CHEMICAL AND ECONOMIC ANALYSIS OF A VALUE-ADDED PRODUCT FROM MUSCADINE GRAPE POMACE

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To my family and all the people that have believed in me
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Polyphenolics are recognized for their antioxidant capacity and their contribution to flavor and color of several fruits and vegetables. Health benefits of these compounds are still under investigation and their use is currently based on observed experiments both in vitro and in vivo. The sources of natural antioxidants such as polyphenolics include fruits, vegetables, spices, and herbs. Muscadine grapes (Vitis rotundifolia) are an important fruit crop in the southeastern region of U.S. due to their tolerance to Pierce’s disease. These grapes have a unique polyphenolic profile compared to other Vitis species that has sustained efforts to develop a value-added product from them. By-products (skin and seeds) from muscadine grape processing have presented processors with an inexpensive source of polyphenolics to produce valuable food ingredients. This research study evaluated isolation techniques and subsequent stability of compounds recovered from muscadine grape pomace. Methods to reduce or eliminate sugars were also explored by the use of yeast fermentation and solid-phase isolation. Following fermentation, methods to remove residual water were explored using freeze drying, spray drying and vacuum evaporation. Additionally, processes were assessed to determine the efficiency and profitability for the muscadine grape industry. Target compounds extracted from muscadine grape pomace showed high antioxidant activity (34.3 ± 0.57 μmol Trolox Equivalents/g).
Although processing positively influenced polymerization and stability of polyphenolics, antioxidant capacity was significantly decreased. Initially, high temperature, low concentration of polyphenolics, oxygen exposure, and high pH environment were considered major factors that affected polyphenolic content and bioactive characteristics. Latter information indicated that such factors had a significant effect on the polyphenolics antioxidant capacity but only a small change on their concentration. Vacuum drying showed the best results for maintaining polyphenolics concentration and preserving their antioxidant capacity following fermentation. Fermentation proved to be a more practical procedure than solid phase isolation to eliminate or reduce sugars without putting the valuable nutritional characteristics of the final product at risk. Fermentation following a simple concentration step was an economical way to obtain polyphenolics from muscadine grape skins. Results from three isolation techniques suggested that a simple muscadine grape by-product concentration followed by a drying operation can be suitable and profitable for a typical muscadine producer. This process could be adjusted and implemented by any fruit or vegetable processor to estimate the potential increase in profit of their additional by-product operation.
Polyphenolics are plant metabolites that are recognized for their antioxidant capacity and their contribution to flavor and color of several fruits and vegetables (Croft 1999). In recent years, polyphenolic characteristics such as enzyme inhibition and radical scavenging have captured consumer’s attention because of the association of these compounds, and their activity, with long-term human health (Parr and Bowell 2000).

Since the knowledge of how polyphenolics behave in the body is still limited, the efficiency of natural antioxidant products is not easy to estimate. Furthermore, phenolic compounds could act synergistically or antagonistically which complicates the antioxidant product assessment. Therefore, the use of most natural antioxidant products is currently based on empirical knowledge from research conducted in model systems and some existing products (Meyer and others 2002).

The sources of natural antioxidants such as vitamin C, tocopherols, polyphenolics, and organic acids include fruits, vegetables, spices, and herbs. The share of polyphenolics in the market of antioxidants has increased as the demand for antioxidants from natural sources grows steadily. In 1996, 26% of the food antioxidant market was occupied by natural antioxidants with a yearly growing rate of 6-7% (Meyer and others 2002). In berries and fruits, the most abundant antioxidants are vitamin C and polyphenolics. Companies such as Optiture (USA), Chr. Hansen (Denmark), Overseal Natural Ingredients (GB), Quim Dis (France), Inheda (France), and Folexco (USA) share the market of extracts and concentrates from these sources (Meyer and others 2002).

Muscadine grapes (*Vitis rotundifolia*) are unique among grape species due to the presence of polyphenolics such as anthocyanin diglucosides and ellagic acid and its derivatives (Lee and
Talcott 2004). Moreover, muscadine grapes are an important native fruit crop to the south and southeastern U.S. due to their remarkable tolerance to Pierce’s disease. Pierce’s disease is caused by a bacterium (*Xylella fastidiosa*) that invades the vascular system of grape vines causing decay, and this microorganism is commonly transported by glassy-winged sharpshooters (Pooling, 1996; Mizell and others 2003). Due to the resistance to Pierce’s disease, muscadine grapes have sustained a commercial industry in the southeast region of the U.S. (Ruel and Walker 2006).

A growing concern for grape juice and wine producers is the handling of by-products (skin and seeds) and the production of food-grade products with added value. The importance of compounds in grape pomace that may have significance to the food industry due to their association to human health has sustained efforts to produce valuable food ingredients (Sort 2003). In the case of muscadine grapes, almost half of the fresh fruit weight constitutes skin that is a very rich source of phytochemicals such as resveratrol, ellagic acid and some flavonols (Pastrana-Bonilla and others 2003; Morris and Brady 2004). However, unique characteristics of muscadine grapes have presented technological challenges to extract polyphenolics from the pomace.

This research study assessed isolation techniques and subsequent stability of polyphenolics recovered from muscadine grape pomace (target compounds from the grape skins only) using various extraction and processing techniques to obtain a dry powder or a low water concentrate. Methods to reduce or eliminate sugars were explored by the use of aerobic yeast fermentation and partitioning from solid-phase supports with specific affinities to the compounds of interest. Processing methods to reduce or remove residual water were explored using freeze drying, spray drying and vacuum evaporation techniques. It was hypothesized that extract processing would
affect polyphenolic stability. Processes were sought to optimize the concentration and stability of target polyphenolics in an effort to determine the most efficient and profitable process for the industry. The specific objectives of this study were:

- To evaluate the antioxidant capacity, polyphenolic composition and pigment stability of muscadine grape pomace extract as affected by concentration, sugar elimination, and dehydration processes.

- To propose protocols suitable for the Muscadine grape industry to develop an extract rich in polyphenolics.

- To evaluate the profitability of best proposed protocols to manufacture an extract from the muscadine grape pomace.
CHAPTER 2
LITERATURE REVIEW

2.1 Muscadine Grape

Muscadine grapes (*Vitis rotundifolia*) are an important native fruit crop to the south and southeastern U.S and have been cultivated and utilized by people in these regions before European colonization (Poling 1996). Due to the high pressure of insect vectors and environmental routes for plant diseases, it is not possible to commercially cultivate most *Vitis* species, other than *Vitis rotundifolia*. Because of their adaptation, many muscadine grape cultivars have shown remarkable tolerance to pests and diseases (Poling 1996). More specifically, muscadine grapes are resistant to Pierce’s disease caused by *Xylella fastidiosa*, a bacteria that is commonly spread by glassy-winged sharpshooters (*Homalodisca coagulate*) that invade the vascular system of grape vines and cause a significant vine decline over time (Mizell and others 2003). Due to the severity of Pierce’s disease in the southeastern region of U.S., *Vitis rotundifolia* and *Vitis arizonica*, both native to these regions, have demonstrated resistance to Pierce’s disease sufficient to create a commercial industry (Ruel and Walker 2006).

Muscadine grapes are found in tight small clusters of 3 to 10 berries that may not ripen uniformly, thus, they are harvested as single berries instead of bunches (Himelrick 2003; Takeda and others 1983). The fruit possess a much thicker skin than other grape species, has large seeds, is very turgid, and has musky-flavored pulp. The fruit is found in black to bronze colors (Croker and Mortensen 2001; Himelrick 2003). The production area of muscadine grapes is around 5,000 acres. Georgia and North Carolina encompass more than half of the total acreage, and, Arkansas and Florida also hold an important portion of the cultivated area (Table 2-1). Commercial production of muscadine grapes is divided mainly into fresh fruit and wine. According to Halbrooks (1998), muscadine juice manufacture has been positively evaluated, but
its production has still not been exploited. Fresh fruit is marketed as pick-your-own and packaged berries. In 1979, 95% of the grape production of Florida was sold directly to customers (Degner and others 1981). A recent report divided the muscadine market into juice, wine, vinegar, sweet spreads, dry products, and by-products and nutraceuticals (Morris and Brady 2004). The most common product manufactured with muscadine grapes is wine, which is attractive due to its fruity flavor. The shelf life of these wines may be shorter than other wines due to changes in their pigments during aging. If there is an oversupply of muscadine wine, high quality vinegar might be produced to create an extra product of this industry (Sims and Morris 1985; Morris and Brady 2004).

Table 2-1. Estimated muscadine grape acreage in the southeastern United States (Cline and Fisk 2006).

<table>
<thead>
<tr>
<th>State</th>
<th>Acreage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>&lt; 75</td>
</tr>
<tr>
<td>Arkansas</td>
<td>400 – 500</td>
</tr>
<tr>
<td>Florida</td>
<td>600 – 1,000</td>
</tr>
<tr>
<td>Georgia</td>
<td>1,400</td>
</tr>
<tr>
<td>Lousiana</td>
<td>70</td>
</tr>
<tr>
<td>Mississippi</td>
<td>300</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1,300</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>South Carolina</td>
<td>300</td>
</tr>
<tr>
<td>Tennessee</td>
<td>160</td>
</tr>
<tr>
<td>Texas</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Virginia</td>
<td>&lt; 50</td>
</tr>
</tbody>
</table>

Since 40% of the fruit is skin (Pastrana-Bonilla and others 2003), only about half of the fruit is used in conventional products such as juice and wine. After pressing, processors must decide the best way to handle their waste and such a substantial volume of residue can only mean significant costs to the overall procedure. Further processing of pomaces rich in polyphenolics, such as muscadine grapes, can lead to an increase in economic value per ton of fruit and the decrease of waste material (Ector 2001). Pigments could be extracted from the skins and be used
as food ingredients; these compounds could then contribute to the overall product color and increase its nutraceutical content (Morris and Brady 2004).

2.2 Polyphenols

There is substantial interest in polyphenolic compounds in foods due to their effects on food quality and their association with human health benefits against coronary heart disease and cancer (Parr and Bowell 2000). Polyphenolic compounds are not only recognized for their bioactive properties but also for their contribution to flavor and color of several fruits and vegetables (Croft 1999). Approximately 8000 phenolic compounds have been identified that possess a common aromatic structure with at least one hydroxyl group (Robbins 2003). These phenolic compounds originate as secondary plant metabolites, from phenylalanine and tyrosine precursors and the phenylpropanoid pathway, and are essential for plant reproduction, stability, and growth processes in plants (Croft 1999; Shahidi and Naczk 2003).

2.2.1 Structure and Classification

According to the number of phenol subunits, phenolic compounds can be divided into simple phenols and polyphenols and are further divided into other categories depending on their structure and activity (Robbins 2003; Shahidi and Naczk 2003). The term “polyphenolics” is commonly used to describe compounds of this nature. Phenolic compounds are formed by the release of ammonia from phenylalanine and tyrosine due to the action of phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) to form \textit{trans}-cinnamic acid and \textit{p}-coumaric acid, respectively. Subsequently, these two compounds serve as precursors in the formation of several phenolic compounds (Shahidi and Naczk 2003). De Bruyne and others (1999) explained that phenolic compounds are products of a plant aromatic pathway: the shikimate section that generates the aromatic amino acids phenylalanine, tyrosine and tryptophan; the phenylpropanoid pathway that produces the cinnamic acid derivatives; and the flavonoid route that produces a
diversity of flavonoid compounds. Phenolic compounds are divided into the following groups: hydroxylated derivatives of benzoic or cinnamic acids (phenolic acids); coumarins; flavonoids and stilbenes; lignans and lignins; suberins and cutins; and tannins (Shahidi and Naczk 2003).

### 2.2.1.1 Phenolic acids and simple phenols

Phenylpropanoids are typically known as “phenolic acids”. These compounds are a group of aromatic secondary plant metabolites that have one carboxylic acid functional group. While the basic skeleton in all these compounds is similar, the number and positions of the hydroxyl or carboxyl groups on the aromatic ring generate a diversity of compounds (Shahidi and Naczk 2003; Robbins, 2003).

Current research relates phenolic acids with various plant functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, among others (Wu and others 2000). In foods, phenolic acids are related to sensory characteristics and nutritional properties. Moreover, these compounds are intimately related with enzymatic browning thus affecting food quality and shelf life (Robbins 2003).

### 2.2.1.2 Flavonoids

Flavonoids are widely known for their red, purple, and blue color and their association with health in diets rich in fruits and vegetables. Currently more than 6,400 flavonoids have been identified from diverse plant species. Flavonoids include a broad group of compounds that share a similar diphenylpropane (C6-C3-C6) basic structure (Figure 2-1) and depending on the position of the association of the aromatic ring and the benzopyrano, flavonoids could be divided in three main groups: flavonoids, isoflavonoids, and neoflavonoids (Marais and others 2006; Winkel 2006).

Flavonoids are formed from the condensation of phenylpropane with coenzyme A to form chalcones that will then form other end products. The differences within flavonoids are dictated
by the level of oxidation of the central pyran ring of the main diphenylpropane structure; the number and distribution of hydroxyl, carboxyl groups; and the degree of alkylation or glycosilation. Flavones, flavanones, flavonols, flavanonols, flavan-3-ols and other related compounds may be formed due to all these substitutions (Shahidi and Naczk 2003). From these compounds, flavonols, flavones, flavanones, catechins, anthocyanidins and isoflavones are commonly consumed (Le Marchand 2002).

Flavones and flavonols are the most abundant flavonoids in foods with approximately 100 flavones and 200 flavonols identified in plants. The most common flavonols are myricetin, quercetin and kaempferol which are found in many important fruits and vegetables (Table 2-2). Flavonols differ from flavones because of the presence of a hydroxyl group on the 3-position and are also known as 3-hydroxyflavones (Shahidi and Naczk 2003; Le Marchand 2002). Flavanones and flavanonols have a saturated C-ring. Flavanonols differ from flavanones due to the presence of a hydroxyl group on the 3-position. Flavanones are mainly found in citrus fruits, these compounds are frequently glycosylated in the 7-position with disaccharides (Tomás-Barberán and Clifford 2000). Catechins and anthocyanins are also known as flavans and are an important group of flavonoids. Catechins are primarily found in tea and red wine while anthocyanins are generally found in many berries and flowers (Le Marchand 2002; Shahidi and Naczk 2003).
Due to their bright colors, flavonoids can act as visual attractants for pollinating insects. Some flavonoids might have a protective mechanism against predatory insects. UV protection and growth and development are among other functions and processes associated with flavonoids in plants (Winkel 2006; Pietta 2000). In addition to their physiological functions in plants, flavonoids are also significant components of the human diet. Flavonoids are present in most edible fruits and vegetables, and depending on their source, their bioavailability may vary tremendously. Thus, dietary intake of flavonoids is variable, ranging from 3 to 800 mg/day (Erlund 2004; Le Marchand 2002; Pietta 2000).

Table 2-2. Classes and dietary sources of flavonoids (Shahidi and Naczk 2003).

<table>
<thead>
<tr>
<th>Class</th>
<th>Dietary Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalcone</td>
<td>Miscellaneous</td>
</tr>
<tr>
<td>Flavone</td>
<td>Fruit skins, parsley, celery, buckwheat, citrus, red pepper, red wine,</td>
</tr>
<tr>
<td></td>
<td>tomato skin</td>
</tr>
<tr>
<td>Flavanone</td>
<td>Citrus</td>
</tr>
<tr>
<td>Flavonol</td>
<td>Leek, broccoli, endives, grapefruit, tea, onion, lettuce, tomato, beeries,</td>
</tr>
<tr>
<td></td>
<td>apples, olive oil</td>
</tr>
<tr>
<td>Flavanonol</td>
<td>White grape skins, soybean, fruits</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>Soybean</td>
</tr>
<tr>
<td>Flavanol</td>
<td>Tea</td>
</tr>
<tr>
<td>Anthocyanidin</td>
<td>Berries, dark fruits</td>
</tr>
</tbody>
</table>

2.2.1.3 Tannins

Tannins are a group of oligomeric and polymeric water-soluble polyphenols. Tannins are divided in two main groups (Figure 2-2) due to their structure and susceptibility to acid hydrolysis: condensed and hydrolyzable tannins (Meyers and others 2006). The name “tannins” comes from the ability of these compounds to tan animal skin into leather by protein precipitation (Lei 2002). These compounds are usually found in the bark of trees and can precipitate proteins from aqueous solutions (Shahidi and Naczk 2003). Condensed tannins are oligomers or polymers of flavan-3-ols. Approximately 50 proanthocyanidins have been identified in the literature. Based on the monomer units, condensed tannins could be further
divided in procyanidins, propelargonidins, and prodelphinidins. These proanthocyanidins are large molecules ranging from 2000 to 4000 Da. Hydrolyzable tannins are formed by the glycosylation of gallic or ellagic acids, so, they are further divided in two groups: gallotannins and ellagittannins. These tannins range from 500 to 2800 Da (Kraus and others 2003; Meyers and others 2006; Shahidi and Naczk 2003).

Tannins have anti-nutritional properties due to the relation with proteins to form complexes making them unavailable for digestion (Shahidi and Naczk 2003). In contrast, tannins are associated with health benefits possessing antimutagenic and anticarcinogenic properties, reduction of serum cholesterol, and many other biological functions in herbivore animals (Meyers and others 2006; Kraus and others 2003).

![General structure of hydrolyzable tannins (left) and condensed tannins (right)](Shahidi and Naczk 2003; Meyers and others 2006).

**2.2.2 Polyphenolics as Antioxidants**

Polyphenolics are naturally occurring antioxidants that prevent oxidation of substrates containing these compounds. This prevention occurs not only in foods but also in humans, relating phenolic compounds with the control of many degenerative diseases. Antioxidants protect oxidative substrates by reducing the concentration of oxygen, intercepting singlet
oxygen, or scavenging initial radicals to prevent the activity of reactive oxygen, nitrogen and chlorine species that are related with diseases such as arthritis, diabetes, atherosclerosis, among others (Shahidi and Naczk 2003; Le Marchand 2002).

