>
<td>36.965 a</td>
</tr>
<tr>
<td>3m</td>
<td>2.379±0.028 a</td>
<td>35.761±0.385 cb</td>
<td>0.480±0.003 ab</td>
<td>30.514 ab</td>
</tr>
<tr>
<td>3+1m</td>
<td>2.363±0.027 a</td>
<td>38.484±0.545 a</td>
<td>0.477±0.006 ab</td>
<td>29.835 ab</td>
</tr>
<tr>
<td>3+2m</td>
<td>2.257±0.029 b</td>
<td>35.977±0.706 ab</td>
<td>0.461±0.004 bc</td>
<td>21.982 b</td>
</tr>
<tr>
<td>3+3m</td>
<td>2.039±0.022 c</td>
<td>37.306±0.577 bc</td>
<td>0.449±0.001 c</td>
<td>19.234 b</td>
</tr>
</tbody>
</table>
In terms of antioxidant capacity, both the Nesbitt and the Triumph varieties significantly increased with processing time (Figure 2.3). The Nesbitt variety increased starting at the 3+1 min time point and then significantly decreased at 3+3 min of processing time (Table 2.3). There was 106.3% antioxidant capacity retention between the unprocessed and 3+3 min samples (Figure 2.4). The Triumph variety followed a similar pattern, with a significant increase in antioxidant capacity starting at 3+2 min of processing time, but did not significantly decrease in the measured time points (Table 2.4). There was 105.1% antioxidant capacity retention between the 0 min and the 3+3 min time points (Figure 2.5).

One possible explanation for a net increase in antioxidant capacity in both varieties of muscadine grape puree is that a better extraction occurred from the food matrix. In addition, this suggested that the muscadine grape puree is relatively robust, as previous research has reported only 74.1% antioxidant capacity retention in muscadine grape juice.35

Table 2.4. Changes in Bioactive Components of Triumph Muscadine Grape Puree During Processing at 95°C. *Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Process Time</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>1.695±0.014 a</td>
<td>27.185±0.339 b</td>
</tr>
<tr>
<td>3m</td>
<td>1.693±0.015 a</td>
<td>27.923±0.345 b</td>
</tr>
<tr>
<td>3+1m</td>
<td>1.506±0.012 b</td>
<td>27.077±0.370 b</td>
</tr>
<tr>
<td>3+2m</td>
<td>1.557±0.015 b</td>
<td>30.227±0.658 a</td>
</tr>
<tr>
<td>3+3m</td>
<td>1.516±0.010 b</td>
<td>28.568±0.621 ab</td>
</tr>
</tbody>
</table>
Figure 2.3. Total Antioxidant Capacity vs. Processing Time at 95°C for Nesbitt and Triumph Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

Figure 2.4. Percent Retention of Bioactive Components of Nesbitt Muscadine Grape Puree During Processing at 95°C. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity, TMA= Total Monomeric Anthocyanin Content, PPC= Percent Polymeric Color) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).
In contrast to the total antioxidant capacity, the total monomeric anthocyanin content was the most labile component of the parameters studied. The total monomeric anthocyanin content data is only shown for the black, Nesbitt variety because the total monomeric anthocyanin content in the Triumph variety was negligible (Table 2.3). The lack of anthocyanins in the bronze varieties of muscadine grapes has been previously reported\textsuperscript{41}. These measurements showed a decrease in total monomeric anthocyanin content between the 0 min and the 3+2 min processing time points and again with the 3+3 min time point (Figure 2.6). The percent retention of the total monomeric anthocyanins at 3+2 min was 94.3% and at 3+3 min was 91.8% (Figure 2.4). Similar monomeric retention values have been reported in bench-top processed muscadine grape juice\textsuperscript{33}, but other studies have reported 75.9-78.4% retention\textsuperscript{34,35} during high temperature-short time pasteurization of muscadine grape juice, suggesting that the muscadine grape puree was fairly robust.
In order to understand changes in the total monomeric anthocyanin content, percent polymeric color was measured (Table 2.3). The percent polymeric color was again solely reported for the Nesbitt variety of muscadine grapes due to negligible levels of anthocyanins present in the Triumph variety (Figure 2.7). The percent polymeric color significantly decreased with processing between the 0 min, 3 min, and 3+1 min samples and the 3+2 min sample (40.5% decrease from 0 min) and then again with the 3+3 min time point (48.0% decrease from 0 min). While previous studies have reported an increase in percent polymeric color with processing\(^{42-44}\), due to the pH of the Nesbitt variety of 3.4, the monomeric anthocyanins likely divided into their phenolic acid and aldehyde constituent parts\(^{45}\).
Figure 2.7. Percent Polymeric Color vs. Processing Time at 95°C for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

2.4.2 Storage Study

Samples that had been processed for 3+3 min at 95°C were stored in a 37°C incubator for up to 8 weeks. Color readings were taken throughout the storage study. The Nesbitt data showed that the samples became, lighter, greener, and yellower with storage (Table 2.5). Visible color differences represented by the ΔE value suggested a difference between the 0 min and all of the processed samples. In addition, this value showed a difference between 2 and 4 weeks and 6 and 8 weeks. This resulted in a visual browning of the samples. In the Triumph variety, there was a significant difference in color between the 0 week time point and the rest of the weeks, between 2 weeks and the rest of the weeks, and between 8 weeks and the rest of the weeks (Table 2.6).
<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔEb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.3±0.1 b</td>
<td>18.5±0.1 a</td>
<td>1.4±0.1 d</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27.5±0.6 a</td>
<td>16.2±0.4 b</td>
<td>5.7±0.1 c</td>
<td>7.9</td>
</tr>
<tr>
<td>4</td>
<td>24.0±0.1 ab</td>
<td>12.9±0.1 c</td>
<td>5.9±0.1 c</td>
<td>7.6</td>
</tr>
<tr>
<td>6</td>
<td>26.0±0.2 ab</td>
<td>10.5±0.2 d</td>
<td>8.5±0.1 a</td>
<td>11.7</td>
</tr>
<tr>
<td>8</td>
<td>28.2±0.2 a</td>
<td>10.9±0.1 d</td>
<td>7.7±0.1 b</td>
<td>12.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔEb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.7±0.1 a</td>
<td>6.0±0.1 b</td>
<td>17.5±0.1 a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>41.4±0.2 b</td>
<td>6.0±0.1 b</td>
<td>15.7±0.1 b</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td>37.2±0.4 c</td>
<td>6.3±0.2 ab</td>
<td>14.2±0.1 c</td>
<td>8.2</td>
</tr>
<tr>
<td>6</td>
<td>36.8±0.5 c</td>
<td>6.5±0.1 a</td>
<td>14.1±0.1 c</td>
<td>8.6</td>
</tr>
<tr>
<td>8</td>
<td>34.8±0.1 d</td>
<td>6.5±0.1 a</td>
<td>13.7±0.1 d</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Bioactive component analysis was also conducted throughout the storage study. The total phenol levels generally decreased with storage time (Figure 2.8). In the Nesbitt variety, there was a significant decrease in total phenol content between 0, 2, and 4 weeks of storage time and 6 and 8 weeks of storage time (Table 2.7). This resulted in 95.4% retention of the total phenol content between the 0 week and the 6 week time point (Figure 2.12). In the Triumph variety, phenol content significantly decreased between 0, 2, and 4 weeks and 6 weeks of storage, but increased significantly at the 8 week storage time point (Table 2.8).
Table 2.7. Changes in Bioactive Components of Nesbitt Muscadine Grape Puree During Storage at 37°C. *Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
<th>Monomeric Anthocyanin Content (mg equiv/g FW)</th>
<th>Percent Polymeric Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.039±0.022 a</td>
<td>35.963±0.582 a</td>
<td>0.449±0.001 a</td>
<td>19.234 e</td>
</tr>
<tr>
<td>2</td>
<td>2.013±0.018 a</td>
<td>25.616±0.579 c</td>
<td>0.225±0.004 b</td>
<td>56.697 d</td>
</tr>
<tr>
<td>4</td>
<td>2.064±0.015 a</td>
<td>23.368±0.731 c</td>
<td>0.146±0.001 c</td>
<td>67.008 c</td>
</tr>
<tr>
<td>6</td>
<td>1.865±0.015 b</td>
<td>20.291±1.127 d</td>
<td>0.097±0.003 d</td>
<td>89.586 b</td>
</tr>
<tr>
<td>8</td>
<td>1.807±0.021 b</td>
<td>29.943±0.336 b</td>
<td>0.082±0.002 e</td>
<td>102.516 a</td>
</tr>
</tbody>
</table>

