

QUALITY OF NIAGARA GRAPE JUICE AND NEW YORK WINES: EFFECT OF
ADDITIVES, THERMAL PROCESSING, COLD STABILIZATION, AND
FILTRATION

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by

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FILTRATION

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We aimed to improve the quality of grape juices and wines by using natural antimicrobials and processing techniques to reduce sulfite usage and anthocyanin losses, and by utilizing crossflow filtration to increase sustainability.

We evaluated harvest and processing methods to improve the quality of bottled Niagara grape juice stored at 18°C. Handpick vs mechanical harvest, early and late harvest date, potassium metabisulfite vs ascorbic acid (AA) addition (antioxidants), aeration and fining agents effect, hot-break prior pressing, filtering conditions and hot-pack temperature, were evaluated.

Late harvest juices had better quality. AA juice quality was comparable to sulfited juices, thus AA could be used as sulfite substitute. Hot-break juices had significantly higher phenolic and antioxidant capacity, lower turbidity and brown color than traditional cold-pressed juices; however, hot-break juices darkened faster over time. Harvesting method, hot-pack and hot-break temperature did not affect juice quality.

Compared to traditional diatomaceous earth (DE) filtration, 500 kDa polymeric crossflow membrane filtered-juices had lower turbidity and brown color. Ceramic membranes produced juices with comparable quality to DE. Fining agents partially removed browning precursors but were not as effective as sulfite in browning

prevention.

The effectiveness of traditional and natural antimicrobials for shelf-life extension in cold-filled Niagara juices (still and carbonated) inoculated with yeast was assessed. Best results were obtained with 250 ppm dimethyl dicarbonate alone or in combination with 5-10 ppm natamycin for shelf-life extension comparable to juices with 0.05% sorbate/benzoate of about 160 days.

Ceramic and polymeric crossflow membrane microfiltrations for 4 NY red and white wines were evaluated against DE. All filters produced microbiologically stable wines with comparable quality, but only ceramic membrane wines were perceived similarly to DE. For tank bottoms (higher solids content), ceramic membrane filtration represented a more sustainable operation.

Losses of anthocyanins with potassium bitartrate (KHT) coprecipitation during cold-stabilization were largely controlled by pH of model solutions. Losses were minimized when $\text{pH} \leq 2.95$ which was likely due to neutralization of negatively charged KHT crystal surface. Anthocyanin coprecipitation decreased as potassium concentration increased, indicating both compounds were competing for coprecipitation. Rutinosides were less likely to coprecipitate than glucosides.

BIOGRAPHICAL SKETCH

Passaporn Siricururatana was born in Bangkok, Thailand where she received her early education at Kasetsart University Laboratory School. After high school, she attended the Science Department at Chulalongkorn University, Bangkok, Thailand, where she obtained her Bachelor of Science Degree in Food Technology and graduated with 1st class honors in 2005.

In 2006, Passaporn received the Fulbright Scholarships and started her Master's program in Food Science and Technology at Cornell University under the supervision of Dr. Olga Padilla-Zakour. Her research focused on quality and stability of pulp-rich fruit juices made from selected varieties of tart cherries and apples and Concord grapes. Upon completion of her master's degree in 2008, Passaporn was then awarded with a graduate research assistantship from the Department of Food Science to continue working with Dr. Olga Padilla-Zakour for her Ph.D. degree at Cornell.

While a student, Passaporn took active part in various extracurricular activities, including the Cornell Thai Association, Chocolate & Confections Technology Club at Cornell, Student Association of the Geneva Experiment Station, Western New York Institute of Food Technologists, and various food science product development teams. She also received a great opportunity to intern with Research and Development Division at PepsiCo in Valhalla, NY and Frito-Lay in Plano, TX during the summer of 2009 and 2010, respectively.

Her research for Ph.D degree focused on the effect of processing on the quality of Niagara grape juices and New York State wines. Her area of concentration was Food Processing, with minors in Food Chemistry and Microbiology.