Even though the exact association of polyphenolics with some diseases is not fully understood, flavonoids have proven not only to inhibit enzymes directly related in the generation of reactive oxygen species, but also chelate metals which are important in the oxygen metabolism (Pietta 2000). Moreover, since polyphenolics have a wide range of hydrophobicity, both the hydrophilic and lipophilic environments could be protected by these compounds (Parr and Bowell 2000).

The most predominant method of antioxidant activity seems to be the hydrogen donation, also known as radical scavenging (Robbins 2003). Free radicals could cause extensive damage to macromolecules in the body. Free radicals remove a proton from macromolecules, generating highly reactive radicals of high molecular weight. This creates a chain degradation reaction where radicals are trying to stabilize by removing a proton from a neighboring molecule. Polyphenolics donate a hydrogen atom, breaking the degradation cycle. Furthermore, if phenolic compounds react with the initial forms of free radicals, they donate their proton, thus quenching the free radical and producing a less reactive radical that will be subsequently stabilized by resonance delocalization (Parr and Bowell 2000; Shahidi and Naczk 2003).

Many studies have suggested that the antioxidant properties of phenolic compounds, specifically flavonoids, are generally located in the B ring of the molecule, more specifically in the number of hydroxyl groups present in that ring (Reviewed by Pietta 2000). However, flavonoids that do not possess a hydroxyl group in the B ring still have antioxidant activity. In
the case of tannins, the antioxidant capacity is closely related to the degree of polymerization due to the amount of radicals that can be quenched per molecule (De Bruyne and others 1999).

2.2.3 Polyphenolics in Muscadine Grapes

Muscadine grapes are unique among grapes species due to the presence of anthocyanin 3,5-diglucosides, free ellagic acid and numerous derivatives of ellagic acid such as ellagic acid glycosides and ellagitannins (Lee and Talcott 2004). As an isolated compound, anthocyanin diglycosides are more resistant to oxidative and thermal processes than a respective monoglycoside, however in vivo they exhibit less color, enhanced formation of polymers, and a greater susceptibility to exhibit brown color than foods that contain anthocyanin monoglucosides (Lee and Talcott 2004). Other unique compounds in muscadine grapes are ellagic acid and its derivatives which are commonly associated with fruits such as blackberries, raspberries, pomegranates, certain tree nuts and strawberries (Rommel and Wrosltad 1993; Lee 2004).

Characterization and quantification of phenolic compounds present in muscadine grapes has been extensively studied. The polyphenolics, other than anthocyanins, found in muscadine are flavanols (catechin and epicatechin), ellagic acid, flavonols (myricetin, quercetin and kaempferol), gallic acid, and resveratrol ranging from 0.1 to 86.1 mg/100g of whole fruit in 10 different muscadine cultivars (Pastrana-Bonilla and others 2003). These compounds are mainly located in the skins and the pulp. The skins contain ellagic acid, flavonols and resveratrol while the seeds contain flavanols and gallic acid (Pastrana-Bonilla and others 2003). In another study (Yi and others 2005) five anthocyanin aglycones were identified after hydrolysis. However, two studies at the University of Florida (Talcott and Lee 2002; Talcott and others 2003) have identified six main forms of anthocyanidins in muscadine grapes including pelargonidin.
2.3 Anthocyanins

Interest in natural food colorants and additives continues to increase in response to consumer demands and the potential health benefits they impart (Del Pozo and others 2004). Anthocyanins are the most important class of water-soluble pigments responsible for the red, blue and violet colors in many fruits, vegetables, roots, tubers, bulbs, legumes, cereals, leaves and flowers (Bridle and Timberlake 1997). Many fruits contain high concentrations of anthocyanins and several studies have shown a relationship between fruit consumption and reduction of certain diseases attributable to the presence of antioxidant polyphenolics (Rommel and Wroslstad 1993; Parr and Bowell 2000; Aaby and others 2005). Polyphenolics such as anthocyanins are effective radical scavengers and can break free radical reactions through their electron donation, metal chelation, enzyme inhibition, and oxygen radical quenching capabilities (Pastrana-Bonilla and others 2003).

2.3.1 Structure and Occurrence

Anthocyanins are flavonoids formed by condensation of phenylpropane compounds with participation of three molecules of malonyl coenzyme A that form a chalcone that is cyclated under acidic conditions (Shahidi and Naczk 2003). Currently, 17 naturally occurring anthocyanidins have been identified, and only six of them (Figure 2-3) are common in higher plants: pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp). From those six, Cy, Dp, and Pg are the most widespread in nature (Kong and others 2003). The six different anthocyanin base forms vary based on their hydroxyl (OH) and methoxyl (OCH₃) substitutions on the anthocyanidin base (B ring). Moreover, anthocyanins are exclusively found as glycosides in undisturbed tissues of flowers and fruits, where they are bound to one or more molecules of sugar yielding around 200 different anthocyanins that have been identified (Shahidi and Naczk 2003).
The anthocyanidin form or flavylium cation (2-phenylbenzopyrilium) is the basic structure of the anthocyanin molecule which has conjugated double bonds responsible for light absorption around 500 nm, thus becoming visible with a red hue to the human eye (Rein 2005). Depending on the presence of hydroxyl or carboxyl, the hue of the color will be either blue or red, respectively (Shahidi and Naczk 2003).

![Chemical structures of anthocyanidins](image)

Figure 2-3. Chemical structures of anthocyanidins (Shahidi and Naczk 2003).

Anthocyanins are usually glycosylated with glucose, galactose, arabinose, xylose, or rhamnose as 3-glycosides (monoglycosides), 3,3, 3,5 or 3,7-diglycosides (diglycosides) or triglycosides (Rein 2005). Anthocyanins can also be acylated with organic acids that are usually aromatic or aliphatic dicarboxyl acids bound to the anthocyanin through ester bonding. The most common acylating agents are: hydroxycinnamic acids such as p-coumaric, ferulic, caffeic and sinapic acids, hydroxybenzoic acids like gallic acid, and aliphatic acids including malonic, acetic, malic, succinic and oxalic acids (Francis 1989; Bruneton 1995; Cabrita and Andersen 1999).

The most important role of anthocyanins is their ability to impart color to the plants and plant products in which they occur. This color establishment plays a crucial role in the attraction
of animals for pollination and seed dispersal. They also act as antioxidants, phytoalexins or antibacterial agents, possess known pharmacological properties, and are used for therapeutic purposes (Kong and others 2003).

2.3.2 Color Stability

Anthocyanins are unstable compounds and the color loss may depend on the hydrolysis of the unstable aglycone form. The stability of anthocyanins is closely related to self-association, concentration and structure, pH, organic chemicals, temperature, light, enzymes, oxygen, copigments, metallic ions, ascorbic acid, sugars, and processing (Shahidi and Naczk 2003; Stingzing and others 2002). Glycosylation, acylation, and hydroxylation contribute to the anthocyanin stability. It has been shown that the acyl groups maintained color when bound to anthocyanins. Polyacylated anthocyanins are more stable than monoacylated ones. Hydroxylation in positions C-4 and C-5 prevents water addition to form colorless species (Saito and others 1995; Rein 2005; Turker and others 2004; Shahidi and Naczk 2003). Copigmentation enhances the color of anthocyanins by increasing the absorbance due to pigment concentration and association with other compounds by hydrophobic interaction between aromatic bases of the molecules involved (Shahidi and Naczk 2003). Copigmentation occurs through numerous interactions, such as intermolecular and intramolecular complexes, self-association, and metal complexation. Anthocyanin copigmentation results in a stronger and more stable color than a singular anthocyanin molecule. Furthermore, overlapping association of copigmentation results in a prevention of nucleophillic attack of water to the molecule (Rein 2005), thus providing the new molecule with higher color intensity and more stability. Copigments are colorless or slightly yellowish natural molecules in plant cells that exist along with anthocyanins, the most common copigments are flavonoids. Other copigments could be organic acids, amino acids, and metal ions (Brouillard and others 1989).
Anthocyanins are very sensitive to pH shifts. In solutions they exist in four different forms: blue neutral and ionized quinonoidial base, red flavylium cation or oxonium salt, colorless pseudobase, and colorless chalcone (Figure 2-4). Each of the four species has a variety of tautomeric forms and the chalcone could exist as cis or trans forms (Shahidi and Naczk 2003; Clifford 2000).

![Anthocyanin equilibria: quinonoidal base (A), flavylium cation (B), carbinol base or pseudobase (C) and chalcone (D) (Clifford 2000; Shahidi and Naczk 2003).](image)

Even though anthocyanins can exhibit a diversity of color tones in the pH range from 1-14, they are more stable at acidic media showing an intense red coloration in the pH range of 1 to 3 (Rein 2005; Shahidi and Naczk 2003). The increase of pH decreases the concentration of the flavylium cation, thus, decreasing the intense red color to form the carbinol base. This compound does not have a conjugated double bond between the rings A and B so there is no absorption of visible light. As the pH continues to increase (Figure 2-5), the colored
quinonoidal form is produced by the loss of a hydrogen atom. If pH continues to rise, the cabinol base yields the colorless chalcone form (Rein 2005).

Figure 2-5. Effect of pH value on anthocyanin equilibria (Clifford 2000).

Anthocyanin stability is also affected by temperature. This degradation process follows first order kinetics (Kirca and others 2006). Elevated temperatures alter the anthocyanin equilibria or hydrolyze the glycosidic bonding to form unstable chalcones or aglycone forms, respectively, as the first step in thermal degradation. Ultimately, thermal degradation leads to the formation of brown pigments (Rein 2005; Clifford 2000). On the other hand, extremely low temperatures also affect the quality of anthocyanins. Quinonoidal formation is favored by low temperatures, therefore if a product was frozen red, it might appear blue after thawing due to the change of flavylium cation to quinonoidal form during that low temperature exposure (Bridle and Timberlake 1997).

Oxygen intensifies the degradation of anthocyanins. Even though the formation of unstable chalcones due to pH or thermal changes is reversible, the presence of oxygen during these procedures impedes the normal reconversion of these compounds (Bridle and Timberlake
1997). The effect of oxygen on anthocyanins occurs as direct oxidative mechanisms or through indirect oxidation, yielding colorless or brown end products (Rein 2005). Active forms of oxygen are highly reactive, as described in the pecking order table (Buettner 1993), and can degrade any type of molecule with a lower one–electron reduction potential ($E^0$).

Although light is needed in the biosynthesis of anthocyanins, once formed light damages these compounds (Markakis 1982). Visible and UV light are harmful to anthocyanins, and a study conducted on Berberis species illustrated the damaging effect of light on their anthocyanin profile (Laleh and others 2006). Specifically, shorter wavelengths are more harmful than longer ones (Skrede and Wrolstad 2000; Wang 2006). In a photochemical study, Furtado and others (1993) found that aqueous solutions of anthocyanins submitted to irradiation help with the disappearance of the flavylium cation due to the formation of the chalcone form.

Enzymes also have an important effect on anthocyanin stability, thus, inactivation of enzymes is a key process in the production of a variety of fruit and vegetable products (Fang and others 2006). The most common enzymes related to the degradation of anthocyanins are glycosidases. Glycosidases are not specific in the structural requirements of the aglycone portion of a molecule (Huang 1955), therefore they cleave the anthocyanins separating the sugar from the unstable aglycone form. Peroxidases (POD) and polyphenol oxidases (PPO) are enzymes naturally present in fruits that degrade phenolics compounds resulting in the formation of precursors of brown pigments (Kader and others 1997). PPOs degrade anthocyanins indirectly by the formation of quinones that subsequently will react with anthocyanins to form colorless products (Kader and others 1998). Another investigation (Fang and others 2006) explained that PPO oxidizes chlorogenic acid to form a quinone that will eventually react with anthocyanins to form brown pigments.
Substances such as sugars and organic acids can react with other solutes like polyphenolics and influence their stability as well. Sugars play a double role in anthocyanin stability. Sugars and syrups could be used as cryoprotectants by associating with plant water by osmosis. The process of sugar addition is also known as osmotic dehydration (Wang 2006). Syrups have proven to work better than dry sugars because the sucrose, which is commonly the osmotic agent, is dissolved (20-65%) and ready to migrate to the fruits or vegetables. Dry sugars are used with fruits that contain a high percent of juice where sugars can be dissolved. An osmotic step could protect color against degradation during drying (Torreggiani and Bertolo 2001). However, once some browning derived products have been produced from sugar caramelization, the degradation of anthocyanins is enhanced. The browning products responsible for the degradation effect in anthocyanins are furfural and Maillard reaction products (Tsai and others 2005).

Ascorbic acid fortification has commonly been used in fruit juices as antioxidant protection and to increase the nutritional value. Ascorbic acid has proven to retard enzymatic browning by reducing the \( o \)-quinones to \( o \)-diphenols that no longer produce brown pigments or degrade anthocyanins (Gregory 1996; Kader and others 1998). Another study (Rababah and others 2005) showed that ascorbic acid addition in fruit products did not change the phenolic or anthocyanin concentration, but dehydration together with ascorbic acid addition slightly reduced the amount of anthocyanins. However, addition of ascorbic acid was shown to degrade anthocyanins in pomegranate (Marti and others 2001) and Açaí juice (Pacheco 2006).

Bisulfite and other sulfur compounds are also used to protect color and phenolic compounds. These compounds are usually used in wine production. \( \text{SO}_2 \) acts as an antioxidant and bacteriostatic agent (Morata and others 2006). Bisulfite, like ascorbic acid, reacts with the \( o \)-
quinone to eliminate the basic compound to form brown pigments (Lindsay 1996). Nevertheless, SO₂ prevents the formation of visitins. Visitins are compounds formed by condensation of anthocyanins and pyruvic acid or acetaldehyde released by certain strains of yeast. Visitins are more stable than anthocyanins and do not affect the desired color of these compounds (Morata and others 2006).

2.3.3 Anthocyanins in Muscadine Grape

Five main anthocyanin forms were reported showing the absence of pelargonidin aglycone form (Yi and others 2005; Yi and others 2006). However, all six major anthocyanidins were reported in muscadine grapes (Talcott and others 2003; Talcott and Lee 2002), and all of them were present as diglucosides (Figure 2-6). These diglucosides are more resistant to oxidative and thermal processes than a respective monoglycoside as isolated compounds. However, in vivo they exhibit less color, enhanced formation of polymers, and a greater susceptibility to exhibit brown color than foods that contain anthocyanin monoglucosides (Lee and Talcott 2004).

Figure 2-6. Anthocyanin diglycoside structure (Cyanidin-3,5-diglucoside).
2.4 Ellagic Acid

2.4.1 Structure and Occurrence

Ellagic acid (EA) is formed through the conjugation of two molecules of gallic acid which is a derivative of hydroxyl benzoic acid (Figure 2-7). Ellagic acid is primarily found as ellagitannins (ETs). The formation of free ellagic acid is a result of the spontaneous conversion of both ester groups of hexahydroxydiphenoyl group (HHDP) into EA following its hydrolysis from ETs (Rommel and others 1993). In the presence of tannase, tannins are hydrolyzed into HHDP’s and a sugar molecule. Subsequently, HHDP is converted to EA through coupled oxidation and spontaneous lactonization (Shi and others 2005; Lei 2002).

EA is a polyphenolic located in the vacuole and represents the main phenolic compound in the Rosaceae family (Atkinson and others 2005). EA is an important polyphenolic compound in fruits such as raspberries, strawberries and blackberries. These fruits contain as much as three times the concentration of EA of some nuts (Tomás-Barberán and Clifford 2000; Rommel and others 1993). EA could also be found in pomegranate (Gil and others 2000), oak (Lei 2002) and other woody plants (Lee 2004).

![Chemical structure of ellagic acid.](image)

Figure 2-7. Chemical structure of ellagic acid.

2.4.2 Ellagic Acid Derivatives

Ellagic acid derivatives could be divided in two main groups: Ellagic acid glycosides (EAG) and ellagitannins (ETs). EAG are compounds that consist of a sugar moiety bound to an
EA molecule (Figure 2-8). Usually the sugars involved in the formation of EAG are pentoses such as xylose, rhamnose, and arabinose. Glucose may also be present in the formation of EAGs. The linkage between sugars and EA typically occurs in the 4-position since only ellagic acid-4-glycosides have been reported (Lee 2004; Lee and others 2005; Zafrilla and others 2001; Mullen and others 2003).

Figure 2-8. Ellagic acid glycosides A) Ellagic acid-4-arabinoside, B) Ellagic acid-4-acetylarabinoside, C) Ellagic acid-4-acetylxyloside (Mullen and others 2003).

ETs (Figure 2-9) are water soluble polyphenolics of molecular weights up to 4000 Da that represent one of the largest groups of tannins. ETs are likely derived from a gallotannin precursor (penta-\(O\)-galloyl-\(\beta\)-D-glucose) by oxidative coupling of at least two galloyl units yielding a HHDP unit that is the base of an ET (Clifford and Scalbert 2000; Khanbabaee and Ree 2001). ETs are also categorized as hydrolysable conjugates, including one or more HHDP groups esterified to a sugar molecule (Lee 2004). Currently, 500 different types of ETs were reported in nature that differ from each other in the number of HHDP units, the conformation of
the glucose ring, and the location of the galloyl ester groups (Feldman and Sambandam 1995; Helm and others 1999).

ETs can be divided in monomeric or oligomeric depending on the amount of glucose molecules present. Monomeric ETs are HHDP ester groups bound to one molecule of glucose. The coupling between HHDP groups and glucose generally occurs at the 4,6- carbon position and/or 2,3- carbon position of the glucose molecule. 1,6-, 1,3-, 3,6-, and 2,4- arrangements can also take place. These diverse combinations generate numerous monomeric ellagitannins. The most prominent ellagitannins are 4,6-HHDP (tellimagrandin I and II), 2,3-HHDP (sanguin H4 and H5) and 4,6-2,3-HHDP (pedunculagin and potentellin) (Lei 2002).