Table 2.8. Changes in Bioactive Components of Triumph Muscadine Grape Puree During Storage at 37°C. *Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.516±0.010 b</td>
<td>28.533±0.621 a</td>
</tr>
<tr>
<td>2</td>
<td>1.557±0.014 b</td>
<td>22.543±0.368 b</td>
</tr>
<tr>
<td>4</td>
<td>1.503±0.016 b</td>
<td>23.547±0.517 b</td>
</tr>
<tr>
<td>6</td>
<td>1.400±0.028 c</td>
<td>20.755±0.295 c</td>
</tr>
<tr>
<td>8</td>
<td>1.677±0.016 a</td>
<td>22.118±0.312 c</td>
</tr>
</tbody>
</table>

Figure 2.8. Total Phenol Content with Storage Time at 37°C for Nesbitt and Triumph Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.
Figure 2.9. Total Antioxidant Capacity with Storage Time at 37°C for Nesbitt and Triumph Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

The total antioxidant capacity data followed a similar trend to that of the total phenols (Figure 2.9). In the Nesbitt variety, there was a significant decrease in antioxidant capacity between the 0 week and the 2 and 4 week storage time points and then again at the 6 week time point (Table 2.7). There was then a significant increase at the 8 week time point, rendering 83.3% antioxidant capacity retention (Figure 2.10). In the Triumph variety, there was a significant decrease in antioxidant capacity between 0 weeks of storage and the 2 and 4 week time points. There was another significant decrease at the 6 and 8 week time points (Table 2.8). The percent antioxidant capacity retention between 0 weeks and 8 weeks of storage time was 77.5% (Figure 2.11).
Figure 2.10. Percent Retention of Bioactive Components of Nesbitt Muscadine Grape Puree During Storage at 37°C. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity, TMA= Total Monomeric Anthocyanin Content, PPC= Percent Polymeric Color) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

Figure 2.11. Percent Retention of Bioactive Components of Triumph Muscadine Grape Puree During Storage at 37°C. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).
Figure 2.12. Total Monomeric Anthocyanin Content with Storage Time at 37°C for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

The total monomeric anthocyanin content is again only displayed for the Nesbitt variety (Figure 2.12). There was a significant decrease in monomeric anthocyanin content at each 2 week time point measured (Table 2.7). This represented 50.1% monomeric anthocyanin retention after 2 weeks of storage, 32.5% retention after 4 weeks of storage, 21.6% retention after 6 weeks, and 18.3% retention after 8 weeks at 37°C (Figure 2.10). In contrast to the processing study data, the percent polymeric color increased with storage time, until the samples reached around 100% polymeric color after 8 weeks of storage (Figure 2.13). There was a significant increase in percent polymeric color at each 2 week time point measured, in direct opposition to the trend shown for total monomeric anthocyanins throughout storage (Table 2.7). Therefore, the monomeric anthocyanins likely polymerized during storage.
It is important to understand the changes that occur during processing and storage, from a food quality perspective. The visual aspect of a food is a consumer’s first clue as to whether or not they would like to consume that food. Because of consumer demand for natural colorants, such as anthocyanin-based colorants, it is essential to investigate their stability. For both the processing study and the storage study, the color data was congruent with the total monomeric anthocyanin content and the percent polymeric color data in the black, Nesbitt variety. While processing samples resulted in a lighter color in the samples, the total monomeric anthocyanin content and the percent polymeric color decreased. In contrast, the samples that were stored at 37°C became browner with storage time. This was indeed reflected in a decreased monomeric content and an increased percent polymeric color, which is associated with browning. In order to try to prevent color change and anthocyanin degradation, the addition of a nitrogen-flushing step during the packaging could be useful, preventing excessive presence of oxygen in the package.
2.5 Conclusions

The results from this study including color data, total phenol content, total antioxidant capacity, total monomeric anthocyanin content, and percent polymeric color, largely suggested that muscadine grape puree was a robust food matrix. Phenol content generally decreased throughout processing and storage of both varieties of muscadine grape puree, but still experienced a minimum of 85.8% total phenol retention. Similarly with total antioxidant capacity there was a minimum of 56.4% antioxidant capacity retention, with most of the processed samples actually increasing in antioxidant capacity. In both processing and storage, the largest changes occurred in anthocyanins, with total monomeric anthocyanin levels decreasing through both processing and storage. These results are important for the muscadine grape industry in order to minimize waste by creation of a robust food product that is rich in bioactive components that are important for food quality and human health. These results also show changes that occur during processing and storage, in order to gain understanding of the bioactive components that would be available for consumption in a finished food product.

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(44) Brownmiller, C.; Howard, L.R.; Prior, R.L. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products. *J. Food Sci.* **2008**, *73*(6), H134-H140.

CHAPTER 3- Dielectric Properties of Muscadine Grapes
3.1 Abstract

Continuous flow microwave processing is an alternative processing technique that shows promise due to its superior bioactive component retention and attenuation of off-flavors caused by over-heating. In order to understand the potential for muscadine grapes to be processed using this technology, the dielectric properties of three varieties of whole muscadine grape puree and the skin, pulp, and seeds of one variety were measured. The dielectric constants generally decreased with increasing temperatures, whereas the dielectric loss factors increased with increasing temperature. Similarly, the loss tangent increased with increasing temperature and the depth of penetration decreased with increasing temperature. Results were similar between all varieties of whole muscadine grape puree. For the muscadine grape components, the seeds were different from the other parts, likely due to its lower water content. These results suggest that whole muscadine grape puree is compatible with microwave processing.

KEYWORDS: muscadine grapes, dielectric
3.2 Introduction

Microwave processing is an emerging alternative food processing technique, whereby microwaves are sent through a given food or biomaterial, exciting the polar molecules within the sample. These polar molecules then rotate, resulting in friction that creates heat. The heat created by the microwaves serves to process the samples, but is a very efficient form of energy transfer that requires a much shorter ‘come up’ time than traditional processing. In contrast to traditional heat processing, which heats from the outside of a sample towards the inside, microwave processing can generate heat wherever polar molecules exist within a given matrix. This results in a much more efficient process that minimizes overcooking of a sample, while still resulting in a similar or higher level of safety\(^1\). This technology therefore has the potential of better nutrient and flavor preservation than traditional forms of processing, as previously seen in applications such as apple juice, red cauliflowers, and purple sweet potatoes\(^1\)\(^-\)\(^3\).

Efficient microwave processing is highly dependent upon a given food matrix, parameters that can partially be quantified by the measurement of dielectric properties\(^1\),\(^2\),\(^4\). These properties are characterized by two categories: dielectric constant (\(\epsilon'\)), being efficiency of energy storage, and dielectric loss factor, being the ability to convert electromagnetic energy into thermal energy and the energy that is dissipated in the system (\(\epsilon''\)). Interactions between the dielectric properties and a material can be described using the loss tangent, utilizing both the dielectric constant and the dielectric loss factor. In addition, depth of penetration, the distance (m) required to dissipate 63% of the microwave energy, can be...
calculated using these measures. This calculation can be used to understand the uniformity in processing a sample\(^5\).

The frequency is essential for understanding dielectric properties, with lower frequencies having a higher dielectric constant and a lower dielectric loss factor than higher frequencies\(^6\). Temperature of the sample has a similar effect, with higher temperatures typically resulting in a lower dielectric constant and higher dielectric loss factor than lower temperatures. The industry standard frequency, 915 MHz, can be chosen in order to take advantage of these dielectric properties, resulting in a safe product with superior food quality. The dielectric properties of grape juice and wine (\textit{Vitis vinifera}) have been studied\(^7\), but not those of whole, skins, pulp, or seeds of muscadine grapes.

The purpose of this study was to gain understanding of the dielectric properties of muscadine grape puree and the puree of its component parts. Therefore whole grape purees of two black varieties (Nesbitt and Supreme) and one bronze variety (Tara) along with separate purees of the skins, pulp, and seeds of the Tara variety were measured. Dielectric constant and dielectric loss values were measured under static conditions between 15\(^\circ\)C and 110\(^\circ\)C at frequencies between 300 and 3000 MHz. These values were used to understand the possibility of processing muscadine grapes in an industrial scale microwave processing system at 915 MHz.
3.3 Materials and Methods

3.3.1 Materials

Muscadine grapes were donated by Cottle Farms (Faison, NC, USA). Three varieties were chosen including two black varieties, Nesbitt and Supreme, and one bronze variety, Tara. These grapes were received frozen and then stored at -80°C (Kelvinator Series 100 Manitowac, WI, USA). The Tara grapes were separated by hand for testing of their skins, seeds, and pulp.

3.3.2 Sample Preparation

Whole Nesbitt, Supreme, and Tara varieties of muscadine grapes and Tara skins, pulp, and seeds were blended separately in a food processor (Kitchenaid Chef’s Chopper, T. Joseph, MI, USA) for 2 min. They were then each blended in a spice grinder (Waring Professional Spice Grinder, Torrington, CT, USA) for 2 min in order to achieve a small particle size.