Oligomeric ETs are created by the polymerization of monomeric ETs. The most predominant oligomeric ETs are dimeric and tetrameric ETs. The polymerization of monomeric ETs occurs through oxidative C-O couplings between galloyl and HHDP moieties or the C-C interactions between glucose and HHDP. Examples of oligomeric ETs with C-O pairing, coriariin and rugosin D, which are dimmers of tellimagrandin I. Oligomeric ETs with C-C pairing, roburin A and D, which are dimmers of vescagalin/castalagin (Lei 2002).

Figure 2-9. Ellagitannins: Tellimagrandin II (monomeric ET) (left), Sanguin H-6 (oligomeric ET) (right) (Meyers and others 2006, Clifford 2000, Lei 2002).
2.4.3 Ellagic Acid and Its Derivatives in Muscadine Grape

EA was measured in muscadine grapes and it was found that it is one of the most abundant non-colored polyphenolics in the skins along with myricetin (Pastrana-Bonilla and others 2003). Another investigation (Lee and Talcott 2004) showed an average of 700.5mg/Kg and 1080.9mg/Kg of total EA in the skins of eight different cultivars of unripe and ripe muscadine grapes respectively. Of this total EA, 3.4% was free EA and 5.4% was EAG in unripe fruit. In ripe fruit, 9.2% constituted free EA and 8.7% was EAG of the total EA, proving that a large portion of the ellagic acid was in the form of ETs.

Three EAGs were identified in muscadine grape (EA-rhamnoside, EA-xyloside, and EA-glucoside), and in this study (Lee and others 2005), EAGs represented around 12.7% of the total ellagic acid in Noble muscadine grapes.

Due to lack of chromatographic standards and the diversity of ETs, it is challenging to identify these compounds. Commonly, the concentration of ellagic acid derivatives is measured by the amount of free EA released after acid hydrolysis. ETs are measured by the difference between total EA and EAG and free EA. Investigations have shown that ETs represent around 80%-90% of the ellagic acid present in muscadine grapes and it depends on the stage and maturity of the fruit (Lee and Talcott 2004; Lee and others 2005). Four ETs were reported but not completely identified in muscadine grapes. Moreover, two other ETs were identified as Sanguiin H4 or H5 depending on the position of the galloyl group (Lee and others 2005).

2.5 Processing Effects on Polyphenolics

Numerous changes in properties, both physical and chemical, can occur during fruit and vegetable processing. Some oxidative reactions may occur where electrons are removed from molecules to form oxidized compounds. These oxidative reactions lead to browning, changes in flavor and odor, changes in texture, and most importantly loss of nutritional value. The overall
concentration of nutrients could modify the antioxidant potential of fruit and vegetable products. Therefore, fruit and vegetable processing is directly related to the changes in concentration and functionality of phytochemicals (Wang 2006).

As a result of simple processes such as peeling, grating, cutting, and slicing, commodities that usually had a shelf-life of weeks or months are perishable due to the disruption of plant cells, thus liberating intracellular products and enzymes that will degrade polyphenolics and other compounds of nutritional interest in fruits and vegetables (Laurila and Ahvenainen 2002; Clifford 2000). Some commodities need peeling as part of their process. This step could be accomplished in several ways, but in an industrial scale usually mechanical peeling, chemical peeling, or high-pressure steam peeling is used. If this process is not gentle, the cell walls near the peel may be disrupted and some compound may cause browning, and other degradation procedures (Laurila and Ahvenainen 2002). Studies have shown that simple procedures should be conducted with stainless steel materials that won’t oxidize the compounds in fruits and vegetables. Furthermore, these materials should be disinfected constantly during operation (Laurila and Ahvenainen 2002).

2.5.1 Heat Procedures

Although the main objective of heat processing is safety, it is commonly known that this procedure negatively affects the original properties of raw materials. Specifically, thermal processing is responsible for the decrease of compounds of nutritional value such as ascorbate, tocopherols and anthocyanins (Wang 2006). However, thermal process can be beneficial. Heat treatments have proven to enhance the availability of some compounds due to thermal destruction of cell walls and subcellular compartments with the release of nutritional compounds and the denaturation of degrading enzymes such as polyphenol oxidases (PPO) and peroxidases (PO) (Takamura and others 2002; Wang 2006).
Food canning is an important heat treatment widely used in the food industry to prevent the presence of dangerous microorganisms such as *Clostridium botulinum*, which is an anaerobic organism that creates a lethal toxin. This microorganism grows at pH over 4.6 and is thermolabile. Even though the possibility of *C. botulinum* occurrence in high acid foods is uncommon, an F-value of 5D must be accomplished to properly eliminate the risk of *C. botulinum* activity. To accomplish this task, canned products have to be exposed to a high temperature retort that will have detrimental effects on its nutritional compounds (Pflug and Esselen 1979).

Pasteurization is one of the most common heat procedures used in food products. The most common pasteurization protocol, used to ensure proper heat transmission while reducing the time of exposure, is “high temperature short time” system (HTST) used as a continuous system for pasteurization in milk products and juices. A study (Klopotek and others 2005) showed that pasteurization was highly influential in the total phenolic and anthocyanin concentration of strawberry products.

### 2.5.2 Drying Procedures

Drying is one of the most ancient processes used to preserve foods. The purpose of this procedure is to reduce the water activity of a fruit or vegetable to a level where growth of spoilage microorganisms and occurrence of degrading chemical reactions are halted or slowed down. Furthermore, drying was used to reduce the volume and weight of commodities for easier distribution and extended shelf-life (Barbosa-Cánovas and others 2005). However, drying can diminish the nutritional content in foods. Due to thermal degradation, polyphenolics may be lost or their bioactivity may be reduced. A study (Schmidt and others 2005) showed that different processes did not decrease the phenolic content significantly but their bioactivity was reduced. Another investigation (Rababah and others 2005) showed that even though concentration of
polyphenolics in strawberries, peaches and apples was not significantly changed after
dehydration, their antioxidant capacity decreased significantly.

Spray drying is one of the most well-known and widely used drying procedures. This
process take places at temperatures between 150-200°C (Orsat and Raghavan 2006). The
particles have a spherical shape. Due to the diminutive size of the particles, the drying procedure
is shorter compared to other drying protocols and is a suitable method for sensitive compounds
to heat deterioration. Powdered milk and whey concentrates are among the most common
products produced by spray drying. Other products include coffee, tea extracts, baby formula,
enzymes, and yeast (Barbosa-Cánovas and others 2005).

It is important to control the feed rate, drying air temperature, and pressure of the air in the
nozzle in spray drying as these characteristics determine the final quality of the powder.
Moreover, carriers such as maltodextrins can be used to improve agglomeration and provide
stability (Orsat and Raghavan 2006). The best spray drying characteristics for higher yields of
roselle extract were analyzed (Andrade and Flores 2004) and results showed that the extract did
not differ in pH from the original liquid feed, but the flavor was lost. The best results for color,
pH, and yield recovery were detected at a temperature range of 178-190°C, and a pressure of 5-6
bar in the atomizer.

Also known as liophilization, freeze drying is another major drying procedure.
Liophilization was introduced to the food industry in 1954. Later, in 1964 coffee was subjected
to freeze drying. Freeze-dried products maintain most of their physico-chemical and sensory
characteristics due to the lack of heat exposure (Barbosa-Cánovas and others 2005). Freeze
drying consists of two main steps: freezing the product and drying it under vacuum to cause
sublimation. For water, sublimation is accomplished when the temperature is lower or equal to
0°C and the pressure is below 672 Pa. This is called the triple point where ice could be evaporated without melting (Figure 2-10). It is important to realize that the amount and nature of solids in food play an important role in the sublimation temperature. Commercial freeze drying is carried out at -10°C and absolute pressure of 2mm Hg or less (Barbosa-Cánovas and others 2005).

Figure 2-10. Phase diagram of water (adapted from Barbosa-Cánovas and others 2005).

Characteristics such as reconstitution, retention of volatile compounds, rehydration and others are maintained by freeze drying. An investigation (van Golde and others 2004) showed that the polyphenols of wine that were freeze dried had around a 70% recovery with the same qualities as the polyphenols from the original wine. Freeze-drying appears to be a good process for the conservation of large polyphenols like tannins whereas small polyphenols might not be protected (Abascal and others 2005). Another investigation (Tambunan and others 2001) demonstrated that the quality of freeze-dried herbal products was slightly decreased but the overall quality was still higher than oven-dried samples. Freeze drying is considered the best drying procedure because the initial material is frozen and the atmosphere around the sample has
a low concentration of oxygen. However, this procedure can be expensive and time consuming (Barbosa-Cánovas and others 2005).

Drum drying is a technique where heat is transferred to a material by conduction from a rotating drum. After the product is dried, it is separated from the drum with a scraper (Orsat and Raghavan 2006). This protocol is one of the cheapest drying methods. It is energy efficient, saves space and is more economical than spray drying for small volumes. The disadvantages of this procedure are: the product needs to be liquid; it has to adhere to a metal surface, resist an oxygen exposure and high temperatures (Barbosa-Cánovas and others 2005). Furthermore, a study (Hsu and others 2003) showed that drum drying had the highest losses in antioxidant capacity compared to freeze drying and hot air drying.

Other drying procedures, not as common as the ones previously discussed, can be found in the industry. Convective drying is a procedure where a layer of product is exposed to heated air. Vacuum drying is a procedure where steam heats the products under low pressure. Due to the use of low pressure, vacuum drying improves the quality of a product by using lower temperature (Orsat and Raghavan 2006).

### 2.5.3 Extraction Procedures and Storage

Pressing is a common procedure used for extracting juice from fruits. In this procedure a significant concentration of polyphenolics may remain in the fruit solids. An investigation (Klopotek and others 2005) showed step by step how the phenolic profile was changing during strawberry processing. Total phenolics were reduced by 44% during mashing and pressing. On the other hand, the anthocyanin content was maintained during initial steps of strawberry processing.

Storage can also have severe effects on food quality if its temperature is not properly controlled. Numerous studies have explored the detrimental effect of storage on polyphenolics.
Storage had more impact than thermal process in guava juice production (Fender 2005). Turker and others (2004) showed a decrease in anthocyanin content and color density were decreased during a 90 days storage study at 40°C. Another study (Kirca and others 2006) showed similar results where the largest lost of compounds was at 37°C, followed by 20°C.

2.5.4 Enzymatic Procedures

The use of enzymes to achieve desirable changes in food products has been utilized for centuries. Enzymes are catalysts that aid in the increase of yield, facilitate processes, and play an important role on sensory characteristics. Pectinases are used to increase yield of pigments extracted from grapes in wine production, Naringinases are used to reduce the bitter flavor in citrus juices, pectin methyl esterase (PME) is use to increase the yield and clarify citrus juices, and the list of enzymes and processes they are used in is vast (Whitaker 1994; Wang 2006).

Enzymes could be classified as endogenous and exogenous depending on whether the enzyme was found in the substrate or was intentionally added to accomplish an activity. Enzymes could be further divided into six main groups depending on the reaction they catalyze (Whitaker 1994). However, not all enzymes are beneficial in the food industry. There are enzymes that are found naturally in fruits and vegetables that need to be inactivated in order to preserve the quality and prolong the shelf-life of a product. The main enzymes studied that are closely related to fruit and vegetable deterioration are PPO and PO which are oxidoreductases (Whitaker 1994; Kader and others 1997; Kader and others 1998).
3.1 Introduction

Interest in phytochemicals has increased in recent years due to their association with human health benefits as well as their role in foods as functional ingredients (Talcott and Lee 2002; Wang 2006). The major mechanism by which these compounds enhance food quality and aid human health is radical scavenging (Robbins 2003), other mechanisms include enzymatic inhibition, enzymatic co-factoring, growth selectivity and inhibition for deleterious gastrointestinal bacteria, and essential nutrients absorption enhancement (Reviewed by Dillard and German 2000). Radical scavenging stops a degradation chain reaction caused by free radicals that are formed inside and outside the body. Around 100 radicals have been associated with degenerative diseases such as cancer, atherosclerosis, arthritis, and cataracts, and polyphenolics donate a hydrogen atom, obstructing the development of such diseases (Shahidi and Naczk 2003; Parr and Bowell 2000).

Muscadine grapes (*Vitis rotundifolia*) are a significant fruit crop in the south and southeastern U.S. and are unique among *Vitis* species not only for their increased tolerance to Pierce’s disease (*Xylella fastidiosa*), but also in their chemical composition. These grapes are known to possess a diversity of polyphenolics, such as anthocyanin diglycosides, ellagic acid and its derivatives, numerous phenolic acids and flavonoids (Pooling 1996; Mizell and others 2003; Lee and others 2005). The severity of Pierce’s disease in the southeastern region of U.S. has limited the production of *Vitis* species other than *Vitis rotundifolia* which has demonstrated to be suitable for a commercial industry (Ruel and Walker 2006).

A growing concern for muscadine grape juice and wine producers is the handling of by-products (skin and seeds) and the desire to produce a food-grade product with added value.
There are numerous phytochemical compounds in grape pomace that may have significance for the food industry due to their association to human health, thus sustained efforts are currently underway to produce value-added food ingredients from these otherwise waste products (Sort 2003). The unique properties of muscadine grapes that have presented technological challenges include their thick pectin-laden skins, the selective recovery of polyphenolics, and, for juice pomace, their high residual sugar content since wine by-product contains little or no sugar concentration after fermentation. Recently, the literature on antioxidant compounds from residual sources has been increasing steadily with investigations on grape pomace, leaves, and skins (Monagas and others 2006; Bonilla and others 1999), olive mill waste (Visioli and others 1999), wine industry (Makris and others 2007) and several other fruits and vegetables (Peschel and others 2006).

Therefore, the purpose of this study was to determine isolation techniques and subsequent stability of polyphenolics recovered from muscadine grape skins using various extractions and processing techniques to obtain a dry powder or a concentrate.

3.2 Materials and Methods

3.2.1 Materials and Processing

Grape pomace was obtained from Paulk Vineyards (Wray, GA) from deseeded muscadine grapes grown in 2006. The pomace was obtained following a freeze-thaw cycle and a hydraulic pressing for juice recovery in the absence of rice hulls as a pressing aid. The resulting pomace was frozen and transported overnight to the Food Science and Human Nutrition Department at the University of Florida, Gainesville, FL and held in frozen storage at -20°C until further processing.

Upon thawing and removal of residual free-run juice grape skins were thawed and mixed (1:1, 1:2, 1:5, and 1:10 w/w) with hot water (90-95°C). Extracts were manually stirred three or
four times a day to improve contact between the skins and water for a three-day assessment period. Extracts were pressed and filtered through cheesecloth daily and mixed with new grape skins to increase the phenolic concentration. For the first extraction grape skins were mixed with hot water while the second and third extraction were not submitted to heat to minimize degradation in compounds already extracted. After the third day of concentration the extract was pressed and filtered to get rid of the skins. Extracts were analyzed for total anthocyanin content as a marker for completion of the polyphenolic extraction (appendix A).

The disadvantage of a long concentration process was that the juice already started undesired fermentation process and the color extraction in the second and third day was not as important as the first one. Due to the long time spent in compound concentration, the mixture process was reduced to only one day with hot water and likewise assessed for total anthocyanin content as a marker for polyphenolic extraction (appendix B). At this point, the ratios of pomace to water 1:1, 1:2, 1:5 were assessed. The high volume of water in dilution 1:10 would have represented extensive drying in a future processing and was eliminated. After a one-day extraction, dilution 1:5 was also eliminated due to a low concentration of compounds and high volume of water. On the other hand, the most concentrated sample (1:1) did not have enough water to facilitate handling of the product and minimal extraction of compounds, thus excluded from further experimentation. Consequently, the dilution (1:2) was used for further processing and investigation.

After the 24-hour process was conducted and the grape skin to water ratio was selected, the mixture was assessed with samples every 30 minutes to determine the maximum compound extraction time by total anthocyanin evaluation (appendix C). The extract was manually stirred every 15 minutes to improve contact between the skins and water, and allowed to extract. The
maximum color extraction was accomplished at 3.5 hours and samples collection was conducted for 90 more minutes to assure this maximum extraction. Therefore, the process was reduced from 24 hours of water skin exposure to about 3.5 hours at which, a maximum concentration of 1,200 mg/kg total anthocyanins was accomplished. After this point the content of anthocyanins was relatively stable.

Based on previous results, an experiment was run at a semi-industrial scale. Grape skins were thawed and mixed (1:2 w/w) with 45.4 Kg of hot water (90-95°C) for 3.5 hours. The free run extract was collected and skins pressed in a hydraulic press at (500 bar) to obtain an aqueous extract. This extract was filtered through cheesecloth and a 1-cm bed of diatomaceous earth to remove insoluble agents and to clarify the extract. This clarified extract was used as the starting material for subsequent procedures to eliminate or reduce the presence of soluble sugars. Three isolation methods were utilized including two solid phase extractions and a fermentation procedure followed by three concentration protocols that included spray drying, freeze drying and vacuum concentration. All handling and processing methods were compared to a control of the starting clarified extract for calculation of phytochemical recovery of changes due to process techniques. Upon completion of each isolation or processing protocol, samples were held at -20°C until analysis.