3.3.3 Dielectric Properties

Dielectric properties of whole Nesbitt puree, whole Supreme puree, whole Tara puree, Tara skin puree, Tara pulp puree, and Tara seed puree were measured under static conditions using an open-ended coaxial probe (Model HP 85070B Agilent Technologies, Palo Alto, CA, USA) connected to a network analyzer (HP 8753C, Agilent Technologies, Palo Alto, CA, USA) in duplicate.\textsuperscript{8} The network analyzer was calibrated by running the
analyzer while it was in contact with air, metal, and deionized water. Samples were then placed into a sealed metal cell (35 mm inner diameter, 36.5 mm height) and attached to the coaxial probe and a thermocouple (Figure 3.1). The cell was placed in a mineral oil bath and heated from 15°C to 110°C. Dielectric properties were measured constantly during this heating process at frequencies between 300 and 3000 MHz, with an increment of 5MHz.

**Figure 3.1.** Metal Cell for Dielectric Measurement.

Values were reported for 915 MHz because this is the frequency used in continuous flow microwave processing systems. Dielectric property values are reported as dielectric constant ($\varepsilon'$) and dielectric loss factor ($\varepsilon''$), loss tangent ($\tan\delta$) (Equation 1), and depth of penetration ($d_p$) (Equation 2).

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'}$$  \hspace{1cm} [1]

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon'}} \left[ 1 + \frac{\varepsilon''}{\varepsilon'} \right]$$  \hspace{1cm} [2]

Where $c$ is the speed of light in free space ($3\times10^8$ ms$^{-1}$) and $f$(Hz) is frequency.
3.4 Results and Discussion

Three varieties of muscadine grapes, 2 black (Nesbitt, Supreme) and one bronze (Tara) were ground and their dielectric properties were measured. In addition, Tara skins, pulp, and seeds were ground and evaluated by the same measurements.

3.4.1 Whole Muscadine Grape Purees

For each variety of whole muscadine grape puree, the dielectric constants decreased with temperature and the dielectric loss factor increased with temperature. The dielectric constant for the whole Nesbitt variety decreased from 69.71 at 22°C to 57.15 at 102°C (Figure 3.2). For the Supreme variety, the dielectric constant was 65.20 at 22°C and 51.97 at 102°C (Figure 3.3). The dielectric constant of the Tara variety, decreased from 69.94 at 22°C and 58.34 at 102°C (Figure 3.4). Therefore, the dielectric constant exhibited an inverse relationship with temperature, where the dielectric constant decreased with increasing temperature in all of the studied varieties. This reflects the results exhibited in previous studies that were conducted in other food matrices. The dielectric constants were similar in each of the varieties.
Figure 3.2. Dielectric Properties of Whole Nesbitt Muscadine Grape Puree.

Figure 3.3. Dielectric Properties of Whole Supreme Muscadine Grape Puree.

Figure 3.4. Dielectric Properties of Whole Tara Muscadine Grape Puree.
The dielectric loss factor for the Nesbitt variety increased from 9.81 at 22°C to 21.51 at 102°C (Figure 3.2). For the Supreme variety, the dielectric loss factor went from 9.06 at 22°C to 20.69 at 102°C (Figure 3.3). The dielectric loss factor for the Tara variety increased from 9.91 at 22°C to 20.78 at 102°C (Figure 3.4). In each of the varieties studied, there was a trend of increasing dielectric loss factor with increasing temperature. Again, all of the values for each of the varieties were fairly similar.

In order to effectively compare the dielectric properties of the muscadine grape purees, the loss tangent is a useful tool. The loss tangents of the whole Nesbitt, Supreme, and Tara purees were 0.13, 0.14, and 0.15 at 22°C and 0.38, 0.40, and 0.36 at 102°C, respectively (Figure 3.5). Again, these values were within the same range, but increased with temperature. Depth penetration measurements were calculated to represent energy dissipated. The d(p) values for the whole Nesbitt, Supreme, and Tara purees were 0.0041, 0.0041, and 0.0037 m at 22°C and 0.0017, 0.0018, and 0.0018 m at 102°C, respectively (Figure 3.6). This showed that energy was dissipated faster at higher temperatures, but that the various varieties were fairly similar. All varieties were suggested to be compatible with microwave processing.
3.4.2 Muscadine Part Purees

The dielectric properties of Tara muscadine grape parts (whole, skins, pulp, seeds) were studied. The dielectric constants for each of the studied parameters decreased with increased temperature. The dielectric constant of the whole Tara puree, decreased from 69.94 at 22°C and 58.34 at 102°C (Figure 3.4). For the skins, it decreased from 66.00 at 22°C to 55.52 at 102°C (Figure 3.7). The dielectric constant of the pulp was 70.97 at 22°C
and 55.75 at 102°C (Figure 3.8). The dielectric constant of the seeds was much lower than those of the other components tested and stayed relatively constant, going from 30.64 at 22°C to 30.11 at 102°C (Figure 3.9). The results were similar for all of the muscadine grape components except for the seeds. This deviation in pattern could be due to the absence of large amounts of free water that can cause decreases in dielectric constants at higher temperatures.

Figure 3.7. Dielectric Properties of Tara Muscadine Grape Skin Puree.

Figure 3.8. Dielectric Properties of Tara Muscadine Grape Pulp Puree.
The dielectric loss factor for the whole Tara puree increased from 9.91 at 22°C to 20.78 at 102°C (Figure 3.4). That of the skin increased from 9.18 at 22°C to 34.19 at 102°C (Figure 3.7). The dielectric loss factor of the pulp increased from 8.54 at 22°C to 16.59 at 102°C (Figure 3.8). For the seeds, the dielectric loss factor increased with temperature from 7.88 at 22°C to 16.74 at 102°C (Figure 3.9). Therefore, the dielectric loss factor increased with increasing temperature in all of the muscadine grape parts.

The loss tangent was calculated in order to compare the Tara muscadine grape components. For the whole, skins, pulp, and seed purees, the loss tangents were 0.14, 0.14, 0.12, and 0.25 at 22°C and 0.36, 0.60, 0.29, and 0.56 at 102°C, respectively (Figure 3.10). The loss tangent of the seed puree was higher than for the other samples, possibly related to its lower water content. The loss tangent of the skin puree increased with temperature, potentially due to release of certain minerals or other factors from the food matrix. The depth of penetration for the same components was 0.0037, 0.0039, 0.0043, and 0.0047 at 22°C and 0.0018, 0.0011, 0.0023, and 0.0022 at 102°C, respectively (Figure 3.11). These
results were relatively similar, but decreased with increasing temperature, suggesting that a higher temperature results in more dissipation of energy.

**Figure 3.10.** Loss Tangent of Whole, Skins, Pulp, and Seeds of Tara Muscadine Grape Pure

**Figure 3.11.** Depth of Penetration of Whole, Skins, Pulp, and Seeds of Tara Muscadine Grape Puree.
3.5 Conclusion

The results from this study showed a trend of decreasing dielectric constant with increasing temperature and increasing dielectric loss factor with increasing temperature. The loss tangent also increased with temperature, while the depth of penetration decreased. This was true for all of the whole muscadine grape puree samples and most of the muscadine grape components. The seeds proved to be the most drastically different, likely due to their lower water content. The results from these experiments provide necessary insight into the applicability of muscadine grapes for thermal processing using a continuous flow microwave processing system.

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CHAPTER 4- Changes in Bioactive Components of Muscadine Grape Puree (*Vitis rotundifolia*) During Continuous Flow Microwave Processing and Storage
4.1 Abstract

Microwave processing is a novel processing technique that has been used for superior nutrient retention and flavor preservation, while still maintaining a safe product. This study utilized 2 varieties of muscadine grapes (Nesbitt, Frye) and their bioactive component profiles were measured throughout grinding into a puree, microwave processing, and accelerated storage. Both the Nesbitt and Frye varieties maintained high retention of total phenol content through grinding (115.9, 93.1%), microwave processing (140.7, 82.5%), and storage at 37°C for 4 weeks (100.4, 87.5%), respectively. These varieties also retained most of their antioxidant capacity through grinding (100.9, 90.7%), microwave processing (118.2, 100.4%), and storage (88.6, 61.1%). There were substantial decreases in total monomeric anthocyanins in the Nesbitt variety during grinding (68.8%), microwave processing (31.6%), and storage (38.4%). These results show that whole muscadine grape puree is a viable food matrix for microwave processing.
4.2 Introduction

Muscadine grapes (*Vitis rotundifolia*) are rich in bioactive components including phenolic compounds and anthocyanins that confer antioxidant capacity\(^1\). The bioactive components are important because they have the potential to cause disease resistance in muscadine grape vines\(^2,3\), antibacterial activity\(^4\text{-}10\), health benefits\(^11\text{-}19\), and can contribute to food quality including flavor preservation and color. Therefore, the preservation of these naturally-present components is of the utmost importance. Inclusion of the seeds and skins into a puree product can be highly beneficial due to their bioactive profile\(^1\). While the skins and seeds of the grapes are normally discarded during fresh consumption, they can be incorporated into a whole muscadine grape puree in order to create a value-added product.