3.2.2 Solid Phase Isolation

A batch of extract was separated as a control and for affinity column isolation treatments (Amberlite XAD-4, reverse phase C_{18}). This batch was clarified through diatomaceous earth and no further processing was done to a control sample of the extract that was stored frozen at -20°C until analysis. Reversed phase C18 is a model system used commonly at a laboratory scale due to its high cost. Moreover, particle size is also an impediment for its usage on a larger scale (Kraemer-Schafhalter and others 1998). SEP-PAK C_{18} columns proved to efficiently separate
major phenolic compounds to improve analysis by HPLC and be very practical and easy to clean (Jaworski and Lee 1987; Oszmianski and others 1988; Kraemer-Schafhalter and others 1998). Amberlite copolymers, on the other hand, are used at laboratory scale but could also be used as industrial alternatives for affinity column isolation due to their commercial availability and price (Pietrzyk and Chu 1977b). Various types of Amberlite XAD copolymers have been industrially utilized in the removal of impurities from waste and potable water as well as isolation of carotenoids, steroids, and other biologically important compounds (Pietrzyk and Chu 1977a; Fritz and Willis 1973). Amberlite copolymers, which vary in surface area, porosity, and activity, where shown to vary in their mode of separation, quality of compound retention based upon nature of the target compounds, pH of the environment, amount of Amberlite resin used as adsorbent and the type of copolymer present in the resin (Kraemer-Schafhalter and others 1998; Pietrzyk and Chu 1977a, Pietrzyk and Chu 1977b). Amberlite resin XAD-2 was partially satisfactory in retaining polar solutes from aqueous extract and it was shown that Amberlite XAD-7 could adsorb substances with both lipophillic and polar interaction even though Amberlite XAD-2 showed greater affinity with aromatic compounds (McRae and others 1982). Another work illustrated the efficiency and feasibility of Amberlite XAD-8 in the extraction of polyphenolics because such resin was used for 2 years showing reproducible results. However, when the Amberlite resin was overloaded with compounds, the solvent started to remove material from the resin (Lalaguna 1993). Kraemer-Schafhalter and others (1998) explained that a type of Amberlite XAD-7 showed poor separation and was a difficult column to clean, while other Amberlite resins showed better separation but still showed cleaning complications. Furthermore, Amberlite XAD-2 showed insufficient pigment retention. For present experiments,
reversed phase \( C_{18} \) column was used as a comparison to Amberlite XAD-4 as an industrial solid-phase separation and isolation technique.

Amberlite XAD-4 resin (10g) previously washed with methanol and thoroughly cleaned with deionized water was loaded into a small column whereby 2mL of extract was loaded and allowed to adsorb for 1 hour. Following adsorption, unbound compounds were washed with water (200mL) and desorbed with 100% methanol. Following evaporation to dryness, compounds were re-dissolved in a known volume of 0.1M citrate buffer at pH 3.0. Those compounds not retained on the resin were subsequently adsorbed onto 1-gram of Sephadex LH-20 (normal phase) placed in a mini-column, washed with water, eluted with 100% methanol, evaporated, and likewise re-dissolved in citrate buffer.

Likewise, 5mL of extract was loaded onto a 5g reversed phase \( C_{18} \) mini-column and allowed to adsorb by gravity feed. The column was then washed with water (200mL) and phytochemicals desorbed with 100% methanol. Following evaporation, compounds were re-dissolved to a known volume of the citrate buffer. Non-retained compounds were likewise adsorbed onto a Sephadex LH-20 (normal phase) mini-column and re-dissolved in citrate buffer.

A third procedure to remove residual sugars involved fermentation of simple sugars by inoculation with wine yeast (\textit{Saccharomyces cerevisiae} strain Premium Cuveé) at a rate of 2.5g/L and allowing aerobic fermentation to occur at 20-25°C until soluble solids content was decreased to a constant amount by monitoring \(^{\circ}\text{Brix} \) values every 12 hours. After fermentation, the extract was clarified by passing through a 1-cm bed of diatomaceous earth with the aid of vacuum.

Each of the three phytochemical isolation techniques that also served to remove residual sugars was compared to a non-isolated control. To determine the effects of common processing
or concentration steps, three processing techniques were evaluated for the fermented isolate. The isolate was sub-divided into equal portions for vacuum concentration, spray drying, and freeze drying. A control sample was retained and frozen at -20°C until analysis. For spray drying, 2L of the fermented extract was spray dried (Anhydro, Copenhagen, Denmark) at a temperature of 220-230°C and an exhaust temperature of 100-110°C over a 4 hour period. The resulting powder obtained was re-dissolved in a known volume of 0.1M citrate buffer at pH 3.0 for subsequent analysis. A freeze dried sample of the same fermented extract (300mL) was accomplished in a Freeze Drier 5 unit (Labconco, Kansas City, MO) at -100°C and 1 Torr inside the drying chamber over an 8 hour period. The resulting powder was likewise re-dissolved in citrate buffer for analysis. Lastly, 15mL of fermented extract was evaporated at 60°C using a rotary evaporator over a 40 minute period and re-dissolved in a known volume of citrate buffer for analysis.

3.2.3 Chemical Analysis

3.2.3.1 Spectrophotometric determination of total anthocyanins

Total anthocyanin content was determined spectrophotometrically by the pH differential method (Wrolstad 1976). Isolation and processing treatments were appropriately diluted with buffer solutions at pH 1.0 and pH 4.5. Absorbance was read on a UV-Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale, CA) at a fixed wavelength of 520 nm and total anthocyanin concentration calculated and reported in mg/kg equivalents of cyanidin-3-glucoside with an extinction coefficient of 29,600 (Jurd and Asen 1966).

3.2.3.2 Determination of polymeric anthocyanins

The percentage of polymeric anthocyanins was determined based on color retention in presence of sodium sulfite (Rodriguez-Saona 1999). Treatments were diluted in pH 3.0 buffer and each sample subdivided into two fractions. A solution containing 5% sodium sulfite was
added to one fraction while an equivalent volume of pH 3.0 buffer was added to the remaining fraction. Absorbance at 520 nm was recorded for each on a UV-Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale, CA). Concentration of polymeric anthocyanins was calculated and reported as the percentage of absorbance remaining after the addition of sodium sulfite.

3.2.3.3 Determination of total soluble phenolics

Total soluble phenolics were determined by the Folin-Ciocalteu assay (Singleton and Rossi 1965). Samples were diluted in water and 100μL of each were loaded into test tube for reaction with 0.25N Folin-Ciocalteu reagent (Sigma Chemical Co. St. Louis, MO). After a 3 min reaction of the reagent and the sample, 1N sodium carbonate was added to form a blue chromophore that was read after 30 minutes at 726 nm on a UV-Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale, CA). Total soluble phenolics were quantified in equivalents of a gallic acid standard with data expressed in mg/kg of gallic acid equivalents.

3.2.3.4 Quantification of antioxidant capacity

Antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) method (Cao and others 1996), adapted to be performed with a 96-well Molecular Devices fmax® fluorescent microplate reader (485 nm excitation and 538 nm emission). The assay measures the ability of an antioxidant to inhibit the decay of fluorescein induced by the peroxyl radical generator 2,2-azobis (2-amidinopropane dihydrochloride) as compared to Trolox, a synthetic, water-soluble vitamin E analog. For analysis, samples were diluted in pH 7.0 phosphate buffer and 50μL of each sample was then transferred to a microplate along with a Trolox standard curve (0, 6.25, 12.5, 25, 50μM Trolox) and phosphate buffer blanks. 100μL of fluorescein and 50μL of peroxyl radical generator were added to all samples, standard curve, and blanks. Readings were taken every 2 min over a 70 min period at 37°C. Antioxidant capacity
was quantified by linear regression based on the Trolox standard curve and results were expressed in μmol of Trolox equivalents per gram (μmol TE/g).

### 3.2.3.5 Half life determination

Samples were diluted with pH 3.0 citrate buffer and placed in a 96-cuvette microplate subdivided into two fractions. A solution containing 3% hydrogen peroxide was added to one fraction while an equivalent volume of pH 3.0 buffer was added to the remaining fraction. Absorbance at 520nm was recorded using a UV-Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale, CA). The assay was carried out at 45°C for 60 minutes with readings every 2 minutes to quantify color loss over time. Results were expressed as minutes of half-life.

### 3.2.3.6 Analysis of polyphenolics by HPLC

Polyphenolic compounds were analyzed by reverse phase HPLC using modified chromatographic conditions (Lee and others 2005) with a Dionex system equipped with an ASI-100 Autosampler injector, a P-680 HPLC pump, and a PDA-100 Photodiode Array Detector. Separations were performed on a 250 x 4.6 mm Acclaim 120-C18 column (Dionex, Sunnyvale, CA) with a C18 guard column. Mobile phase A consisted of water acidified with o-phosphoric acid (pH 2.4) and Mobile phase B consisted of 60:40 methanol and water acidified with o-phosphoric acid (pH 2.4). Samples were hydrolyzed in 2N HCl (adjusted to contain 50% methanol) for 90 min at 95°C before injection. The gradient solvent program held Phase A for 3 min; then phase B from 0 to 30% in 3 min; 30 to 50% in 2 min, 50 to 70% in 5 min, 70 to 70.63% in 3 min, 70.63 to 70.7% in 1 min, 70.7 to 70.81% in 0.5 min, 70.81 to 71.2% in 2.1 min, 71.2 to 71.3% in 2 min, 71.3 to 85% in 1.4 min and 85 to 100% in 10 min for a total run time of 30 minutes for both set of samples at a flow rate of 1mL/min. Polyphenolics were identified by UV/VIS spectral interpretation, retention time and comparison to authentic standards (Sigma
Chemical Co., St. Louis, MO). All treatments were filtered through a 0.45μM filter and directly injected into the HPLC. Data was reported as mg/L of each compound. Anthocyanins were compared to a cyanidin aglycone standard, flavonols were compared to a quercetin standard, and ellagic acid was compared to an ellagic acid standard.

### 3.2.4 Statistical analysis

The study was designed as a completely randomized design (CRD) that included seven treatments (Amberlite, C18, spray drying, freeze drying, vacuum drying, and controls for both the fermented and non-fermented extracts). Data for each treatment is the mean of three replicates. Analysis of variance and means separations by LSD test (P < 0.05) were conducted using JMP software (SAS Institute, Cary, NC).

### 3.3 Results and Discussion

The effects of processing were evaluated and results for all analyses were reported in units from each assay mentioned earlier in the method section (Table 3-1).

Table 3-1. Quality analyses of muscadine pomace extract (polyphenols) as affected by various processing protocols.

<table>
<thead>
<tr>
<th>Process</th>
<th>Total Phenolics(^1)</th>
<th>Total Anthocyanins(^2)</th>
<th>Polymeric Anthocyanins(^3)</th>
<th>Half life(^4)</th>
<th>ORAC(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E(^8)</td>
<td>1640 a(^6)</td>
<td>1470 a</td>
<td>7.65 e</td>
<td>17.2 d</td>
<td>34.3 a</td>
</tr>
<tr>
<td>F</td>
<td>1580 a</td>
<td>1520 a</td>
<td>9.75 de</td>
<td>17.9 cd</td>
<td>28.7 b</td>
</tr>
<tr>
<td>BC(_{18})</td>
<td>1130 bc</td>
<td>996 c</td>
<td>25.8 a</td>
<td>24.7 a</td>
<td>16.7 d</td>
</tr>
<tr>
<td>UBC(_{18})</td>
<td>4.55 e</td>
<td>0.25 e</td>
<td>NA(^7)</td>
<td>NA</td>
<td>&gt;0.1 f</td>
</tr>
<tr>
<td>BA</td>
<td>1030 c</td>
<td>873 d</td>
<td>18.6 b</td>
<td>21.4 b</td>
<td>22.6 c</td>
</tr>
<tr>
<td>UBA</td>
<td>186 d</td>
<td>1.65 e</td>
<td>NA</td>
<td>NA</td>
<td>1.91 e</td>
</tr>
<tr>
<td>SD</td>
<td>1240 b</td>
<td>1060 c</td>
<td>14.3 bcd</td>
<td>20.5 b</td>
<td>22.0 c</td>
</tr>
<tr>
<td>FD</td>
<td>1270 b</td>
<td>1230 b</td>
<td>11.3 cde</td>
<td>19.8 bc</td>
<td>18.1 d</td>
</tr>
<tr>
<td>VD</td>
<td>1620 a</td>
<td>1450 b</td>
<td>14.8 bc</td>
<td>20.5 b</td>
<td>28.8 b</td>
</tr>
</tbody>
</table>

\(^1\)Expressed in gallic acid equivalents (mg/kg).  \(^2\)Expressed in cyaniding-3-glucoside equivalents (mg/kg).  \(^3\)Expressed in percentage of polymeric anthocyanins (%).  \(^4\)Expressed in time (min).  \(^5\)Expressed in Trolox equivalents (μmol TE/g).  \(^6\)Similar letters within columns for each analysis are not significantly different (LSD test P < 0.05).  \(^7\)NA (not applicable) samples did not contain the analyte.  \(^8\)Treatment abbreviations: (E) Extract, (F) fermented extract, (BC\(_{18}\)) bound to reversed phase C\(_{18}\) column, (UBC\(_{18}\)) unbound to reversed phase C\(_{18}\) column, (BA) bound to Amberlite XAD-4, (UBA) unbound to Amberlite XAD-4, (SD) spray dried, (FD) freeze dried, and (VD) vacuum dried.
3.3.1 Anthocyanin Color Stability

Anthocyanin stability was assessed spectrophotometrically and is shown in Figure 3-1. Anthocyanin content was maintained during fermentation and vacuum drying (Table 3-1). Initial anthocyanin concentration in extract (1470 ± 102 mg/kg) was somewhat affected by freeze drying (1230 ± 114 mg/kg), but experienced a 28% decrease after spray drying (1060 ± 26.3 mg/kg). Samples after affinity column isolation (reversed phase C$_{18}$ column and Amberlite resin) also showed significant decrease in their anthocyanin content (996 ± 24.4 mg/kg and 873 ± 78.9 mg/kg respectively). The extensive loss of color in both reversed C$_{18}$ column and Amberlite XAD-4 procedures (32 and 41% respectively) presumably occurred mainly while anthocyanins were exposed to high pH environment since less than 0.03% of the initial material’s anthocyanins were found in the unbound fraction from reversed phase C$_{18}$ and less than 0.2% was found in the unbound fraction from the Amberlite resin. Another possible reason for loss of anthocyanin by solid phase isolation might be column efficiency. Perhaps, a single elution with methanol was not sufficient to dissociate polyphenolics from either column and, after desorption, a fraction of these compounds remained in association with the column and was washed away during cleaning of the resin and not collected for analysis. Thus, recovery from reversed phase C$_{18}$ bound anthocyanins were more efficient than those from Amberlite resin. Another possible explanation is that Amberlite XAD-4 might have higher affinity with polyphenolics than reversed phase C$_{18}$ thus, making it difficult to recover compounds on a simple desorption step. Furthermore, in both affinity column isolation techniques, anthocyanins were subjected to copious amounts of solvent to separate polyphenolics from sugars and other compounds that were washed away. After adsorption, anthocyanins were desorbed with 100% methanol followed by evaporation. Although evaporation uses mild temperatures, in the last phase when most of the solvent has been evaporated, compounds could have been subjected to
heat. Stability of anthocyanins is known to be jeopardized by lowering concentration of anthocyanins in a medium (Giusti and Wrolstad 2003), decreasing acidity (Clifford 2000), and applying heat (Klopotek and others 2005; Clifford 2000), explaining why both affinity column isolation protocols significantly decreased anthocyanin concentration in muscadine pomace extract.

![Figure 3-1](image-url)  

Figure 3-1. Total anthocyanin content of muscadine pomace extract as affected by various processing methods. Error bars represent the standard error of each mean, n=3.

Reasons for color loss were oxygen exposure, low acidity levels in the environment and heat. Vacuum drying maintained the concentration since some temperature was substituted by low pressure and oxygen was removed from the medium to generate vacuum, thus making this process mild and effective. On the other hand, freeze drying showed more than 16% anthocyanin content loss despite the fact that no heat was applied to the extract. The exposure time for the sample to freeze dry, and possibly errors at the recovery phase, might have had a negative effect on anthocyanin concentration. Affinity column isolation techniques showed the highest color losses due not only to heat and oxygen exposure, but also to higher pH
environment and low anthocyanin concentration due to massive amount of solvent used during adsorption and desorption, thus illustrating the important role of pH in anthocyanin stability.

Polymerization of anthocyanins present in muscadine pomace extract was significantly influenced by processing. The bound fraction of the reversed C\textsubscript{18} column showed the highest polymerization index (25.8 ± 3.25%) followed by bound fraction of Amberlite resin, vacuum dried, and spray dried that showed no significant difference between each other. At the same time, the freeze dried, spray dried and the fermented extract samples showed no difference between each other (Table 3-1). The extract showed the lowest polymerization index (7.65 ± 0.52%) due to the minimum processing (concentration and clarification) it was subjected to (Figure 3-2).

When comparing the concentration of anthocyanins to polymeric anthocyanin content, it was concluded that both affinity column isolation techniques had the lowest anthocyanin concentration and yet had the highest polymerization index (Table 3-1). Therefore, anthocyanins might be forming new high molecular weight compounds by association with other polyphenolics such as condensed tannins and other anthocyanins. Processing was directly related to the polymerization process since processed samples had higher polymerization index while the starting material (extract) showed the lowest polymer formation, and as processing was milder, polymer formation was decreased. A correlation between anthocyanin concentration and polymeric index (r = -0.73) confirmed that as processes were conducted, polymers were forming in the media. As compared to other studies (Weinert and others 1990), processing induced polymerization which explained some of the losses in total anthocyanins attributed to this process.
Half life determinations were made under accelerated conditions of storage (45°C) in the presence of hydrogen peroxide, a strong oxidizing agent. Concentrations of peroxide and holding temperatures were determined to create a slow decay curve, suitable to testing a wide-range of anthocyanin concentrations with varying degrees of stability. Analogous to the formation of the polymeric pigments, the effects of anthocyanin isolation and processing slightly increased protection against hydrogen peroxide-induced oxidation. The bound fraction of C18 showed the highest resistance (24.7 ± 1.01 min) followed by all three drying protocols and the bound fraction of Amberlite resin (Table 3-1). The initial extract showed shortest half life of all treatments (17.2 ± 0.55 min) and was due to the predominance of monomeric anthocyanins in this fraction. Correlations between anthocyanin concentration and half life ($r = -0.67$) and half life and polymeric index ($r = 0.86$) confirmed that as polymerization index increases, anthocyanins become more stable, thus, increasing their durability (Figure 3-3). Stability of anthocyanins has been enhanced due to polimerization (Weinert and others 1990; Rein and Heinonen 2004), and copigmentation of anthocyanins showed reduction in pigment degradation.
in grape anthocyanins (Brenes and others 2005), which explains the increase in anthocyanin half life in processed muscadine extract samples.

![Figure 3-3. Half life (min) of muscadine pomace extract as affected by various processing methods. Error bars represent the standard error of each mean, n=3.](image)

Although processing reduced the concentration of anthocyanins, the anthocyanins remaining in the matrix were more resistant. Polymeric anthocyanin proved to be more stable to processing but that may affect the final quality of a food product since the color of the anthocyanin might change due to this process. An investigation showed that some copigments enhanced the a* value by retaining more red but also increased the b* value showing a yellowing in the sample during storage (Rein and Heinonen 2004). Moreover, polymerization could decrease the antioxidant capacity of phenolic compounds by variations in the hydroxyl groups arrangement and availability which are related to their radical scavenging ability (Miller and Ruiz-Larrea 2002), thus polymeric anthocyanins may not have as important benefit as compared to monomeric anthocyanins. In addition, absorption of phenolic compounds might be conducted by hydrolysis to obtain aglycone forms or simple phenols are likely to be absorbed more efficiently in the human body (Reviewed by Miller and Ruiz-Larrea 2002).
3.3.2 Polyphenolic Concentration and Stability

Total soluble phenolics were assessed spectrophotometrically as affected by isolation and food processes (Figure 3-4). Polyphenolic concentration in muscadine pomace extract and processed samples experienced somewhat similar behavior as compared to the anthocyanin content. Polyphenolic content was maintained during fermentation and vacuum dehydration while affected by freeze drying and spray drying (Table 3-1 & Figure 3-4). Affinity column isolation protocols (reversed phase C\textsubscript{18} column and Amberlite resin) significantly decreased the concentration of polyphenolics in the extract (1130 ± 43.8 and 1030 ± 17.8 mg/kg respectively). The unbound fraction of C\textsubscript{18} did not show significant polyphenolic concentration (4.55 ± 2.72 mg/kg) while the unbound fractions from Amberlite resin processing showed an important concentration of polyphenolics (186 ± 122 mg/kg). Amberlite was less effective in binding polyphenolics than reversed phase C\textsubscript{18} since the unbound fraction showed a much higher concentration (15.3%) than the unbound fraction from C\textsubscript{18} (<0.5%). The unbound fraction of Amberlite resin contained mostly polyphenolics that did not absorb light at 520nm since previous analysis showed a small concentration of anthocyanins in this fraction. Such compounds might tentatively be ellagitannins since previous investigations have shown occurrence of ellagitannins in unbound fractions of solid phase separation techniques (Lee 2004).

Polyphenolic losses in solid phase extraction techniques were possibly due to low acidity medium, oxygen exposure, and heat exposure. Only vacuum drying maintained the concentration due to temperature substitution and removal of oxygen by vacuum. On the other hand, freeze drying showed almost 23% loss in polyphenolic content even though the product was frozen during the procedure. Furthermore, there were no significant differences between freeze drying, spray drying and reversed phase C\textsubscript{18} column process.
Reversed phase C\textsubscript{18} column effectively bound most polyphenolics since less than 0.5% was detected in the unbound fraction. On the other hand, the Amberlite resin showed compound losses due to the presence of polyphenolics that did not bind with the resin and were washed away together with sugars and some other organic compounds. The unbound fraction had more than 15% of the phenolics that were exposed to the Amberlite resin and more than 11% of the polyphenolics from the starting material (extract).

![Graph showing total phenolic content in muscadine pomace extract](image)

Figure 3-4. Total phenolic content in muscadine pomace extract as affected by various processing methods. Error bars represent the standard error of each mean, n=3.

### 3.3.3 Polyphenolics by HPLC

Polyphenolics present in muscadine pomace extract and various processing methods were analyzed and monitored by HPLC at 360 and 520 nm. Analysis was focused on total ellagic acid, anthocyanins, and flavonols. Compounds were identified and quantified in hydrolyzed samples.

#### 3.3.3.1 Anthocyanins by HPLC

HPLC analysis of muscadine pomace extracts confirmed the occurrence of six anthocyanin aglycone forms (Figure 3-5). Previous investigations reported similar results (Talcott and Lee
2002; Talcott and others 2003), although some reports on the specific identity of anthocyanins in muscadine grapes differed (Lee and Talcott 2004; Yi and others 2005; Yi and others 2006), detecting only five anthocyanins aglycones, excluding pelargonidin. Since samples from this study were hydrolyzed and six peaks eluted, it was concluded that the six main forms of anthocyanin aglycones were present and thus, anthocyanidins could be identified by their elution order based on their structure and polarity.

![HPLC chromatogram of anthocyanidins present in muscadine pomace: delphinidin (A), cyanidin (B), petunidin (C), pelargonidin (D), peonidin (E), malvidin (F). Identification (520 nm) was done based on spectral characteristics and comparison to cyanidin aglycone.](image)

Addition of the corresponding peak areas for all six anthocyanidins yielded the total anthocyanin content for each treatment which was used for general comparison between treatments (Table 3-2). Delphinidin, cyanidin, petunidin, and peonidin constituted most of the muscadine skin extract anthocyanin profile while pelargonidin accounted for only 1.1% and malvidin for 8.2%. Low content of malvidin in the extract may be explained by its lower polarity compared to the other five anthocyanidin bases. Possibly malvidin was not as well extracted with hot water from the skin as other anthocyanidins. In muscadine grape juice, the concentration of malvidin is somewhat similar peonidin (Del Pozo-Insfran 2006).

The unbound fraction from the Amberlite resin had small amounts of delphinidin and cyanidin on its compound profile. The presence of these two anthocyanidins in the unbound
fraction from Amberlite can possibly be explained by their higher solubility in water as they are the two most polar anthocyanidins. Due to such polarity, both anthocyanidins might not have bound to the Amberlite resin as well as the others.

Data indicated that peonidin and malvidin were the most resistant anthocyanidins to degradation under the processing conditions since only the spray dried sample indicated significant differences with the starting material (extract). On the other hand, pelargonidin showed the highest instability since only freeze drying and vacuum drying maintained the concentration of this compound while other processing techniques significantly reduced it. The Amberlite resin technique indicated the lowest concentrations of three of the four major anthocyanidins (delphinidin, cyaniding, and petunidin), while spray drying affected pelargonidin, peonidin, and malvidin the most. Fermentation preserved all anthocyanidins except pelargonidin which had lower concentration compared to the extract. Previous investigations have illustrated the unstable nature of pelargonidin during processing (Garzón and Wrosltad 2001; Garzón and Wrosltad 2002; Kammerer and others 2007) while malvidin has been proven to resist thermal processing (Del Pozo-Insfran 2006) and showed great stability in general due to the presence of only one hydroxyl group in the B ring (Hradzina and others 1970, Talcott and others 2003; Lee and Talcott 2004).

HPLC analysis results showed a slight discrepancy compared to the total anthocyanins assay. Such discrepancy might be explained by the limitation of colorimetric assays to account for copigmentation compared to single compounds examination (Talcott and Lee 2002; Talcott and others 2003). Moreover, after HPLC results, data suggested that heat was not a major issue in degradation since only Amberlite and spray dried samples indicated significantly lower concentration of total anthocyanins compared to the starting material (Table 3-2). These results
illustrated the divergence of previous results compared to HPLC analysis data, opposing to early conclusions mentioning vacuum drying being the only processing protocol that maintained anthocyanin concentration. High variation on HPLC results and slight higher concentrations of some anthocyanidins in processed samples were due to the difficulties of analyzing anthocyanidins instead of anthocyanin glycosides, once the sugar molecule is separated from the anthocyanidin, the aglycone form is very susceptible to degradation (Dao and others 1998). Furthermore, consistency at the hydrolysis step prior to HPLC analysis and poor compound separation might also explain these results.

Table 3-2. Anthocyanidin concentrations in the muscadine pomace extract as affected by various processing protocols.

<table>
<thead>
<tr>
<th>Process</th>
<th>Anthocyanin concentration¹</th>
<th>Dp²</th>
<th>Cy</th>
<th>Pt</th>
<th>Pg</th>
<th>Pn</th>
<th>Mv</th>
<th>Total³</th>
</tr>
</thead>
<tbody>
<tr>
<td>E⁴</td>
<td>316 ab⁵</td>
<td>333 bc</td>
<td>258 bc</td>
<td>15.7 a</td>
<td>367 ab</td>
<td>115 abc</td>
<td>1400 ab</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>401 a</td>
<td>411 a</td>
<td>329 ab</td>
<td>12.2 b</td>
<td>449 a</td>
<td>143 a</td>
<td>1750 a</td>
<td></td>
</tr>
<tr>
<td>BC₁₈</td>
<td>243 bc</td>
<td>264 cd</td>
<td>219 cd</td>
<td>11.5 b</td>
<td>301 bc</td>
<td>95.2 bcd</td>
<td>1130 bc</td>
<td></td>
</tr>
<tr>
<td>UBC₁₈</td>
<td>ND⁶</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>165 c</td>
<td>186 e</td>
<td>169 d</td>
<td>9.27 b</td>
<td>280 bc</td>
<td>87.6 cd</td>
<td>897 c</td>
<td></td>
</tr>
<tr>
<td>UBA</td>
<td>12.7 d</td>
<td>7.74 f</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20.4 d</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>207 c</td>
<td>216 de</td>
<td>185 d</td>
<td>5.40 c</td>
<td>250 c</td>
<td>75.2 d</td>
<td>939 c</td>
<td></td>
</tr>
<tr>
<td>FD</td>
<td>313 ab</td>
<td>335 bc</td>
<td>283 abc</td>
<td>16.0 a</td>
<td>396 a</td>
<td>127 ab</td>
<td>1470 ab</td>
<td></td>
</tr>
<tr>
<td>VD</td>
<td>401 a</td>
<td>374 ab</td>
<td>336 a</td>
<td>16.2 a</td>
<td>439 a</td>
<td>135 a</td>
<td>1700 a</td>
<td></td>
</tr>
</tbody>
</table>

¹Expressed in cyanidin aglycone equivalents (mg/kg). ²Anthocyanidin abbreviations: (Dp) Delphinidin, (Cy) Cyanidin, (Pt) Petunidin, (Pg) Pelargonidin, (Pn) Peonidin, (Mv) Malvidin. ³Sum of all anthocyanidin bases. ⁴Treatment abbreviations: (E) Extract, (F) fermented extract, (BC₁₈) bound to reversed phase C₁₈ column, (UBC₁₈) unbound to reversed phase C₁₈ column, (BA) bound to Amberlite XAD-4, (UBA) unbound to Amberlite XAD-4, (SD) spray dried, (FD) freeze dried, and (VD) vacuum dried. ⁵Values with similar letters within columns are not significantly different (LSD P < 0.05). ⁶Compounds were not detected.

3.3.3.2 Ellagic acid and flavonols by HPLC

HPLC analysis of non-anthocyanin polyphenolics in muscadine pomace extract confirmed the presence of free ellagic acid, as previously characterized in other studies (Lee and Talcott 2002; Talcott and Lee 2002; Lee and Talcott 2004; Lee and others 2005; Pastrana-Bonilla and others 2003; Yi and others 2006). However, only myricetin and quercetin could be detected after hydrolysis, while previous investigations (Talcott and Lee 2002; Lee and Talcott 2004; Pastrana-
Bonilla and others 2003; Yi and others 2005; Yi and others 2006) have shown three flavonols in muscadine grapes (myricetin, quercetin, and kaempferol). Identification was possible since the retention time and spectroscopic attributes concurred with those of the authentic standards of ellagic acid and quercetin aglycones (Figure 3-6).

Figure 3-6. HPLC chromatogram of polyphenolics present in muscadine pomace: ellagic acid (A), myricetin (B), and quercetin (C). Identification (360 nm) was done based on spectral characteristics and comparison to authentic standards of ellagic acid and quercetin.

Total ellagic concentration was fairly stable and only affected by spray drying and Amberlite treatments (Table 3-3). The content of ellagic acid was expected to be maintained in most of the treatments since it has been proven to be quite stable to processing and increment its concentration due to hydrolysis of ellagitannins which are unstable to heat and acidic conditions (Amakura and others 2000; Zafrilla and others 2001; Tomás-Barberán and Clifford 2000). However, another investigation showed that ellagic acid was decreased after cooking probably due to oxidation reactions (Häkkinen and others 2000). The unbound fraction from the Amberlite resin showed an important concentration of total ellagic acid (8.6%) that was not bound to the resin and thus thought to be ellagitannins (Lee 2004). Similar to the total soluble phenolics assay, reversed phase C18 was an efficient separation technique since negligible concentrations of free ellagic acid was detected in the unbound fraction from this technique.

In the case of flavonols, myricetin and quercetin were observed in the muscadine pomace extract (10.2 mg/kg and 5.44 mg/kg respectively). No significant differences were detected in
flavonols due to processing (Table 3-3). Remaining isolation protocols showed higher concentrations of flavonols, thus, the only conclusion that could be derived from this data is that processes had no effect on the flavonol concentration of the muscadine pomace product. Such results differed with some investigations that illustrated the loss of flavonols due to heat processing (Amakura and others 2000; Häkkinen and others 2000). However, flavonols have indicated better stability than anthocyanindins in storage at 20 and 37°C (Talcott and Lee 2002). Such results could be explained by the high variation detected in HPLC analysis due to the use of aglycone forms of flavonoids that are know to be very unstable making this assay difficult.

Neither myricetin or quercetin were detected in the unbound fractions (C18 and Amberlite), thus flavonols might bind more effectively to both columns compared to other polyphenolics.

Table 3-3. Ellagic acid and flavonol concentrations in the muscadine pomace extract as affected by various processing protocols.

<table>
<thead>
<tr>
<th>Process</th>
<th>Ellagic acid1</th>
<th>Myricetin2</th>
<th>Quercetin2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>168 ab4</td>
<td>10.2 c</td>
<td>5.44 bc</td>
</tr>
<tr>
<td>F</td>
<td>176 ab</td>
<td>22.2 ab</td>
<td>7.26 a</td>
</tr>
<tr>
<td>BC18</td>
<td>123 cd</td>
<td>18.6 b</td>
<td>6.54 ab</td>
</tr>
<tr>
<td>UBC18</td>
<td>ND5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BA</td>
<td>113 d</td>
<td>11.3 c</td>
<td>5.16 bc</td>
</tr>
<tr>
<td>UBA</td>
<td>9.77 e</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SD</td>
<td>122 cd</td>
<td>13.8 c</td>
<td>4.94 c</td>
</tr>
<tr>
<td>FD</td>
<td>155 bc</td>
<td>20.7 ab</td>
<td>7.13 a</td>
</tr>
<tr>
<td>VD</td>
<td>202 a</td>
<td>22.8 a</td>
<td>7.67 a</td>
</tr>
</tbody>
</table>

1Expressed in ellagic acid equivalents (mg/kg). 2Expressed in quercetin equivalents (mg/kg). 3Treatment abbreviations: (E) Extract, (F) fermented extract, (BC18) bound to reversed phase C18 column, (UBC18) unbound to reversed phase C18 column, (BA) bound to Amberlite XAD-4, (UBA) unbound to Amberlite XAD-4, (SD) spray dried, (FD) freeze dried, and (VD) vacuum dried. 4Values with similar letters within columns are not significantly different (LSD P < 0.05). 5Compounds were not detected.

3.3.4 Antioxidant Capacity

Antioxidant capacity of muscadine pomace extract (34.3 ± 0.57 μmol Trolox equivalents (TE)/g) was found to be relatively high and comparable to other fruits such as cherries (33.4 ± 3.4 μmol TE/g) and strawberries (35.4 ± 4.2 μmol TE/g), and vegetables such as red cabbage.
(31.5 ± 6 µmol TE/g) (Wu and others 2004). Processing in general had a negative effect on the antioxidant capacity of the extract (Figure 3-7). Fermentation, which maintained the concentration of polyphenolics from the starting extract, had significant differences in antioxidant capacity (28.7 ± 2.53µmol TE/g). Following the fermentation procedure only vacuum drying maintained antioxidant capacity (28.8 ± 1.23µmol TE/g) while spray and freeze drying significantly reduced it (22.0 ± 0.75µmol TE/g and 18.1 ± 3.13µmol TE/g respectively). Solid phase isolation process also had a detrimental effect on the antioxidant capacity of the starting material since both affinity column techniques decrease the antioxidant capacity by more than 34% (Amberlite) and 51% (reversed phase C18). The unbound fraction of Amberlite showed a comparatively low antioxidant capacity probably due to the presence of ellagitannins in the fraction (1.91 ± 3.64µmol TE/g) since more than 15% of polyphenolics that were subjected to the Amberlite were found in the unbound fraction and were thought to contain comparable content of antioxidant capacity that was washed away. Data of the unbound fraction only indicated 8% of antioxidant capacity which confirmed the previous assumption of ellagitannins occurrence in the fraction since its antioxidant capacity is considered to be lower than ellagic acid aglycone as explained by Lee (2004) where antioxidant capacity was higher in aglycone forms and gradually reduced by polymerization of ellagic acid. Data from the unbound fraction of reversed phase C18 did not indicate presence of antioxidant compounds. Furthermore, HPLC, total anthocyanins, and total phenolics analyses confirmed a negligible concentration of anthocyanins and polyphenolics in this fraction (Table 3-1).