In order to create a shelf-stable food product, a processing step is required. Unfortunately, processing can result in degradation of phenols\(^20\), antioxidants capacity\(^21,22\), and anthocyanins\(^23\text{-}30\). While all of these components have exhibited pseudo-first order or first order degradation patterns throughout processing and storage, research has been conducted to prevent some of these bioactive component losses. Alternative processing techniques have been utilized in order to diminish degradation of bioactive components, color changes, and flavor changes that occur during traditional processing and storage. Dense phase CO\(_2\) processing\(^31\text{-}33\), high hydrostatic pressure processing\(^34\), and copigmentation\(^35\) techniques have all been applied to muscadine grapes juice, with positive results. Continuous flow microwave processing has emerged as a promising alternative processing technique in terms of processed food quality and safety, but has not been applied to muscadine grape products.
Continuous flow microwave processing utilizes microwaves at a frequency of 915 MHz to excite polar molecules within a sample, resulting in friction that creates heat and microbial destruction. This method has advantages over traditional processing because instead of heating from the outside to the inside of a sample, it can heat the sample volumetrically. This diminishes the requirement for over-heating of the outside parts of a sample in order to safely heat any cold spots, better preventing bioactive compound destruction and off-flavors caused by over-cooking than traditional heat processing. In addition the microwave frequency used in industrial microwave processing, 915 MHz, yields better control of the pasteurization process than the frequency used in home microwaves. Industrial scale microwave processing has been successfully applied in various food matrices including orange fleshed sweet potato puree, purple sweet potato puree\textsuperscript{36}, apple juice\textsuperscript{37}, strawberry puree\textsuperscript{38}, and red cauliflower\textsuperscript{39}.

The purpose of this study was to investigate the changes in bioactive components in muscadine grape puree during continuous flow microwave processing and storage at 37°C. Two varieties of muscadine grape puree were sterilized in a 100 kW continuous flow microwave processing system and then aseptically packaged. Packaged purees were then stored in an incubator at 37°C for up to 8 weeks. Samples were taken at every stage of this process and analyzed for color, total phenol content, total antioxidant capacity, total monomeric anthocyanin content, and percent polymeric color.
4.3 Materials and Methods

4.3.1 Materials

Muscadine grapes, including one black variety, Nesbitt, and one bronze variety, Frye were donated by Hinnant Farms (Pine Level, NC, USA). These grapes were received frozen and then stored at -20°C (Kelvinator Series 100 Manitowac, WI, USA). Sodium acetate, sodium phosphate, and sodium carbonate were obtained from Fisher Scientific (Waltham, MA, USA). All other chemicals and solvents were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and solvents were analytical grade.

4.3.2 Sample Preparation

The grapes were taken out of frozen storage 24 hours before processing in order to be thawed, preventing flashing during the grinding process. The grapes were then put through a slicer/dicer (Louis Allis Company, Milwaukee, WI, USA) two times in order to reduce their size. The whole muscadine grape puree was then run through a comitrol (Model 1700 Comitrol, Urschel laboratories, Valparaiso, IN, USA) to achieve a small particle size. The final puree was then placed in buckets and stored in the refrigerator overnight before microwave processing. Samples were taken at each step of this process including frozen (F), after thawing (T), after each of the two passes through the slicer/dicer (SD1, SD2), after the comitrol (C), after sitting overnight and before being run through the microwave (BM), and finally after the muscadine grape puree was microwave processed (AM) (Figure 4.1).
4.3.3 Microwave Processing

Muscadine grape purees were processed in a 100 kW continuous flow microwave system (UltrAseptics Inc., Raleigh, N.C., U.S.A.) operating at 915 MHz as described by Pascua Cubides\textsuperscript{40}. The entire microwave run included a sterilization step, processing of other fruit purees, processing of four batches of whole muscadine grape puree, and finally the reprocessing of other fruit purees to push the muscadine grape puree through the system. Temperature profiles are shown for the whole microwave run, and the four batches of muscadine grape puree, but analytical analysis was only done on the first two batches of muscadine grape puree.

The purees were treated with microwaves and were pumped through three segments
of heating tubes. Muscadine grape puree was pumped through the system using a positive
displacement pump (Model A7000, Marlen Research Corp., Overland Park, Kans., U.S.A.)
and then exposed to the microwaves in the first heater. After travelling through all three
heating tubes, the muscadine puree passed through a holding tube. The minimum heat
treatment required for destruction of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*
was 82.8 °C for 0.1 min. The puree was cooled using four consecutive tubular heat
exchangers to 40 °C then aseptically packaged in aluminum-polyethylene laminated bags
(Scholle Corp, Chicago, Ill., U.S.A.) using a bag-in-box aseptic filler unit (Model PT A.F.,
Astepo, Parma, Italy). Samples were then placed in a refrigerator at 4°C. Temperature of the
product was monitored by Type T thermocouples (OMEGA Engineering, Inc., Stamford, CT,
USA) positioned at the inlet and exit of three heaters, hold tube, chiller, and filler. These
thermocouples were in the tubes and readings were taken at the center of the tube, the wall,
and the middle between these two points (Figure 4.2).
4.3.4 Storage Conditions

Samples that had been microwave processed and then aseptically packaged were placed in an incubator (Isotemp Oven 630G Fisher Scientific) at 37°C. An individual packaged sample was taken out of the incubator at 0, 2, 4, 6, and 8 weeks after processing for further analysis.

4.3.5 Colorimeter Measurement

The samples were measured on a Hunter L a b colorimeter after calibration (Hunter DP-9000; Reston, VA) directly after the pouches were opened. The ΔE value was calculated
as follows (Equation 1) with all samples compared to the 0 min time point and with the understanding that a difference of greater than 1 signifies a difference that is detectable to the naked eye.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

[1]

4.3.6 Extraction Parameters

In order to extract the muscadine grape bioactive components, 10 g of each blended sample was weighed into a conical centrifuge tube and combined with 30 ml of 0.5% acetic acid in methanol. The filled centrifuge tubes were vortexed and allowed to sit for 10 min before centrifugation at 4200 G for 20 min (IEC CL31 Multispeed Centrifuge, Thermo Electron Corporation). The supernatant was then poured into an Erlenmeyer flask and 20 ml of acidified methanol was added, vortexed, and allowed to rest for 20 min. This last step was repeated an additional 2 times. The extracted supernatant was evaporated in a TurboVap II (Upssala, Sweden) under a nitrogen stream of 7-12 psi and a water bath temperature of 45°C until the volume was less than 25 ml. This was then brought to a 25 ml volume with acidified methanol. Extractions were done in triplicate.

4.3.7 Phenol Content

Total phenol content was measured by the Folin Ciocalteu assay. A high-throughput method designed for a 96-well plate was utilized, including a gallic acid standard curve. Samples were diluted using deionized water. Folin Ciocalteu reagent (1M) was then added
to the wells and mixed in a plate reader (EnSpire 2300 Multilabel Reader, Perkin Elmer, Waltham, MA, USA). After standing for 2 min, 20% sodium carbonate solution was added and again the plate was mixed in the plate reader. After 10 min, the samples were read in the plate reader at 690 nm. This analysis was conducted in triplicate at room temperature and reported as mg gallic acid equivalents/g fresh weight muscadine (mg GAE/g FW).

### 4.3.8 Antioxidant Capacity

Total antioxidant capacity was measured by the Hydrophilic-Oxygen Radical Absorbance Capacity assay (H-ORAC) using a trolox standard. Samples and standards were diluted using 0.075 M phosphate buffer. After addition of the samples and standard curve to a 96-well plate, 70 nM fluorescein was added. After equilibration of the pipetted plate to 37°C for 15 min, 153 mM 2, 2’-azobis(2-amidinopropane) dihydrochloride (AAPH) was added. An excitation wavelength of 483 nm and an emission wavelength of 525 nm were used to tabulate oxygen radical absorbance capacity over a period of 90 min at 37°C in a Tecan Safire plate reader (Tecan Group Ltd., Männedorf, Switzerland). Samples were tested in triplicate and reported as µmol trolox equivalents/g fresh weight muscadine (µmol trolox equiv/g FW).

### 4.3.9 Total Monomeric Anthocyanin Content

Total monomeric anthocyanin content was analyzed using the pH Differential assay\(^4^3\). Samples were diluted at 1:10 using 0.025M potassium chloride buffer (pH 1.0) and 0.4M
sodium acetate buffer (pH 4.5), separately, and allowed to sit for 15 min before reading. The
spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA) was zeroed using
derionized water and then samples were read at both 520 nm and 700 nm. Absorbance (A) of
the diluted samples was calculated using Equation 2.

\[ A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}} \]  \[2\]

The diluted absorbance was used to find final anthocyanin concentrations (mg/g fresh
weight muscadine) expressed as cyanidin-3-glucoside equivalents according to Equation 3.