All treatments affected the initial antioxidant capacity from the extract which agrees with Schmidt and others (2005), who suggested that even if the polyphenolic concentration of any sample is maintained after processing, its bioactive characteristics are modified. Vacuum
concentration resulted in a greater retention of antioxidant capacity compared to spray drying and freeze drying which resulted in losses of 23% and 37% respectively. Also, vacuum concentration was the only isolation procedure that preserved the antioxidant capacity following fermentation. Consequently, the vacuum drying process did not have a negative effect on the quality of the final extract.

![Graph](image)

Figure 3-7. Antioxidant capacity of muscadine pomace extract as affected by various processing protocols. Error bars represent the standard error of the mean, n=3.

### 3.4 Conclusions

Target compounds extracted from muscadine grape pomace showed fairly high antioxidant activity (34.3 ± 0.57 μmol TE/g) that is comparable to some fruits and vegetables. Processing positively influenced polymerization and stability of anthocyanins. In the first section of this work, high temperature together with low concentration of polyphenolics, oxygen exposure, and high pH environment were thought to be the most harmful factors that affected polyphenolic content and bioactive characteristics. However, later information from HPLC analysis illustrated the impact of polymerization was not accounted by colorimetric analysis and heat was not a major problem, thus showing biased results. Although processing did not show significant
compound losses in all treatments except Amberlite and spray drying (by HPLC analysis), antioxidant capacity was significantly affected by processing. Vacuum drying proved to be the best treatment of all since it best maintained both anthocyanins and other polyphenolics concentration and also preserved the antioxidant capacity following fermentation. Fermentation showed better results than solid phase isolation to get rid of sugars without jeopardizing the quality of the final product. Moreover, solid phase isolation, specifically the Amberlite resin technique, could not bind all polyphenolics efficiently. Drying procedures following an aerobic fermentation were more practical than solid phase isolation followed by methanol evaporation, thus drying processes were not only statistically different but also showed practicality to be considered for implementation by the industry. Furthermore, the use of methanol, a non edible alcohol, in both affinity column isolation techniques could represent an environmental downside and a potential operative cost compared to use of water in the fermentation procedure.
CHAPTER 4
ECONOMIC ANALYSIS OF AN ISOLATED PRODUCT OBTAINED FROM MUSCADINE GRAPE POMACE

Functional foods and beverages consumption is growing due to major consumer trends toward health consciousness (Milo 2005). As a result of consumer awareness, natural products such as food colorants and antioxidants have gained substantial attention in the market. In 1996, 26% of the food antioxidant market was occupied by natural antioxidants with a growth rate of 6-7% annually. The sources of natural antioxidants such as vitamin C, tocopherols, polyphenolics, and organic acids include fruits, vegetables, spices, and herbs (Meyer and others 2002). In berries and fruits, the most abundant antioxidants are vitamin C and polyphenolics.

Companies such as Optiture (USA), Chr. Hansen (Denmark), Overseal Natural Ingredients (GB), Quim Dis (France), Inheda (France), and Folexco (USA) share the market for extracts and concentrates from fruits and berries (Meyer and others 2002).

A growing demand for natural products has created an opportunity to substitute synthetic antioxidants by natural compounds. Among the traditional antioxidants used are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which have been associated with potential toxicity. Furthermore, the manufacturing costs of these compounds are more expensive than natural antioxidants from fruits and vegetables (Moure and others 2001).

Polyphenolics can be widely found in pomace or wastes of numerous fruits and vegetables after processing (Koplotek and others 2005; Pastrana-Bonilla and others 2003; Visioli and others 1999; Bonilla and others 1999) and these sources represent an inexpensive material to potentially create food ingredients. As a result, the interest in natural antioxidants and the occurrence of these compounds in by-products have driven fruit and vegetable processors to attempt to extract polyphenolics from their wastes and increase the profitability of their operations. In the case of muscadine grapes, pomace constitutes around 40% of the total fruit and it is an important source
of polyphenolics (Pastrana-Bonilla and others 2003; Morris and Brady 2004) that has not been utilized, but could be an important source to generate food ingredients. Research has focused on converting muscadine and blueberry pomace into nutraceutical products that could be sold for as much as $100/lb. In Georgia alone, around 1.5 million pounds of dry muscadine skins are produced yearly and could be transformed into a very profitable product (Phillips 2006). Therefore, the aim of this part of the study was to analyze the profitability of proposed technologies to extract and concentrate polyphenolics from muscadine grape pomace as an incremental operation to a typical grape juice production facility.

4.2 Materials and Methods

4.2.1 Data Collection

Primary data, regarding the nature of the muscadine industry and its operations, was collected by interviewing muscadine processors of South Georgia. Other primary information, regarding equipment specification, capacity and pricing, was collected by interviewing some companies responsible for selling used equipment for food industries. Assumptions used in this study were drawn from interview comments and educated estimations.

4.2.2 Economic Analysis

An economic analysis was conducted based on profitability, sensitivity, and economic return of three alternatives of polyphenolic isolation following fermentation. Potential profitability was evaluated by comparing total revenue and total costs by a breakeven analysis of both prices and volumes of production. Results were reported as minimum price per pound ($/lb) and minimum volume (lb) needed to cover total annual costs respectively. A ratio between production volume of extract and the volume of dried residue from the skins following fermentation was used for relevant calculations. A ratio between prices of the extract and the dried skins was also applied assuming that prices would fluctuate proportionally. Variations in
the output of the model were assessed by a sensitivity analysis conducted by modifying the production and price of both the extract and the dried skins (±40%). The gross revenue and the profit were displayed in the analysis for better illustration of the outcome. Results were reported in US dollars ($) per season. Finally, the economic return to the business was assessed over a period of ten years by a cash flow analysis. Net Present Value (NPV) was calculated based on a rate of 12% which was compared to a calculated Internal Rate of Return (IRR). Inflation was estimated as 3.43%. Depreciation of equipment was conducted by the single line method with a 10% salvage value. A loan is calculated based on a fixed interest rate with higher payments towards interest at the beginning, paying the loan in 10 years. Results were expressed as US currency per season.

4.2.2.1 Description of the operation

The intended operation is an extension of a juice process facility illustrated in Figure 4-1. After grapes have been pressed for juice manufacture, the waste is collected and mixed with hot water (1:2) to allow soluble compounds to migrate to water creating a “liquid extract”. A pressing procedure following the initial extraction is necessary to remove skins to facilitate fermentation. Furthermore, skins can be dried and sold as animal feed. Next, sugars which have migrated into the liquid extract together with other compounds must be removed to facilitate further processing. Sugars are removed by an aerobic fermentation followed by a series of filtrations that allow separation of insoluble compounds. Lastly, a drying protocol is necessary to obtain an isolated product rich in polyphenolics. Three concentration protocols have been considered for the process: spray drying, freeze drying, and vacuum evaporation.

4.2.2.2 Economic assumptions

The analyses in this study are based on the following assumptions:

- Land is previously owned and is not included in the capital investment.
The company already has some equipment needed for the operation and it is not included in the capital investment.

Muscadine pomace has no cost since it is a by-product of their own operation and no costs were considered for elimination of this material.

90 tons of fruit will go through juice processing and the pomace will be the by-product operation which will be used as the starting material for polyphenolic extraction.

This operation lasts two months due to the harvesting season of muscadine grapes (61 days).

The final consumer is a processor that will use the product as a food ingredient.

Equipment specifications (Table 4-1) are approximates drawn from interviews with equipment vendors.
• The process needs 4 employees working part time (4h/day) on the process with a wage of $8/h. Employees already work in the company.

• Proposed price for yeast required for fermentation is $5.45/lb and intended to be used at a ratio of 0.25lb yeast/100lb of extract.

• Price for diatomaceous earth (DE) for clarification is quoted at $0.80/lb and planned to be used at a ratio of 1lb of DE/100lb of extract.

• Most of the working time will be spent in the extract manufacturing (70%) while 30% is spent on skin drying.

• Currently no costs in hauling or marketing of the final product are taken into account.

• The final product is intended to be sold to formulator of dietary supplements and/or functional foods.

• The estimated price used for the extract in the analysis is $70/lb.

• The dried skins after this process are going to be sold as animal feed.

• The estimated price of the dried skins in the analysis is $1.5/lb.

• Depreciation is calculated at 5 years for equipment, 20 years for land, and 10 years for other materials.

• The rate used for taxes is 20% for a self employed operation according to IRS.

• There is a loan for the building construction of $70,000 to be paid in ten years at a 7.5% interest rate (Table 4-2).

• For other activities such us cleaning and maintenance needed in the operation, 1% of the total revenue was assigned in the cash flow.

• Installation cost for dryers are 40% of the total cost of the machinery.

• Installation cost for filters are 70% of the total cost of the machinery.

• Pumps have an electrical consumption of 17KW each.

• Operation time was calculated based on the volume produced per day, for electrical calculations, extra time (1 hour) was added for basic maintenance and warming up of equipment.
Table 4-1. Generalities of drying equipments.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Estimated cost ($US)</th>
<th>Estimated capacity (lb/h)</th>
<th>Estimated electrical demand (KW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporator</td>
<td>$150,000</td>
<td>600</td>
<td>39.0</td>
</tr>
<tr>
<td>Spray dryer</td>
<td>$84,000</td>
<td>200</td>
<td>25.0</td>
</tr>
<tr>
<td>Freeze Dryer</td>
<td>$77,000</td>
<td>150</td>
<td>18.4</td>
</tr>
<tr>
<td>Residue dryer</td>
<td>$100,000</td>
<td>500</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Table 4-2. Loan payment plan for ten years at a fixed rate of 7.5%.

<table>
<thead>
<tr>
<th>Initial debt (ID)</th>
<th>Rate ¹</th>
<th>Payment ²</th>
<th>Interest</th>
<th>Amortization (A)</th>
<th>Debt ³ (ID - A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$70,000</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$998</td>
<td>$6,528</td>
<td>$63,473</td>
</tr>
<tr>
<td>$63,473</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$893</td>
<td>$6,633</td>
<td>$56,840</td>
</tr>
<tr>
<td>$56,840</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$788</td>
<td>$6,738</td>
<td>$50,103</td>
</tr>
<tr>
<td>$50,103</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$683</td>
<td>$6,843</td>
<td>$43,260</td>
</tr>
<tr>
<td>$43,260</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$578</td>
<td>$6,948</td>
<td>$36,313</td>
</tr>
<tr>
<td>$36,313</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$473</td>
<td>$7,053</td>
<td>$29,260</td>
</tr>
<tr>
<td>$29,260</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$368</td>
<td>$7,158</td>
<td>$22,103</td>
</tr>
<tr>
<td>$22,103</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$263</td>
<td>$7,263</td>
<td>$14,840</td>
</tr>
<tr>
<td>$14,840</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$158</td>
<td>$7,368</td>
<td>$ 7,473</td>
</tr>
<tr>
<td>$ 7,473</td>
<td>7.50%</td>
<td>$7,525</td>
<td>$ 53</td>
<td>$ 7,473</td>
<td>$ -</td>
</tr>
</tbody>
</table>

¹Rate estimated from a normal bank for a fixed rate loan. ²Payment is constituted by amortization and interest. ³Debt is the result of subtracting annual amortization from previous period debt.

4.3 Results and Discussion

Proposed technologies were economically evaluated and results were reported in US currency ($US).

4.3.1 Economic Analysis

For all three operations (spray, freeze and vacuum drying), the process was identical until the final step where isolation of polyphenolics took place via three different methods. According to results from Chapter 3, the extract has a concentration of polyphenolics (1640 ± 18.1 mg/kg) that is intended to be isolated as a final product (extract). From this number we consider that the final product will be at least 0.16% concentrated and that all calculations are based on this proportion of the liquid extracted from the process. With 90 tons of grapes expected to be used in this process, 40% represent skins (Pastrana-Bonilla and others 2002) that are directly intended
for the secondary process to obtain the desired extract. This results in a season skin production of 72,000 lb of fresh skin that is intended to be mixed with hot water for a production of 144,000 lb of liquid extract and 72,000 lb of residual skins. If 0.16% of the liquid extract represents polyphenolics and we are trying to obtain a product with 5% moisture, the final concentration of extract in a season would be 249 lb. From the remaining skins after fermentation, 30% is considered solids (Phillips, 2006) and is proposed to be sold as animal feed as part of the by-product operation, which will also contain 5% moisture as a final product. From the remaining skins obtained, a total of 22,619 lb of dry skin can be produced in a season. Currently, efforts to process muscadine pomace are still emerging and the amount of time and capital intended for such an operation is limited. Therefore, proposed processes are basic and economically feasible for a typical muscadine producer.

4.3.1.1 Spray drying operation

A used spray dryer with a capacity to remove 200 lb of water per hour was considered for the process. An estimated price ($60,000) was quoted for the equipment which needed to operate 13 hours daily to remove the amount of water required to obtain a 5% moisture isolated product. The initial investment was $120,640 which included the spray dryer, its installation fee ($24,000), and other required materials (Table 4-3). Additional equipment necessary for this specific operation would typically already be owned by a juice processor. As mentioned earlier in the assumptions, the new building construction is intended to be built with a bank loan.

From the seasonal production of extract and dried skins (249 lb and 22,619 lb respectively) and the medium price estimated and expected by an average muscadine grape producer for such extract and dried skins production ($70/lb and $1.5/lb respectively), total income for the incremental operation was calculated. Furthermore, with knowledge of the equipment and material required by this operation, total costs were also calculated and used for further
economic analysis. Once income and cost sources were determined, a production volume
breakeven point established the minimum amount required to sustain the intended by-product
operation. From the break even equation [1], where I represents income from the extract (IE) and
the residue skins (IR), C represents cost both fixed (FC) and variable from the two products (VE
and VR) and the ratio between extract (QE) and dried skins (QR) productions (lb per season) [2],
161 lb of extract and 14,616 lb of dried skins must be produced to cover both annual fixed costs
and variable costs of an operation using a spray dryer with similar technical characteristics as the
method for polyphenolic isolation. Figure 4-2 illustrates that at prices $70/lb for the extract (pE)
and $1.50/lb for the dried skins (pR), the total cost to cover for a season is $33,168 which is
covered by 161 lb of extracts and proportionally by 14,616 lb of dried skins. The production of
dried skins was not shown in the x-axis of this figure to avoid confusion.

Table 4-3. Capital expenditure to initiate a marginal process obtaining extract and dried skins
from muscadine grape skins using spray drying as the isolation technique.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Unit cost</th>
<th>Quantity</th>
<th>Total estimated cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. EQUIPMENT COSTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>Each</td>
<td>$25,000</td>
<td>1</td>
<td>$25,000</td>
</tr>
<tr>
<td>Spray Dryer</td>
<td>Each</td>
<td>$84,000</td>
<td>1</td>
<td>$84,000</td>
</tr>
<tr>
<td>Pumps</td>
<td>Each</td>
<td>$1,200</td>
<td>2</td>
<td>$2,400</td>
</tr>
<tr>
<td>Containers (bins)</td>
<td>Each</td>
<td>$220</td>
<td>42</td>
<td>$9,240</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td></td>
<td></td>
<td>$120,640</td>
</tr>
<tr>
<td><strong>2. CONSTRUCTION COSTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Building</td>
<td>Each</td>
<td>$70,000</td>
<td>1</td>
<td>$70,000</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td></td>
<td></td>
<td>$70,000</td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td>$190,640</td>
</tr>
</tbody>
</table>

1Equipment listed in the table is applied directly to the expenditure of the operation since the rest of equipment
needed for the operation is already owned. 2The total construction cost for the new building includes wiring,
electrical and tubing installations. 3New building will be constructed with a bank loan.

\[ I_E + I_R = FC + VC_E + VC_R \]  \[ Q_E = 0.011Q_R \]

\[ p_E \times Q_E + p_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R \]

\[ 70Q_E + 1.5Q_R = 25,162 + 39.82Q_E + 0.11Q_R \]
Another useful analysis is to keep production volume steady and to vary prices to determine the minimum price needed to cover total costs. For a price break even point calculation, the break even equation [1] and the ratio between extract and dried skins productions [2] were also employed. Furthermore, a ratio between prices ($/lb) [3] was used under the assumption that prices of extract and dried skins would maintain a difference between each other and that such difference would remain proportional. With such assumptions in mind, the prices where the actual production of extract (249 lb) and dried skins (22,619 lb) cover the annual total cost are $51.21/lb and $1.10/lb, respectively. Figure 4-3 illustrates five diverse scenarios with

\[
70(0.011Q_r) + 1.5Q_r = 25,162 + 39.82(0.011Q_r) + 0.11Q_r \\
0.77Q_r + 1.5Q_r = 25,162 + 0.44Q_r + 0.11Q_r \\
2.27Q_r - 0.55Q_r = 25,162 \\
1.72Q_r = 25,162 \\
Q_r = \frac{25,162}{1.72} \\
Q_r = 14,615.56 \text{ lb} \\
Q_E = 160.63 \text{ lb}
\]
different incomes (I) illustrating the significant impact price alterations can have on the operation. Prices ranged from $20/lb (I-1) to $100/lb (I-5) with increments of $20 for the extract and proportionally from $0.43/lb to $2.14/lb with increments of 43 cents for the dried skins. When prices were below $40/lb (extract) and $0.86/lb (dried skins), the minimum production to cover annual costs exceeded 300 lb of extract and more than 27,000 lb of dried skins. On the other hand, when prices reached $100/lb (extract) and $2.14/lb (dried skins), net earnings can be reached after only 100 lb of extract and 9,000 lb of dried skins produced. With such prices and the intended season production volume, gross income of almost $90,000 would be generated and profit would increase up to $48,379 in a single season.

\[ I_E + I_R = FC + VC_E + VC_R \] [1]

\[ p_E \times Q_E + p_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R \]

\[ p_E \times (Q_R \times 0.011) + p_R \times Q_R = FC + VC_E \times (Q_R \times 0.011) + VC_R \times Q_R \]

\[ Q_R (0.011 p_E + p_R) = Q_R \left( \frac{FC}{Q_R} + 0.011 VC_E + VC_R \right) \]

\[ 0.011 p_E + p_R = FC/Q_R + 0.011 VC_E + VC_R \]

\[ p_E = 46.67 p_R \] [3]

\[ 0.011(46.67 p_R) + p_R = \frac{25,162}{22,619} + 0.011(39.82) + 0.11 \]

\[ p_R = \$1.10/lb \]
\[ p_E = \$51.21/lb \]

A sensitivity analysis of the circumstances when a spray dryer (with characteristics previously described) is used as the isolation method was conducted (Table 4-4). Data suggested that a loss of more than $11,000 could be anticipated if production and prices dropped 40%. Losses were still registered when one characteristic, either price or volume, was reduced 40%.
Under initial circumstances (medium price and volume), a profit of almost $14,000 in a season was registered. The remaining situations also showed positive numbers. Therefore, this operation is viable when the combination of production volume and prices surpasses low-low and low-medium combinations.