Monomeric anthocyanin pigment (mg/liter) = \((A \times MW \times DF \times 1000)/(\varepsilon \times 1)\)  \[3\]

Where MW = molecular weight, DF = dilution factor, and \(\varepsilon\) = molar absorptivity

4.3.10 Percent Polymeric Color

Percent polymeric color was determined by the Index for Pigment Degradation,
Polymeric Color, and Browning\(^4\). The samples were diluted to 1:10 with deionized water in
duplicate. Additional deionized water was added to one of these samples as a control and an
equal amount of 0.2 g/ml potassium metabisulfite solution was added to the other. The
samples were allowed to equilibrate for 15 min at room temperature before reading. The
spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA) was blanked with
deionized water and then samples were each read at 420, 520, and 700 nm. Percent
polymeric color was calculated at follows (Equations 4, 5, 6).

Polymeric Color (bisulfite-treated samples) = \([A_{420\text{nm}} - A_{700\text{nm}}] + (A_{520\text{nm}} - A_{700\text{nm}})] \times DF\)  \[4\]

Where DF = dilution factor
Color Density (water-treated samples) = \([A_{420\text{nm}} - A_{700\text{nm}}] + (A_{520\text{nm}} - A_{700\text{nm}})] \times DF\) \[5\]

Percent Polymeric Color = (polymeric color / color density) \times 100 \[6\]

4.3.11 Statistical Analysis

Statistical analysis was done using the Tukey test (P<0.05) on SAS software (Cary, NC, USA). Values were reported as mean ± standard error of the mean. All samples were processed in duplicate and analyses were conducted in triplicate.

4.4 Results and Discussion

4.4.1 Microwave Processing Study

Temperature profiles were taken at several different points throughout the microwave processing process. **Figure 4.3** shows the temperature profile of the entire microwave run, with the muscadine portion between 15690 s and 1900 s. The first portion of the graph represents the temperature profile during the initial sterilization of the microwave machine, followed by several fruit purees, and finally the ending of the microwave run. The remaining temperature profile graphs represent the muscadine grape puree portion of the run.
In all of the microwave temperature profile graphs, thermocouple readings are displayed for the center of the heating tube, for the wall of the tube, at a center point between those two readings, and for ambient temperature outside of the microwave system as a control. The temperature was taken at the inlet for the first heater, before the microwaves came into contact with the muscadine puree. The temperature of the product was around 10°C when it entered into the system (Figure 4.4). The microwaves came into contact with the product in the first heater. At this point, there was extreme temperature variability between the center, middle, and wall of the tubes due to the intense application of microwave power (Figure 4.5).
After this point, the sample was run through a static mixer in order to decrease temperature inconsistencies in the product. **Figure 4.6** shows that this static mixing step was effective in attenuating these temperature inconsistencies between the center, middle, and wall thermocouple readings in comparison to the readings taken in the 2nd heater. This is important from a quality standpoint in order to prevent overcooking of samples, but also from a safety standpoint so as to prevent cold spots. In **Figure 4.7** and **Figure 4.8**, temperature profiles showed a similar heat treatment and consistency for the 3rd heater.
Figure 4.6. Temperature Profile at 2nd Heater Entry- Muscadine.

Figure 4.7. Temperature Profile at 2nd Heater Exit- Muscadine.

Figure 4.8. Temperature Profile at 3rd Heater Entry- Muscadine.
Figure 4.9. Temperature Profile at 3rd Heater Exit/ Hold Tube Entry- Muscadine.

After going through the heaters, the muscadine puree entered the hold tube. Theoretically, the temperature should have been stable in this tube in order to achieve the appropriate kill step. The temperature readings showed a decrease in temperature in the outside if the hold tube in comparison to the center, suggesting that energy was dissipating out of the tubes and a need for further insulation. (Figure 4.9, Figure 4.10) Despite a slight cooling effect, the muscadine grape puree achieved a minimum heat treatment in the holding tube of 85°C for 2 min 5 s. This minimum heat treatment was sufficient for destruction of pathogens including *Salmonella* and *E. coli* O157:H7⁴¹.
After the appropriate heat treatment was achieved, the sample was chilled quickly for improved product quality (Figure 4.11). The temperature profile for the muscadine grape puree showed that it was chilled to around room temperature before being stored in the refrigerator for further cooling. At this point, samples were analyzed for color and bioactive components. While optimization of this process would be required, the muscadine grape puree was effectively sterilized by microwave processing.
Color measurements were conducted directly after opening the pouches. Color differences as assessed by the ΔE values for both the Nesbitt (Table 4.1) and the Frye (Table 4.2) varieties occurred between nearly every processing step tested and the original frozen grapes. In both varieties of the muscadine grape puree, samples became browner, suggesting potential action by enzymes such as polyphenol oxidase. In order to prevent these differences before microwave processing, it would be beneficial to implement a more streamlined grinding approach. In the Triumph variety, there was a large color difference between the BM and the AM samples, suggesting the possibility of some contamination from preceeding samples during the microwave process in the AM samples.

**Table 4.1.** Nesbitt Muscadine Grape Puree L a b Color with Microwave Processing at 95°C.

<table>
<thead>
<tr>
<th>Process</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>18.5±0.1d</td>
<td>16.9±0.2a</td>
<td>-0.5±0.1d</td>
<td>4.4</td>
</tr>
<tr>
<td>T</td>
<td>21.8±0.4c</td>
<td>14.3±0.2b</td>
<td>0.8±0.1c</td>
<td>4.5</td>
</tr>
<tr>
<td>SD1</td>
<td>21.7±0.1c</td>
<td>14.1±0.2bc</td>
<td>0.8±0.1c</td>
<td>5.1</td>
</tr>
<tr>
<td>SD2</td>
<td>22.2±0.3c</td>
<td>13.8±0.3bc</td>
<td>1.0±0.2c</td>
<td>5.7</td>
</tr>
<tr>
<td>C</td>
<td>21.8±0.3c</td>
<td>13.0±0.2c</td>
<td>2.0±0.1b</td>
<td>7.2</td>
</tr>
<tr>
<td>BM</td>
<td>23.5±0.3b</td>
<td>12.9±0.3c</td>
<td>2.6±0.2b</td>
<td>13.2</td>
</tr>
<tr>
<td>AM</td>
<td>29.5±0.3a</td>
<td>14.0±0.4bc</td>
<td>6.2±0.3a</td>
<td>13.2</td>
</tr>
</tbody>
</table>

ΔE Values are all compared to the 0m time point.

**Table 4.2.** Frye Muscadine Grape Puree L a b Color with Microwave Processing at 95°C.

<table>
<thead>
<tr>
<th>Process</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>42.9±0.3ab</td>
<td>1.0±0.1d</td>
<td>16.6±0.1a</td>
<td>1.3</td>
</tr>
<tr>
<td>T</td>
<td>43.9±0.3ab</td>
<td>1.8±0.1c</td>
<td>16.5±0.1a</td>
<td>1.1</td>
</tr>
<tr>
<td>SD1</td>
<td>42.0±0.4bc</td>
<td>1.6±0.1cd</td>
<td>16.4±0.1a</td>
<td>0.9</td>
</tr>
<tr>
<td>SD2</td>
<td>43.4±0.2ab</td>
<td>1.6±0.2cd</td>
<td>16.4±0.2a</td>
<td>2.6</td>
</tr>
<tr>
<td>C</td>
<td>40.9±0.1c</td>
<td>2.7±0.1b</td>
<td>16.6±0.1a</td>
<td>3.2</td>
</tr>
<tr>
<td>BM</td>
<td>40.6±0.3c</td>
<td>3.1±0.4b</td>
<td>16.6±0.1a</td>
<td>3.4</td>
</tr>
<tr>
<td>AM</td>
<td>33.6±0.4d</td>
<td>13.7±0.1a</td>
<td>11.5±0.1b</td>
<td>34.5</td>
</tr>
</tbody>
</table>

ΔE Values are all compared to the 0m time point.
Table 4.3. Changes in Bioactive Components of Nesbitt Muscadine Grape Puree During Microwave Processing at 95°C. Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Process</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
<th>Monomeric Anthocyanin Content (mg equiv/g FW)</th>
<th>Percent Polymeric Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>1.657±0.049 d</td>
<td>29.063±1.778 a</td>
<td>0.392±0.004 a</td>
<td>28.684 d</td>
</tr>
<tr>
<td>T</td>
<td>1.768±0.031 cd</td>
<td>30.042±1.584 a</td>
<td>0.315±0.001 b</td>
<td>41.669 c</td>
</tr>
<tr>
<td>SD1</td>
<td>1.874±0.028 cd</td>
<td>32.218±0.366 a</td>
<td>0.270±0.001 c</td>
<td>53.587 b</td>
</tr>
<tr>
<td>SD2</td>
<td>2.080±0.027 b</td>
<td>32.509±1.813 a</td>
<td>0.305±0.003 b</td>
<td>44.857 c</td>
</tr>
<tr>
<td>C</td>
<td>1.921±0.039 c</td>
<td>29.311±0.939 a</td>
<td>0.270±0.003 c</td>
<td>51.918 b</td>
</tr>
<tr>
<td>BM</td>
<td>1.809±0.038 cd</td>
<td>32.710±2.468 a</td>
<td>0.263±0.003 c</td>
<td>57.981 b</td>
</tr>
<tr>
<td>AM</td>
<td>2.129±0.218 a</td>
<td>34.357±2.434 a</td>
<td>0.124±0.001 d</td>
<td>81.756 a</td>
</tr>
</tbody>
</table>