Figure 4-3. Break-even point as affected by price for a facility using a spray dryer as a final step for product isolation. Abbreviations: I income, TC total costs.

Table 4-4. Sensitivity analysis for a marginal process obtaining extract and dried skins from muscadine grape skins using spray drying as the isolation technique.

<table>
<thead>
<tr>
<th>Production (lb)</th>
<th>Ext / DS</th>
<th>pExt / pDS</th>
<th>Prices ($)</th>
<th>Low&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Medium</th>
<th>High&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low&lt;sup&gt;1&lt;/sup&gt;</td>
<td>178 / 16,156</td>
<td>Sales&lt;sup&gt;3&lt;/sup&gt;</td>
<td>$6,188</td>
<td>$36,664</td>
<td>$51,685</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit&lt;sup&gt;6&lt;/sup&gt;</td>
<td>$(11,363)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>$(887)</td>
<td>$14,133</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>249 / 22,619</td>
<td>Sales</td>
<td>$36,664</td>
<td>$51,329</td>
<td>$72,358</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit</td>
<td>$(887)</td>
<td>$13,778</td>
<td>$34,807</td>
<td></td>
</tr>
<tr>
<td>High&lt;sup&gt;2&lt;/sup&gt;</td>
<td>348 / 31,666</td>
<td>Sales</td>
<td>$51,329</td>
<td>$71,861</td>
<td>$101,302</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit</td>
<td>$13,778</td>
<td>$34,310</td>
<td>$63,750</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Low represent 40% less of both medium prices and volumes.  <sup>2</sup>High represent 40% more of both medium prices and volumes.  <sup>3</sup>Extract and dried skins productions are displayed vertically in the table.  <sup>4</sup>Prices for extract and dried skins are displayed horizontally in the table.  <sup>5</sup>Sales are shown for all 9 different supposed scenarios.  <sup>6</sup>Profits are shown for all 9 different supposed scenarios.  <sup>7</sup>Number in parenthesis indicate profit loss.

The economic return was assessed after 10 years of operation by a cash flow analysis (Table 4-5). With an investment of $120,640 and a bank loan of $70,000, the operation could
return the investment and generate almost $60,000 of net earnings in ten years. With average annual income of more than $30,000, the internal rate of return (IRR) was 10% higher than the rate used for the net present value (NPV) calculation, thus showing thriving results for this additional operation for the muscadine industry. It is important to denote that more compounds could be extracted from the skins if processes are to be optimized resulting in a higher yield, extract production and thus, generating profit for selling more product at $70/lb than $1.5/lb.

4.3.1.2 Freeze drying operation

A used freeze dryer with a capacity to remove 150 lb of water per hour was evaluated for this isolation technique and according to its specifications, it needed 15.7 hours to remove water from the daily liquid extract produced. An estimated price ($55,000) was quoted for the equipment. The initial investment ($113,640) included the freeze dryer, its installation fee ($4,400), and other required materials (Table 4-6). Additional equipment necessary for this specific operation was assumed to be already owned by a typical juice processor. The facilities required to conduct a by-product isolation process were intended to be built with a bank loan as described in the assumption section of this chapter.

Table 4-6. Capital expenditure to initiate a marginal process obtaining extract and dried skins from muscadine grape skins using freeze drying as the isolation technique.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Unit cost</th>
<th>Quantity</th>
<th>Total estimated cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. EQUIPMENT COSTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>Each</td>
<td>$25,000</td>
<td>1</td>
<td>$25,000</td>
</tr>
<tr>
<td>Freeze Dryer</td>
<td>Each</td>
<td>$77,000</td>
<td>1</td>
<td>$77,000</td>
</tr>
<tr>
<td>Pumps</td>
<td>Each</td>
<td>$1,200</td>
<td>2</td>
<td>$2,400</td>
</tr>
<tr>
<td>Containers (bins)</td>
<td>Each</td>
<td>$220</td>
<td>42</td>
<td>$9,240</td>
</tr>
<tr>
<td>Sub-total</td>
<td></td>
<td></td>
<td></td>
<td>$113,640</td>
</tr>
<tr>
<td><strong>2. CONSTRUCTION COSTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Building</td>
<td>Each</td>
<td>$70,000</td>
<td>1</td>
<td>$70,000</td>
</tr>
<tr>
<td>Sub-total</td>
<td></td>
<td></td>
<td></td>
<td>$70,000</td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td>$183,640</td>
</tr>
</tbody>
</table>

1Equipment listed in the table is applied directly to the expenditure of the operation since the rest of equipment needed for the operation is already owned. 2The total construction cost for the new building includes wiring, electrical and tubing installations. 3New building will be constructed with a bank loan.
Table 4-5. Cash flow analysis for a marginal process obtaining extract and dried skins from muscadine grape skins using spray drying as the isolation technique.

<table>
<thead>
<tr>
<th>Periods (years)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investment</strong></td>
<td>$120,640</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>$17,401</td>
<td>$17,998</td>
<td>$18,615</td>
<td>$19,254</td>
<td>$19,914</td>
<td>$20,597</td>
<td>$21,304</td>
<td>$22,035</td>
<td>$22,790</td>
<td>$23,572</td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>$33,928</td>
<td>$35,092</td>
<td>$36,296</td>
<td>$37,540</td>
<td>$38,828</td>
<td>$40,160</td>
<td>$41,537</td>
<td>$42,962</td>
<td>$44,436</td>
<td>$45,960</td>
<td></td>
</tr>
<tr>
<td><strong>Expenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labor</td>
<td>$7,168</td>
<td>$7,412</td>
<td>$7,664</td>
<td>$7,924</td>
<td>$8,194</td>
<td>$8,472</td>
<td>$8,760</td>
<td>$9,058</td>
<td>$9,366</td>
<td>$9,685</td>
<td></td>
</tr>
<tr>
<td>General costs</td>
<td>$1,753</td>
<td>$1,813</td>
<td>$1,874</td>
<td>$1,938</td>
<td>$2,004</td>
<td>$2,072</td>
<td>$2,142</td>
<td>$2,215</td>
<td>$2,291</td>
<td>$2,369</td>
<td></td>
</tr>
<tr>
<td>Depreciation</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$4,424</td>
</tr>
<tr>
<td>Interest (7.5%)</td>
<td>$998</td>
<td>$998</td>
<td>$893</td>
<td>$788</td>
<td>$683</td>
<td>$578</td>
<td>$473</td>
<td>$368</td>
<td>$263</td>
<td>$158</td>
<td></td>
</tr>
<tr>
<td><strong>Gross Income</strong></td>
<td>$19,767</td>
<td>$21,215</td>
<td>$22,710</td>
<td>$24,253</td>
<td>$25,845</td>
<td>$24,367</td>
<td>$23,064</td>
<td>$21,415</td>
<td>$19,622</td>
<td>$18,488</td>
<td></td>
</tr>
<tr>
<td>Tax (20%)</td>
<td>$3,953</td>
<td>$4,243</td>
<td>$4,542</td>
<td>$4,851</td>
<td>$5,169</td>
<td>$5,823</td>
<td>$6,131</td>
<td>$6,923</td>
<td>$9,324</td>
<td>$9,698</td>
<td></td>
</tr>
<tr>
<td>Utilities after Taxes</td>
<td>$15,813</td>
<td>$16,972</td>
<td>$18,168</td>
<td>$19,402</td>
<td>$20,676</td>
<td>$22,049</td>
<td>$23,451</td>
<td>$24,852</td>
<td>$26,298</td>
<td>$27,791</td>
<td></td>
</tr>
<tr>
<td>(+) Depreciation</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$18,303</td>
<td>$4,424</td>
</tr>
<tr>
<td>Amortization</td>
<td>$6,528</td>
<td>$6,633</td>
<td>$6,738</td>
<td>$6,843</td>
<td>$6,948</td>
<td>$7,053</td>
<td>$7,158</td>
<td>$7,263</td>
<td>$7,368</td>
<td>$7,473</td>
<td></td>
</tr>
<tr>
<td><strong>Salvage Value</strong></td>
<td>$7,711</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Net Income</strong></td>
<td>$27,589</td>
<td>$28,643</td>
<td>$29,734</td>
<td>$30,863</td>
<td>$31,972</td>
<td>$30,416</td>
<td>$31,171</td>
<td>$31,801</td>
<td>$31,313</td>
<td>$30,842</td>
<td></td>
</tr>
<tr>
<td><strong>Net Present Value (NPV)</strong></td>
<td>$177,808</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intern Rate of Return (IRR)</strong></td>
<td>22.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
When income and cost sources were determined, a production volume breakeven point was conducted to determine the minimum amount of extract and dried skins required for the by-product operation to be feasible. Analogous to the previous technology (spray drying), the break even equation (US dollars) [1] and the proportion between extract and dried skins productions (lb per season) [2] were used to conduct a break even analysis. Data suggested that 152 lb of extract and 13,847 lb of dried skins were needed to cover total annual costs ($31,424) of the operation when prices for the extract and the dried skins were $70/lb and $1.50/lb respectively (Figure 4-4).

\[ I_E + I_R = FC + VC_E + VC_R \quad [1] \quad Q_E = 0.011Q_R \quad [2] \]

\[ p_E \times Q_E + p_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R \]

\[ 70Q_E + 1.5Q_R = 23,862 + 39.68Q_E + 0.11Q_R \]

\[ 70(0.011Q_R) + 1.5Q_R = 23,862 + 39.68(0.011Q_R) + 0.11Q_R \]

\[ 0.77Q_R + 1.5Q_R = 23,862 + 0.44Q_R + 0.11Q_R \]

\[ 2.27Q_R - 0.55Q_R = 23,862 \]

\[ 1.72Q_R = 23,862 \]

\[ Q_R = \frac{23,862}{1.72} \]

\[ Q_R = 13,847.41 \text{ lb} \quad Q_E = 152.18 \text{ lb} \]
Figure 4-4. Volume break-even point for a facility using a freeze dryer as a final step for product isolation. Abbreviations: FC Fixed costs, VC variable costs, TC total costs, I income.

For the break even price analysis, the break even equation US currency [1] and the ratio between extract and dried skins productions in pounds per season [2] were used for estimations. A ratio between prices ($/lb) [3] was calculated and also used under the assumption that prices would maintain a proportional difference between each other. To cover total annual costs when medium production volumes of extract (249 lb) and dried skins (22,619 lb) are produced, the prices should be at least $39/lb and $1.06/lb, respectively. Five scenarios in which prices varied from $20/lb (I-1) to $/100/lb (I-5) with increments of $20 for the extract and $0.43/lb (I-1) and $2.14/lb (I-5) with increments of 43 cents for the dried skins were analyzed (Figure 4-5). Similar to an operation with spray drying as the isolation method, when prices are below $40/lb (extract) and $0.86/lb (dried skins), the minimum production to cover annual costs would have to be greater than 300 lb of extract and more than 27,000 lb of dried skins. In contrast, when prices just surpass $40/lb (extract) and $0.86/lb (dried skins) profit is reached under such production volumes. This slight difference in the calculation between spray drying and freeze drying operations is attributable to the reduction in the investment. The freeze dryer suggested in this chapter is 10% cheaper.
\[ I_e + I_R = FC + VC_E + VC_R \] [1]

\[ p_E \times Q_E + p_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R \]

\[ p_E \times (Q_E \times 0.011) + p_R \times Q_R = FC + VC_E \times (Q_R \times 0.011) + VC_R \times Q_R \]

\[ Q_R (0.011p_E + p_R) = Q_R \left( \frac{FC}{Q_R} + 0.011VC_E + VC_R \right) \]

\[ 0.011p_E + p_R = \frac{FC}{Q_R} + 0.011VC_E + VC_R \]

\[ p_E = 46.67p_R \]

\[ 0.011(46.67p_R) + p_R = \frac{23.862}{22.619} + 0.011(39.68) + 0.11 \]

\[ p_R = \$1.06/lb \]

\[ p_E = \$49.39/lb \]

Figure 4-5. Break-even point as affected by price for a facility using a freeze dryer as a final step for product isolation. Abbreviations: I income, TC total costs.

A sensitivity analysis of the circumstances where freeze drying is proposed as the isolation method (Table 4-7) indicated loss of $10,025 when production and prices dropped 40%. The low-medium combinations showed a small profit ($450) in a single year. The rest of the sensitivity analysis indicated positive numbers (profit) for the eight remaining situations.
Therefore, the only circumstance under which this operation could not be viable was when both production and prices were 40% lower than the expected values (medium).

Table 4-7. Sensitivity analysis for a marginal process obtaining extract and dried skins from muscadine grape skins using freeze drying as the isolation technique.

<table>
<thead>
<tr>
<th>Prices (SUS)</th>
<th>Low¹</th>
<th>Medium</th>
<th>High²</th>
</tr>
</thead>
<tbody>
<tr>
<td>$50 / $1.07</td>
<td>$26,188</td>
<td>$36,664</td>
<td>$51,685</td>
</tr>
<tr>
<td>$70 / $1.50</td>
<td>$450</td>
<td>$15,471</td>
<td></td>
</tr>
<tr>
<td>$100 / $2.10</td>
<td>$15,116</td>
<td>$36,145</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Production (lb)</th>
<th>Ext / DS³</th>
<th>pExt / pDS⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low¹</td>
<td>178 / 16,156</td>
<td>Sales⁵ $26,188</td>
</tr>
<tr>
<td>Medium</td>
<td>249 / 22,619</td>
<td>Sales $36,664</td>
</tr>
<tr>
<td>High²</td>
<td>348 / 31,666</td>
<td>Sales $51,329</td>
</tr>
</tbody>
</table>

¹Low represent 40% less of both medium prices and volumes. ²High represent 40% more of both medium prices and volumes. ³Extract and dried skins productions are displayed vertically in the table. ⁴Prices for extract and dried skins are displayed horizontally in the table. ⁵Sales are shown for all 9 different supposed scenarios. ⁶Profits are shown for all 9 different supposed scenarios. ⁷Number in parenthesis indicate profit loss.

Economic return of this operation assessed through a ten-year period suggested that the operation was profitable and could return the investment in less than 6 years with and NPV of more than 177,000 with a IRR of almost 24% (Table 4-8). This increase in IRR compared to the spray drying operation is consequence of a reduction in the investment.