After color testing, both Nesbitt (Table 4.3) and Frye (Table 4.4) whole muscadine grape purees were extracted and tested for bioactive component content. For total phenol content, both the Nesbitt and the Frye samples increased in gallic acid equivalents between the F samples (1.657, 2.085 mg GAE/g FW) and the SD2 samples (2.080, 2.171 mg GAE/g FW), respectively (Figure 4.12). From this point, the total phenol content of the Nesbitt variety increased after microwave processing, while there was a significant decrease in the Frye variety. It is possible that the processing parameters allowed for a better extraction of the phenols from the food matrix in the samples where there was an increase in total phenol content. In the Nesbitt variety this resulted in 140.7% retention of total phenol content (Figure 4.16). In the Frye puree, there was 82.5% retention between the F and the AM samples, still indicating substantial phenol preservation (Figure 4.17). These results are similar to those reported in purple sweet potatoes.44.
Table 4.4. Changes in Bioactive Components of Frye Muscadine Grape Puree During Microwave Processing at 95°C. aValues with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Process</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>2.085±0.039 bc a</td>
<td>34.445±0.507 b</td>
</tr>
<tr>
<td>T</td>
<td>2.350±0.016 a</td>
<td>37.054±0.498 ab</td>
</tr>
<tr>
<td>SD1</td>
<td>2.095±0.039 bc c</td>
<td>37.582±0.640 a</td>
</tr>
<tr>
<td>SD2</td>
<td>2.171±0.030 ab</td>
<td>35.744±0.725 ab</td>
</tr>
<tr>
<td>C</td>
<td>1.942±0.059 c</td>
<td>31.229±0.423 c</td>
</tr>
<tr>
<td>BM</td>
<td>2.228±0.055 ab c</td>
<td>36.417±0.775 ab</td>
</tr>
<tr>
<td>AM</td>
<td>1.720±0.222 c</td>
<td>34.570±0.832 b</td>
</tr>
</tbody>
</table>

Figure 4.12. Total Phenol Content During Microwave Processing for Nesbitt and Frye Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

In contrast to the data for total phenol content, there were no large differences tested for total antioxidant capacity (Figure 4.13). In the Nesbitt variety, there no significant differences recorded at any of the processing points (Table 4.1). In the Triumph variety, there were only small differences determined (Table 4.2). After all of the processing steps, the Nesbitt variety still maintained 118.2% of its antioxidant capacity (Figure 4.16) and the Frye variety maintained 100.4% of its antioxidant capacity. This suggests that the
antioxidants in both varieties of muscadine grape puree were stable through all of the processing parameters studied and that the puree was robust.

**Figure 4.13.** Total Antioxidant Capacity During Microwave Processing for Nesbitt and Frye Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

As with the processing study in Chapter 2, the largest differences experienced throughout processing were with the anthocyanins. The results are only reported for the Nesbitt variety because negligible levels of anthocyanins exist in the bronze varieties of muscadine grapes, including Frye. The total monomeric anthocyanin content decreased significantly between the frozen (0.392 mg/g FW) and the thawed samples (0.315 mg/g FW) (**Figure 4.14**). There was again a decrease in monomeric anthocyanin content after grinding (0.263 mg/g FW). After microwave processing, the anthocyanin content decreased significantly (0.124 mg/g FW). In all, there was 31.6% retention of total monomeric anthocyanins between the frozen and the microwave processed samples. A more efficient grinding process could help with the preservation of some of these compounds because the
retention of total monomeric anthocyanin content between the BM and AM samples was 47.0% (Figure 4.16).

Figure 4.14. Total Monomeric Anthocyanin Content During Microwave Processing for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

Figure 4.15. Percent Polymeric Color During Microwave Processing for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).
The browning of the Nesbitt samples, along with the decreased monomeric anthocyanin content lends the possibility that the anthocyanins were polymerizing. Indeed, this is what occurred, based on the percent polymeric color data (Figure 4.15). There were significant increases in percent polymeric color between the F (28.7%), SD2 (44.9%), BM (58.0%), and AM (81.8%) samples. This is congruent with previous studies that have reported polymerization of anthocyanins during processing.46,47.

Figure 4.16. Percent Retention of Bioactive Components of Nesbitt Muscadine Grape Puree During Microwave Processing. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity, TMA= Total Monomeric Anthocyanin Content, PPC= Percent Polymeric Color) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).
Figure 4.17. Percent Retention of Bioactive Components of Frye Muscadine Grape Puree During Microwave Processing. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

While there were significant increases in percent polymeric color and decreases in total monomeric anthocyanin content with processing, the muscadine grape purees were robust in terms of total phenol content and total antioxidant capacity. It is important to investigate changes throughout storage in order to understand the levels of bioactive components throughout the life of the food product.
4.4.2 Storage Study

Table 4.5. Nesbitt Muscadine Grape Puree L a b Color with Storage Time at 37°C. aValues with the same letter within columns are not significantly different (Tukey, P>0.05). bΔE Values are all compared to the 0 week time point.

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.5±0.3 b a</td>
<td>14.0±0.4 a</td>
<td>6.2±0.2 d</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27.1±0.1 d</td>
<td>10.5±0.1 b</td>
<td>8.5±0.1 c</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>28.7±0.1 c</td>
<td>8.3±0.1 c</td>
<td>10.0±0.1 b</td>
<td>6.9</td>
</tr>
<tr>
<td>6</td>
<td>31.9±0.1 a</td>
<td>6.6±0.1 d</td>
<td>11.5±0.2 a</td>
<td>9.5</td>
</tr>
<tr>
<td>8</td>
<td>32.5±0.2 a</td>
<td>6.1±0.1 d</td>
<td>11.6±0.1 a</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 4.6. Frye Muscadine Grape puree L a b Color with Storage Time at 37°C. aValues with the same letter within columns are not significantly different (Tukey, P>0.05). bΔE Values are all compared to the 0 week time point.

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.6±0.4 ab a</td>
<td>13.73±0.1 a</td>
<td>11.5±0.1 b</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31.6±0.1 c</td>
<td>8.88±0.1 b</td>
<td>11.1±0.1 c</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>32.6±0.1 b</td>
<td>7.3±0.1 c</td>
<td>11.9±0.1 a</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>33.5±0.1 ab</td>
<td>6.9±0.1 c</td>
<td>11.9±0.1 a</td>
<td>6.9</td>
</tr>
<tr>
<td>8</td>
<td>30.1±0.1 d</td>
<td>7.0±0.1 cd</td>
<td>10.1±0.1 d</td>
<td>7.7</td>
</tr>
</tbody>
</table>

The color of the stored sampled was significantly different for both the Nesbitt (Table 4.5) and the Frye (Table 4.6) varieties. The largest differences existed in the ‘a’ data point, suggesting that the samples were accelerating towards the green end of the spectrum and away from the red end of the spectrum. These differences could be associated with a decrease in anthocyanin content for the black, Nesbitt variety.
Table 4.7. Changes in Bioactive Components of Nesbitt Muscadine Grape Puree During Storage at 37°C. *Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
<th>Monomeric Anthocyanin Content (mg equiv/g FW)</th>
<th>Percent Polymeric Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.908±0.034 ab</td>
<td>29.558±2.444 a</td>
<td>0.124±0.001 a</td>
<td>81.756 c</td>
</tr>
<tr>
<td>2</td>
<td>1.804±0.051 b</td>
<td>27.753±0.359 a</td>
<td>0.110±0.003 b</td>
<td>50.885 d</td>
</tr>
<tr>
<td>4</td>
<td>1.916±0.019 ab</td>
<td>26.186±1.441 ab</td>
<td>0.047±0.001 c</td>
<td>71.744 cd</td>
</tr>
<tr>
<td>6</td>
<td>2.030±0.049 a</td>
<td>11.166±0.142 b</td>
<td>0.015±0.002 d</td>
<td>132.852b</td>
</tr>
<tr>
<td>8</td>
<td>2.084±0.063 a</td>
<td>18.563±0.309 b</td>
<td>0.021±0.001 d</td>
<td>243.401 a</td>
</tr>
</tbody>
</table>

Table 4.8. Changes in Bioactive Components of Frye Muscadine Grape Puree During Storage at 37°C. *Values with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Storage Time (weeks)</th>
<th>Total Phenol Content (mg GAE/g FW)</th>
<th>Total Antioxidant Capacity (mg trolox equiv/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.960±0.036 a</td>
<td>34.566±0.832 a</td>
</tr>
<tr>
<td>2</td>
<td>1.834±0.064 ab</td>
<td>22.481±0.981 b</td>
</tr>
<tr>
<td>4</td>
<td>1.716±0.046 b</td>
<td>21.104±0.402 bc</td>
</tr>
<tr>
<td>6</td>
<td>1.886±0.036 ab</td>
<td>20.501±0.404 bc</td>
</tr>
<tr>
<td>8</td>
<td>1.824±0.046 ab</td>
<td>18.563±0.650 c</td>
</tr>
</tbody>
</table>

Bioactive components were analyzed at each 2 week time period (Figure 4.18). For the Nesbitt variety, there were minimal significant differences measured in total phenol content (Table 4.7). This resulted in 109.2% total phenol retention by the 8 week time point (Figure 4.22). In the Frye variety, there was a significant difference between the 0 (1.970 mg GAE/g FW) and 2 week (1.834 mg GAE/g FW) time points and the 4 week time point (1.716 mg GAE/g FW) (Table 4.8), but not in the 6 or 8 week time points. There was 93.1% retention of total phenol content by the 8th week (Figure 4.23). The difference between the Nesbitt and the Frye variety could be explained by the degradation of the monomeric anthocyanins in the black, Nesbitt variety into its phenolic acid and aldehyde constituents.
Figure 4.18. Total Phenol Content During Storage at 37°C for Nesbitt and Frye Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

The total antioxidant capacity of the muscadine grape purees followed a similar trend as the total phenol content (Figure 4.19). In the Nesbitt variety, there were no significant differences determined for total antioxidant activity (Table 4.7). There was a significant decrease at the 6 week and 8 week time points, resulting in 37.9% antioxidant capacity retention after 8 weeks of storage in the Nesbitt variety (Figure 4.22). In the Frye variety, there were significant differences between the 0 week (34.566 µmol trolox equiv/g FW) and the 2 week (22.481 µmol trolox equiv/g FW) and 4 week (21.104 µmol trolox equiv/g FW) time points. This resulted in 53.7% antioxidant capacity retention by the 8th week of storage (Figure 4.23). In both varieties there were significant decreases in total phenol content after 6 and 8 weeks of storage.
Figure 4.19. Total Antioxidant Capacity During Storage at 37°C for Nesbitt and Frye Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, $P>0.05$). Error bars represent the standard error of the mean.

There continued to be large changes in anthocyanin content through storage. Results are shown solely for the black, Nesbitt variety because the anthocyanin content of the Frye variety was negligible. For total monomeric anthocyanin content, there was a significant decrease between the 0 week (0.124 mg/g FW) time point, the 2 week time point (0.110 mg/g FW), and the 4 week time point (0.047) (Figure 4.20). There was also a significant decrease at the 6 week and 8 week time points, resulting in 17.2% retention by the 8th week (Figure 4.22). This suggests that the monomeric anthocyanins were either degrading into their phenolic acid and aldehyde constituents or that they were polymerizing with other anthocyanins or phenols such as catechins.
Figure 4.20. Total Monomeric Anthocyanin Content During Storage at 37°C for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

Figure 4.21. Percent Polymeric Color During Storage at 37°C for Nesbitt Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

The percent polymeric color of the Nesbitt variety of muscadine grapes was measured (Figure 4.21). During storage, the percent polymeric color decreased between the 0 week (81.76%) and the 2 week (50.89%) time periods. This then increased significantly at 4 week time period (71.74%). There were significant increases again at the 6 and 8 week time points.
Figure 4.22. Percent Retention of Bioactive Components of Nesbitt Muscadine Grape Puree During Storage at 37°C. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity, TMA= Total Monomeric Anthocyanin Content, PPC= Percent Polymeric Color) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

Figure 4.23. Percent Retention of Bioactive Components of Frye Muscadine Grape Puree During Storage at 37°C. (TPC= Total Phenol Content, TAC= Total Antioxidant Capacity) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

4.5 Conclusion

The Nesbitt and the Frye whole muscadine grape purees proved to be robust throughout microwave processing and storage. There were minimal significant differences in
total phenol content and total antioxidant content in both varieties, even through grinding, microwave processing, and storage. Larger differences existed with respect to total monomeric anthocyanin content and percent polymeric color. Total monomeric anthocyanin content decreased significantly after grinding, microwave processing, and storage. A streamlined processing approach would be beneficial for the preservation of these compounds. Microwave processing is suggested to be a viable processing technique for this application. Further research is required to understand this food matrix during microwave processing and to understand its hedonic qualities.

4.6 Literature Cited


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SUMMARY AND SUGGESTIONS FOR FUTURE WORK
The results from this research suggest that whole muscadine grape puree is a good candidate material to be processed by continuous flow microwave sterilization. The reason for the use of a whole muscadine grape puree is to minimize waste while maximizing bioactive potential of the muscadine grapes. Microwave processing can be used to aid this goal, by enacting a safe processing step to minimize bioactive component destruction. More research would be required to further investigate the potential as whole muscadine grape puree as a potential food product, as sterilized by microwave processing.

Most importantly for consumers, a food product needs to be desirable in terms of flavor, texture, and appearance. Sensory testing of this product would be a logical step in testing its viability as a product to be sold. In addition, optimization of muscadine grape blends and °Brix would aid in achieving this goal.

Further microwave processing studies of the muscadine grape puree is essential in understanding the changes that happen during this process, including enzymatic activity. Addition of natural antioxidants or oxygen barriers could be investigated in order to minimize bioactive component losses. It would also be interesting to conduct a deeper investigation into the anthocyanin end products incurred by muscadine grape processing and storage.

Finally, in order to truly understand the effects that processing and storage, including changes in bioactive components, could have on health, further research in vitro and in vivo are needed. Anti-inflammatory, anti-carcinogenic, and anti-diabetic effects of muscadine grapes have been previously reported. It would be interesting to investigate how processing and storage could affect these properties.
APPENDIX
APPENDIX A - Changes in Bioactive Components of Whole Muscadine Grape Puree During Processing at 85°C in a Model System
A.1 Abstract

Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern United States. There are rich in bioactive components including phenols, antioxidant capacity, and anthocyanins, most notably in their skins and seeds. In order to discover what changes might occur in bioactive components of whole muscadine grape puree during processing, Nesbitt muscadine puree was heated in an oil bath at 85°C. Samples were taken at regular heating intervals and tested for total phenol content, total antioxidant capacity, total monomeric anthocyanin content, ellagic acid content, and (-)-epicatechin content. While significant differences existed in most of the parameters measured, they were small differences. A harsher processing step would be required to understand degradation of the aforementioned compounds during processing.
A.2 Introduction

In order to produce a safe muscadine grape puree product that could be sold in stores, a processing step is required, resulting in a 5-log reduction in the target pathogen. While it is essential to create a safe food product, it is also desirable to create a product that would retain most of its nutritional components and flavor properties. Currently, there is no published data about how processing affects the bioactive components in whole muscadine grape puree. In this study, whole Nesbitt muscadine grape puree was packaged and then processed at 85°C for regular intervals and then analyzed for bioactive component content.

A.3 Materials and Methods

A.3.1 Materials

Nesbitt muscadine grapes were donated by Cottle Farms (Faison, NC, USA). These grapes were received frozen and then stored at -80°C (Kelvinator Series 100 Manitowac, WI, USA). Sodium acetate, sodium phosphate, and sodium carbonate were obtained from Fisher Scientific (Waltham, MA, USA). All other chemicals and solvents were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and solvents were analytical grade.

A.3.2 Sample Preparation

Whole Nesbitt muscadine grapes were blended a food processor (Kitchenaid Chef’s Chopper, T. Joseph, MI, USA) for 2 min. They were then each blended in a spice grinder (Waring Professional Spice Grinder, Torrington, CT, USA) for 2 min in order to achieve a
small particle size. After blending, 40±2 g samples of muscadine puree were immediately weighed into plastic-lined foil pouches. The pouches were then closed and heat sealed both above and below the closure seam (American International Electric Impulse Sealer, Industry, CA, USA).

A.3.3 Processing Parameters

After the samples were prepared, they were heated in a mineral oil bath at 95°C with constant agitation (Barnant Co., Barrington, IL, USA). The come up time, the time it took to get from the temperature of the puree before heating to 95°C, was measured to be 2min30s. Processing time points were taken at 0 min, the come up time of 2min30s and an additional 1, 2, and 3 min. These samples will from here forth be denoted as 0 min, 2m30s, 2m30s+1 min, 2m30s+2 min, and 3m30s+3 min. Samples were then put into an ice water bath. The samples were processed in duplicate. Before chemical analysis, the foil pouches were cut open and then mixed thoroughly to achieve homogeneity.