4.3.1.3 Vacuum drying operation

The evaporator cited in this situation had a capacity of 600 lb of water removed per hour and water produced from the daily operation could be evaporated in less than 4 hours. An estimated price ($107,143) was quoted for the equipment, and initial investment of $186,640 included the evaporator, its installation fee ($42,857), and other required materials (Table 4-9). Additional equipment necessary for the isolation operation was thought to be previously owned by a typical juice processor and thus not included in the current analysis.
<table>
<thead>
<tr>
<th>Periods (years)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investment</strong></td>
<td>$113,640</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>$17,401</td>
<td>$17,998</td>
<td>$18,615</td>
<td>$19,254</td>
<td>$19,914</td>
<td>$20,597</td>
<td>$21,304</td>
<td>$22,035</td>
<td>$22,790</td>
<td>$23,572</td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>$33,928</td>
<td>$35,092</td>
<td>$36,296</td>
<td>$37,540</td>
<td>$38,828</td>
<td>$40,160</td>
<td>$41,537</td>
<td>$42,962</td>
<td>$44,436</td>
<td>$45,960</td>
<td></td>
</tr>
<tr>
<td><strong>Expenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labor</td>
<td>$7,168</td>
<td>$7,412</td>
<td>$7,664</td>
<td>$7,924</td>
<td>$8,194</td>
<td>$8,472</td>
<td>$8,760</td>
<td>$9,058</td>
<td>$9,366</td>
<td>$9,685</td>
<td></td>
</tr>
<tr>
<td>General costs</td>
<td>$1,716</td>
<td>$1,774</td>
<td>$1,835</td>
<td>$1,897</td>
<td>$1,961</td>
<td>$2,028</td>
<td>$2,097</td>
<td>$2,168</td>
<td>$2,242</td>
<td>$2,318</td>
<td></td>
</tr>
<tr>
<td>Depreciation</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td></td>
</tr>
<tr>
<td>Interest (7.5%)</td>
<td>$998</td>
<td>$998</td>
<td>$893</td>
<td>$788</td>
<td>$683</td>
<td>$578</td>
<td>$473</td>
<td>$368</td>
<td>$263</td>
<td>$158</td>
<td></td>
</tr>
<tr>
<td>Gross Income</td>
<td>$20,704</td>
<td>$22,154</td>
<td>$23,650</td>
<td>$25,194</td>
<td>$26,788</td>
<td>$26,888</td>
<td>$41,110</td>
<td>$43,109</td>
<td>$44,862</td>
<td>$46,761</td>
<td>$48,538</td>
</tr>
<tr>
<td>Tax (20%)</td>
<td>$4,141</td>
<td>$4,431</td>
<td>$4,730</td>
<td>$5,039</td>
<td>$5,358</td>
<td>$5,688</td>
<td>$5,822</td>
<td>$5,972</td>
<td>$9,334</td>
<td>$9,708</td>
<td></td>
</tr>
<tr>
<td>Utilities after Taxes</td>
<td>$16,563</td>
<td>$17,723</td>
<td>$18,920</td>
<td>$20,155</td>
<td>$21,430</td>
<td>$33,129</td>
<td>$34,487</td>
<td>$35,889</td>
<td>$37,337</td>
<td>$38,831</td>
<td></td>
</tr>
<tr>
<td>(+) Depreciation</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$17,403</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td>$4,424</td>
<td></td>
</tr>
<tr>
<td>Amortization</td>
<td>$6,528</td>
<td>$6,633</td>
<td>$6,738</td>
<td>$6,843</td>
<td>$6,948</td>
<td>$7,053</td>
<td>$7,158</td>
<td>$7,263</td>
<td>$7,368</td>
<td>$7,473</td>
<td></td>
</tr>
<tr>
<td>Salvage Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$7,211</td>
</tr>
<tr>
<td><strong>Net Income</strong></td>
<td>$27,439</td>
<td>$28,494</td>
<td>$29,586</td>
<td>$30,716</td>
<td>$30,906</td>
<td>$30,501</td>
<td>$31,754</td>
<td>$33,051</td>
<td>$34,393</td>
<td>$35,782</td>
<td></td>
</tr>
<tr>
<td><strong>Net Present Value (NPV)</strong></td>
<td>$177,065</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rate 12.00%</td>
</tr>
<tr>
<td><strong>Internal Rate of Return (IRR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.8%</td>
</tr>
</tbody>
</table>
Table 4-9. Capital expenditure to initiate a marginal process obtaining extract and dried skins from muscadine grape skins using vacuum drying as the isolation technique.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Unit cost</th>
<th>Quantity</th>
<th>Total estimated cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. EQUIPMENT COSTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>Each</td>
<td>$25,000</td>
<td>1</td>
<td>$25,000</td>
</tr>
<tr>
<td>Vacuum Dryer</td>
<td>Each</td>
<td>$150,000</td>
<td>1</td>
<td>$150,000</td>
</tr>
<tr>
<td>Pumps</td>
<td>Each</td>
<td>$1,200</td>
<td>2</td>
<td>$2,400</td>
</tr>
<tr>
<td>Containers (bins)</td>
<td>Each</td>
<td>$220</td>
<td>42</td>
<td>$9,240</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$186,640</strong></td>
</tr>
<tr>
<td>2. CONSTRUCTION COSTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Building</td>
<td>Each</td>
<td>$70,000</td>
<td>1</td>
<td>$70,000</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$70,000</strong></td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$256,640</strong></td>
</tr>
</tbody>
</table>

1Equipment listed in the table is applied directly to the expenditure of the operation since the rest of equipment needed for the operation is already owned. 2The total construction cost for the new building includes wiring, electrical and tubing installations. 3New building will be constructed with a bank loan.

Once revenue and cost sources have been determined, a production volume breakeven analysis was used to determine the minimum amount required to sustain the intended by-product operation. The break even equation [1] and the proportion between extract and dried skins productions [2] were used as part of the analysis. Calculations indicated that in order to cover both annual fixed and variable costs of the operation, 236 lb of extract and 21,516 lb of dried skins should be produced at $70/lb and $1.50/lb respectively. Figure 4-6 illustrates these calculations by showing the point at which the gross income generated covers season costs of $48,828. When using an evaporator of such specifications, the demand of volume of both products is high due to the elevated cost of the equipment (investment). According to these calculations and the medium production, only 13 lb of extract and 103 lb of dried skins represent earnings in the season.

\[
I_E + I_R = FC + VC_E + VC_R \quad [1] \\
Q_E = 0.011Q_R \quad [2]
\]

\[
p_E \times Q_E + p_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R
\]

\[
70Q_E + 1.5Q_R = 37,419 + 37.75Q_E + 0.11Q_R
\]
70(0.011Q_E) + 1.5Q_E = 37,419 + 38.23(0.011Q_E) + 0.11Q_E

0.77Q_E + 1.5Q_E = 37,419 + 0.42Q_E + 0.11Q_E

2.27Q_E - 0.53Q_E = 37,419

1.74Q_E = 37,419

\[ Q_E = \frac{37,419}{1.74} \]

\[ Q_E = 21,516.44 \text{ lb} \quad Q_E = 236.47 \text{ lb} \]

Figure 4-6. Volume break-even point for a facility using a vacuum dryer as a final step for product isolation. Abbreviations: FC Fixed costs, VC variable costs, TC total costs, I income.

A price break even analysis for an operation with an evaporator was also conducted. Similar to previous break even analysis, the break even equation [1], the ratio between extract and dried skins productions [2], and a ratio between prices [3] were used for calculations. Total annual costs could be covered if prices of extract (249 lb) and dried skins (22,619 lb) surpassed $67/lb and $1.44/lb respectively. Figure 4-7 illustrates five scenarios in which prices varied from $20/lb (I-1) to $100/lb (I-5) with increments of $20 for the extract and $0.43/lb (I-1) and $2.14/lb (I-5) with increments of 43 cents for the dried skins. Unlike the two previous analysis
(spray drying and freeze drying), profit with a production under 300 lb of extract and over
27,000 lb of dried skins requires a price of at least $60/lb (extract) and $1.29/lb (dried skins). At
such prices a profit of only $1,203 could be accomplished. In contrast, net season earnings of
$36,600 can be reached when 300 lb of extract and 27,000 lb of dried skins are produced at the
highest prices analyzed ($100/lb and $2.14/lb respectively). Although price of the evaporator
was high, profit can be reached in the first year and cover season costs. Moreover, if the process
is successful and projected to grow, the evaporator analyzed in this scenario has enough capacity
to support such growth while the other two equipments (spray and freeze dryers) operate more
than 10 hours daily while the evaporator works only 4. Thus, while this isolation technique is
capital demanding, if implemented it has room for growth without severe changes in the process
line.

\[ I_E + I_R = FC + VC_E + VC_R \]
\[ P_E \times Q_E + P_R \times Q_R = FC + VC_E \times Q_E + VC_R \times Q_R \]
\[ P_E \times (Q_R \times 0.011) + P_R \times Q_R = FC + VC_E \times (Q_R \times 0.011) + VC_R \times Q_R \]
\[ Q_R (0.011P_E + P_R) = Q_R \left( \frac{FC}{Q_R} + 0.011VC_E + VC_R \right) \]
\[ 0.011P_E + P_R = \frac{FC}{Q_R} + 0.011VC_E + VC_R \]
\[ P_E = 46.67P_R \]
\[ 0.011(46.67P_R) + P_R = \frac{37,419}{22,619} + 0.011(37.75) + 0.11 \]

\[ p_R = $1.44/lb \quad p_E = $67.22/lb \]
Figure 4-7. Break-even point as affected by price for a facility using a vacuum dryer as a final step for product isolation. Abbreviations: I income, TC total costs.

Table 4-10. Sensitivity analysis for a marginal process obtaining extract and dried skins from muscadine grape skins using vacuum drying as the isolation technique.

<table>
<thead>
<tr>
<th>Prices (US)</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext / DS</td>
<td>pExt / pDS</td>
<td>$50 / $1.07</td>
<td>$70 / $1.50</td>
</tr>
<tr>
<td>Low</td>
<td>178 / 16,156</td>
<td>Sales</td>
<td>$ 6,188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit</td>
<td>$ (23,105)</td>
</tr>
<tr>
<td>Medium</td>
<td>249 / 22,619</td>
<td>Sales</td>
<td>$ 36,664</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit</td>
<td>$ (12,630)</td>
</tr>
<tr>
<td>High</td>
<td>348 / 31,666</td>
<td>Sales</td>
<td>$ 51,329</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profit</td>
<td>$ 2,036</td>
</tr>
</tbody>
</table>

1Low represent 40% less of both medium prices and volumes. 2High represent 40% more of both medium prices and volumes. 3Extract and dried skins productions are displayed vertically in the table. 4Prices for extract and dried skins are displayed horizontally in the table. 5Sales are shown for all 9 different supposed scenarios. 6Profits are shown for all 9 different supposed scenarios. 7Number in parenthesis indicate profit loss.

When an evaporator is proposed as the isolation method (Table 4-10), there were losses in the low-low and medium-low combinations of production and prices. The remaining situations showed positive numbers but only the medium-high and high-high combinations showed five-digit figures of profit. At the original circumstances (medium price and volume), resulted in only $2,000 are registered in profit per season. As mentioned before, extract production is likely
to increase if process is optimized and therefore this isolation method is also profitable and convenient.

Vacuum drying, due to the characteristics of the equipment quoted, resulted in a low IRR (12.3%) and NPV of 188,627 (Table 4-11). Although the NPV calculated for this operation was higher than the last two, the investment demanded most of the money earned in a 10 year-operation period. However as mentioned earlier, this process did generate profit and had room for expansion if the process was successfully implemented by a muscadine processor. The machine at the early stages of the operation is going to be sub-utilized while spray dryer and freeze dryer quoted for previous operations are going to be working at almost full capacity. With the actual liquid extract volume, the vacuum evaporator worked less than 4 hours while spray dryer and freeze dryer were working 12 hours and 16 hours respectively.
Table 4-11. Cash flow analysis for a marginal process obtaining extract and dried skins from muscadine grape skins using vacuum drying as the isolation technique.

<table>
<thead>
<tr>
<th>Periods (years)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investment Income</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>$186,640</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extract</strong></td>
<td>$17,401</td>
<td>$17,998</td>
<td>$18,615</td>
<td>$19,254</td>
<td>$19,914</td>
<td>$20,597</td>
<td>$21,304</td>
<td>$22,035</td>
<td>$22,790</td>
<td>$23,572</td>
<td></td>
</tr>
<tr>
<td><strong>Residue</strong></td>
<td>$33,928</td>
<td>$35,092</td>
<td>$36,296</td>
<td>$37,540</td>
<td>$38,828</td>
<td>$40,160</td>
<td>$41,537</td>
<td>$42,962</td>
<td>$44,436</td>
<td>$45,960</td>
<td></td>
</tr>
<tr>
<td><strong>Expenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labor</td>
<td>$7,168</td>
<td>$7,412</td>
<td>$7,664</td>
<td>$7,924</td>
<td>$8,194</td>
<td>$8,472</td>
<td>$8,760</td>
<td>$9,058</td>
<td>$9,366</td>
<td>$9,685</td>
<td></td>
</tr>
<tr>
<td>General costs</td>
<td>$1,357</td>
<td>$1,403</td>
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<td>$1,500</td>
<td>$1,551</td>
<td>$1,604</td>
<td>$1,659</td>
<td>$1,715</td>
<td>$1,773</td>
<td>$1,834</td>
<td></td>
</tr>
<tr>
<td>Depreciation</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$4,424</td>
</tr>
<tr>
<td>Interest (7.5%)</td>
<td>$998</td>
<td>$998</td>
<td>$893</td>
<td>$788</td>
<td>$683</td>
<td>$578</td>
<td>$473</td>
<td>$368</td>
<td>$263</td>
<td>$158</td>
<td></td>
</tr>
<tr>
<td>Gross Income</td>
<td>$11,677</td>
<td>$13,139</td>
<td>$14,648</td>
<td>$16,205</td>
<td>$17,812</td>
<td>$19,366</td>
<td>$20,812</td>
<td>$22,257</td>
<td>$23,714</td>
<td>$25,171</td>
<td>$49,023</td>
</tr>
<tr>
<td>Tax (20%)</td>
<td>$2,335</td>
<td>$2,628</td>
<td>$2,930</td>
<td>$3,241</td>
<td>$3,562</td>
<td>$3,877</td>
<td>$4,192</td>
<td>$4,507</td>
<td>$4,822</td>
<td>$5,137</td>
<td>$9,805</td>
</tr>
<tr>
<td>Utilities after Taxes</td>
<td>$9,342</td>
<td>$10,511</td>
<td>$11,718</td>
<td>$12,964</td>
<td>$14,250</td>
<td>$15,536</td>
<td>$16,822</td>
<td>$18,108</td>
<td>$19,404</td>
<td>$20,700</td>
<td>$39,219</td>
</tr>
<tr>
<td>(+) Depreciation</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$26,789</td>
<td>$4,424</td>
</tr>
<tr>
<td>Amortization</td>
<td>$6,528</td>
<td>$6,633</td>
<td>$6,738</td>
<td>$6,843</td>
<td>$6,948</td>
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<td>$7,158</td>
<td>$7,263</td>
<td>$7,368</td>
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<td>Salvage Value</td>
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<tr>
<td>Net Income</td>
<td>$29,603</td>
<td>$30,668</td>
<td>$31,770</td>
<td>$32,910</td>
<td>$34,076</td>
<td>$35,244</td>
<td>$36,412</td>
<td>$37,580</td>
<td>$38,748</td>
<td>$39,916</td>
<td>$36,170</td>
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<tr>
<td>Net Present Value (NPV)</td>
<td>$188,627</td>
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<tr>
<td>Intern Rate of Return (IRR)</td>
<td>12.00%</td>
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<td>12.3%</td>
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4.4 Conclusions

Aerobic fermentation following a simple concentration step was an inexpensive way to obtain polyphenolics from muscadine grape skins since most of the investment was focused in the latter procedure (isolation). Results from three isolation techniques of polyphenolic from muscadine grape skins suggested that this additional operation can be suitable and profitable for a typical muscadine producer. Further investigation is needed to optimize the polyphenolic concentration procedure prior to fermentation to increment the extract produced and, consequently increase profit. Using initial production conditions (medium price and volume), the profit generated for each of the techniques (in order from most to least) was freeze drying > spray drying > vacuum drying. In contrast, the vacuum evaporator used as an example for this chapter had three times the capacity of the spray dryer and four times the capacity of the spray dryer, thus only the evaporator could support a growth in extract production if the volume processed is increased significantly. Another advantage of vacuum evaporation for a muscadine grape processor is the versatility to obtain both powder and/or concentrate product depending on the purchaser requirements whereas spray drying and freeze drying will produce only a powder limiting the market for the isolated extract. Moreover, to implement a freeze drying line, refrigeration is needed to freeze the liquid extract before it is submitted to drying. This chapter illustrated various assumptions that could be changed to recalculate and to adjust the investment and costs of any processor. Due to limited information regarding equipments, this chapter only contained general information for engineering and equipment specifications, thus, many specifications can be added to predict economic information more accurately. Furthermore, this process could be adjusted and implemented for by-products from various fruits and vegetables sources to estimate the increase in profit of their industries.
CHAPTER 5
SUMMARY AND CONCLUSIONS

Interest in by-product utilization has encouraged fruit and vegetable processors to investigate ways to efficiently and economically add value to otherwise low-benefit processing residues. For muscadine grapes, the potential exists to extract valuable compounds that remain in the by-product and bring these novel products to a revenue-generating market. In parallel, interest in natural antioxidants has driven the production of nutraceuticals from fruit and vegetable sources. Prior studies have focused on extracting polyphenolics from diverse fruit and vegetable sources, but the present studies are the first to explore the potential economic and processing environment impact on polyphenolic content and stability. The studies illustrate the ability to extract polyphenolics from muscadine grape pomace and measuring the effect of various processes on chemical composition, chemical reactivity, and oxidative and thermal stability. Furthermore, these studies tried to illustrate the feasibility of obtaining by-product isolates rich in polyphenolics from muscadine grape pomace. Such feasibility was determined by simple economic indicators, using logical assumptions and the best economic predictors for success of a by-product extraction venue.

Polyphenolics extracted from muscadine grape pomace showed antioxidant activity comparable to fruits and vegetables such as cherries, strawberries, and red cabbage. Fermentation was better than solid phase isolation in getting rid of residual sugars with minimal damage to the chemical profile and antioxidant activity of the final product, as well as showing more practicality on a larger scale. Vacuum drying proved to be the best treatment following fermentation since it best maintained polyphenolic content and preserved the antioxidant capacity. Initial concentration followed by aerobic fermentation was an inexpensive way to obtain polyphenolics from muscadine grape skins. Isolation of polyphenolics from muscadine
grape pomace can be suitable and profitable for a typical muscadine juice producer if their circumstances are somewhat similar with the ones indicted in these studies. Assumptions can be adjusted to any fruit or vegetable processor situation for more accurate calculations. Further research is needed to support the work shown in these studies. Investigations need to be focused on the optimization of polyphenolic concentration prior to fermentation, the impact of processing on phytochemical characteristics at an industrial scale, and more detailed economic evaluation.
Figure A-1. Total anthocyanins during a 3-day extraction procedure.

Figure A-2. °Brix values during a 3-day concentration procedure.
Figure B-1. Total anthocyanin content in a 1-day extraction procedure.

Figure B-2. °Brix values in a 1-day extraction procedure.
Figure C-1. Total anthocyanin content after a 5-hour concentration procedure.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Jorge Cardona was born on October 13, 1983, in Cochabamba, Bolivia. Before graduating from high school in 2000, he traveled to Devon, PA as an exchange student for 11 months with AFS exchange program. He went back to Bolivia to graduate from high school in 2001. After high school, he entered Zamorano University (Escuadra Agricola Panameicana) in Honduras, Central America to obtain his bachelor’s degree in agroindustry. After his graduation in 2005, he was offered an assistantship to pursue his graduate education at the Food Science and Human Nutrition Department at University of Florida, under the supervision of Dr. Stephen Talcott and Dr. Charles Sims. In August 2007, he earned a Master of Science in Food Science and Human Nutrition and will continue his studies towards a doctoral degree.