A.3.4 Extraction Parameters

In order to extract the muscadine grape bioactive components, 10 g of each blended sample was weighed into a conical centrifuge tube and combined with 30 ml of 0.5% acetic acid in methanol. The filled centrifuge tubes were vortexed and allowed to sit for 10 min before centrifugation at 4200 G for 20 min (IEC CL31 Multispeed Centrifuge, Thermo Electron Corporation). The supernatant was then poured into an Erlenmeyer flask and 20 ml of acidified methanol was added, vortexed, and allowed to sit for 20 min. This last step was
repeated an additional 2 times. The extracted supernatant was evaporated in a TurboVap II (Upssala, Sweden) under a nitrogen stream of 7-12 psi and a water bath temperature of 45°C until the volume was less than 25 ml. This was then brought to a 25 ml volume with acidified methanol. Extractions were done in triplicate.

**A.3.5 Total Antioxidant Capacity as Measured by DPPH**

Total antioxidant capacity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, using trolox as a standard. For this assay, 50 µl of sample was pipetted into a test tube and then mixed with 2.95 ml 40 ppm DPPH solution. Samples were allowed to sit in the dark for 1 hour and then were read on a spectrophotometer (Shimadzu UV-2450 Synergy HT Multi-Detection Microplate Reader, BioTek) at 515 nm. Samples were diluted with deionized water and run in triplicate.

**A.3.6 Total Antioxidant Capacity as Measured by ORAC**

Total antioxidant capacity was measured by the Hydrophilic-Oxygen Radical Absorbance Capacity assay (H-ORAC) using a trolox standard. Samples and standards were diluted using 0.075 M phosphate buffer. After addition of the samples and standard curve to a 96-well plate, 70 nM fluorescein was added. After equilibration of the pipetted plate to 37°C for 15 min, 153mM 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added. An excitation wavelength of 483 nm and an emission wavelength of 525 nm were used to tabulate oxygen radical absorbance capacity over a period of 90 min at 37°C in a
Tecan Safire plate reader (Tecan Group Ltd., Männedorf, Switzerland). Samples were tested in triplicate and reported as μmol trolox equivalents/g fresh weight muscadine (μmol trolox equiv/g FW).

A.3.7 Total Phenol Content

The total phenol content was measured by the Folin-Ciocalteu assay using a gallic acid standard curve. Sample (0.1 ml) and deionized water (7.9 ml) were pipetted into test tubes and vortexed. The Folin-Ciocalteu reagent (0.5 ml, 2mM) was added to each tube. After 30 seconds, but before 8 minutes, 20% sodium carbonate (1.5 ml) was added to each test tube and mixed by vortexing. Test tubes were incubated in the dark for 2 hours at room temperature. The absorbance of each sample was read at 765 nm using a spectrophotometer (Shimadzu UV-2450 Synergy HT Multi-Detection Microplate Reader, BioTek). A standard curve was plotted using the gallic acid concentration (milliequivalents) versus the absorbance at 765 nm. Samples were tested in triplicate.

A.3.8 Total Monomeric Anthocyanin Content

Total monomeric anthocyanin content was analyzed using the pH Differential assay. Samples were diluted at 1:10 using 0.025M potassium chloride buffer (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5), separately, and allowed to sit for 15 min before reading. The spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA) was zeroed using deionized water and then samples were read at both 520 nm and 700 nm. Absorbance (A) of the diluted samples was calculated using Equation 1.
\[ A = (A_{\lambda \text{520nm}} - A_{\lambda \text{700nm}}} \text{pH 1.0} - (A_{\lambda \text{520nm}} - A_{\lambda \text{700nm}}} \text{pH 4.5}) \]  

The diluted absorbance was used to find final anthocyanin concentrations (mg/g fresh weight muscadine) expressed as cyanidin-3-glucoside equivalents according to Equation 2.

Monomeric anthocyanin pigment (mg/liter) = \( \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \)  

Where MW= molecular weight, DF= dilution factor, and \( \varepsilon \)= molar absorptivity

A.3.9 HPLC Analysis of Bioactive Compounds

HPLC analyses were performed using an Agilent Technologies 1200 series HPLC (Santa Clara, CA, U.S.A) with a photodiode array (PDA) detector attached to an autosampler (plus Chemstation software as a controller and for data processing). Phenol separation was conducted using a Phenomenex Synergi 4 μ Hydro-RP 80A column (250 mm x 4.6 mm x 5 μm, Torrance, CA, USA). The mobile phase consisted of 2% acetic acid in distilled H₂O (solvent A) and 0.5% acetic acid in 50% acetonitrile in distilled water (solvent B). The flow rate was 1 ml/min with a step gradient of 10%, 15%, 25%, 55%, 100% and 10% of solvent B at 0, 13, 20, 50, 54 and 60 min, respectively. Samples were filtered through 0.2 mm cellulose filters before injecting 10 μl on the HPLC column (25 °C). Quantification of compounds was performed from the peak areas recorded at 254 for ellagic acid and its conjugates in reference to the calibration curve obtained with external reference standards.
A.3.10 Statistical Analysis

Statistical analysis was done using the Tukey test (P<0.05) on SAS software (Cary, NC, USA). Values were reported as mean ± standard error of the mean. All samples were processed in duplicate and analyses were conducted in triplicate.

A.4 Results and Discussion

Nesbitt whole muscadine grape puree was processed at 85°C and then analyzed for bioactive component content at various processing times. The total antioxidant capacity was measured by two different assays, DPPH (Figure A.1) and ORAC (Figure A.2). In both of the assays there was a general trend of stability through at least the 2m30s+1m time point and then a decrease in antioxidant capacity with further processing. The antioxidant capacity was uniformly larger when measured by ORAC when compared to DPPH. Still, in the cases of the most severe processing, there was a 96.6% antioxidant capacity retention in the ORAC assay and 73.4% retention using the DPPH assay (Figure A.5).

Figure A.1. DPPH Total Antioxidant Capacity vs. Processing Time at 85°C for Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.
Figure A.2. ORAC Total Antioxidant Capacity vs. Processing Time at 85°C for Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.

The Nesbitt muscadine grape puree increased in total phenol content during processing (Figure A.3). Increases in phenol content could, in part, be related to a better extraction from the food matrix. This resulted in 116.9% retention of total phenols by the end of the processing period studied (Figure A.5).

Figure A.3. Total Phenol Content vs. Processing Time at 85°C for Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.
The total monomeric anthocyanin content of the Nesbitt puree remained relatively stable through processing (Figure A.4). There were significant decreases in the samples that were heat treated for a longer period of time, but the anthocyanin levels remained at 96.6% of their original levels after the studied processing periods (Figure A.5).

**Figure A.4.** Total Monomeric Anthocyanin Content vs. Processing Time at 85°C for Muscadine Grape Puree. Values with the same letter within each bar series are not significantly different (Tukey, P>0.05). Error bars represent the standard error of the mean.
Figure A.5. Percent Retention of Bioactive Components of Nesbitt Muscadine Grape Puree During Processing at 85°C. (TPC= Total Phenol Content, DPPH= Total Antioxidant Capacity measured by DPPH, ORAC= Total Antioxidant Capacity measured by ORAC, TMA= Total Monomeric Anthocyanin Content) Values with the same letter within each bar series are not significantly different (Tukey, P>0.05).

Levels of both ellagic acid and (-)-epicatechin remained stable through processing (Table A.1). This suggests that these phenols are robust through processing in this food matrix.

Table A.1. Changes in Bioactive Compounds in Nesbitt Muscadine Grape Puree During Processing at 85°C. aValues with the same letter within columns are not significantly different (Tukey, P>0.05).

<table>
<thead>
<tr>
<th>Process Time</th>
<th>Ellagic Acid (mg/g FW)</th>
<th>(-)-Epicatechin (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>0.028±0.001 a</td>
<td>0.530±0.002 b</td>
</tr>
<tr>
<td>2m30s</td>
<td>0.028±0.001 a</td>
<td>0.566±0.007 ab</td>
</tr>
<tr>
<td>2m30s+30s</td>
<td>0.029±0.001 a</td>
<td>0.595±0.006 a</td>
</tr>
<tr>
<td>2m30s+1m</td>
<td>0.028±0.001 ab</td>
<td>0.542±0.003 ab</td>
</tr>
<tr>
<td>2m30s+1m30s</td>
<td>0.027±0.001 ab</td>
<td>0.540±0.012 ab</td>
</tr>
<tr>
<td>2m30s+2m</td>
<td>0.025±0.001 bc</td>
<td>0.559±0.002 ab</td>
</tr>
<tr>
<td>2m30s+2m30s</td>
<td>0.024±0.003 c</td>
<td>0.459±0.066 c</td>
</tr>
<tr>
<td>2m30s+3m</td>
<td>0.028±0.001 a</td>
<td>0.536±0.001 ab</td>
</tr>
</tbody>
</table>
A.5 Conclusion

All of the bioactive components remained relatively stable through processing at 85°C for all of the studied process times. This suggests that the muscadine grape puree is a robust food matrix with respect to phytochemical retention. It would be beneficial to enact a harsher heat-treatment to understand if these components would degrade during processing.