To my beloved family:

Dad, Mum, On, and Oy

(Suporn, Apasra, Pornpas, and Thitiporn Siricururatana)

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CHAPTER 1

LITERATURE REVIEW

Grape varietal

There are three distinct broad classes of grapes grown in the US: the northeastern or native American grape, *Vitis labrusca*, the western grape common in California area, *Vitis vinifera*, and the southern Muscadine grape, *Vitis rotundifolia* (Pederson 1980). *Vitis vinifera* species are commonly used to produce wine, raisins, and table grapes; however, they cannot withstand the severe winters and the attacks of insects and plant diseases. On the other hand, the native grapes, *Vitis labrusca*, thrive despite the insects, disease pressure, or the relatively cold climate. Concord grape, the native American cultivar, is extensively used in the American grape juice industry and famous for their highly purple-red color and distinct aroma and flavor from methyl anthranilate. Niagara, a hybrid of native species, is the leading variety for white grape juice production due to its unique aroma and flavor. Most of the grape juice consumed in the US are made from these two varieties grown chiefly in Washington, New York, Michigan, Ohio, and Pennsylvania.

Grape Production and Utilization

Among non-citrus fruits in the US, grape had the highest utilized production, 40.0% of total production of non-citrus fruits, and the highest utilized production value accounting for 28.9% of the total (NASS 2010). Also, grapes had the highest cash receipt in the US accounting for about a fifth of total fruit cash receipts (ERS 2008). Utilized production of grapes in 2009 was 7.04 million tons, 90% of which

were produced in California. Almost 60% of grapes produced in California were used in the wine industry contributing to 93% of the total amount of grapes used in the wine industry. New York State, the third largest grape producing state, produced 130,000 tons in 2009. Most of grapes utilized in New York State (98.5%) were used in the processing industry and only 1.5% was used for fresh consumption. Of all processed utilization, 34.4% was in the wine industry and 65.6% was in the juice industry. Concord and Niagara grapes (*Vitis labrusca* L.) were the major cultivars in New York State, accounting for 75% of all grapes (NASS 2010); hence making NY the second largest producing state for both varieties.

Juice Market

The total fruit juice and juice drink market value was estimated to be \$14.7 billion in 2007 displaying 16 and 27%, respectively, decline in current and constant process during 2002-2007 (Mintel 2008). Juice drinks accounted for 42% of the total sales, while the remaining 58% came from fruit juices. According to the Fruit and Tree Nuts Situation and Outlook Yearbook 2008 (ERS 2008), Americans consumed 7.63 single-strength equivalent (sse) gallons of fruit juices in 2007. Citrus juices accounted for 57% of juice consumption with orange juice being the largest component. In 2007, orange juice consumption fell by 8% to 3.81 sse gallons per person, the fourth consecutive annual decline. However, non-citrus juice consumption increased 3% to 3.30 sse gallons per person, with apple and grape juice ranking the highest at 68.5 and 16.7% of the total.

During 2005-2009, the overall juice or juice drink consumption remained unchanged but the orange juice consumption declined from 84% in 2005 to 80% in 2009 (Mintel 2010) due to the rising orange juice prices. Furthermore, children aged 6-11 were the biggest juice or juice drinks consumers in the US. In 2009, 98% of children reported drinking any type of juice and the frequency of drinking fruit juice or juice for children had remained unchanged during 2005-2009 (Mintel 2010). The high calorie profile of juice and juice drinks was one of the main reasons for non-consumption (Mintel 2010). The added sugar or containing high-fructose corn syrup (HFCS) or artificial additives were also listed as the cause of non-consumption. Furthermore, the recession had changed consumers' fruit juice or drinks purchasing behavior by drinking less juice, moving to store brands, and purchasing family-size packaging. In addition, the consumer had shifted to other beverages such as flavored water, sport drinks, and energy drinks.

Grape Juice Processing

Juice quality is highly influenced by, but not limited to, preharvest, postharvest and processing factors (McLellan and Padilla-Zakour 2005). Sorting is necessary to remove decay fruits so that the finished product does not have a high microbial load, undesirable flavors, or mycotoxin contamination. Harvested grapes are destemmed and crushed prior to juice extraction. Extraction can be done by either pressing or decanting depending on the type of operation. Juice extraction should be done as rapidly as possible to minimize enzymatic oxidation. Heating and enzyme addition might be included before the extraction to increase yield and juice quality. After

extraction, depectinization by enzyme addition, fine filtration, or high-speed centrifugation are required to achieve the visual clarity for clear juice. After that, juices are subjected to heat treatment or equivalent nonthermal process to achieve safe and stable single-strength juices.

A. Preharvesting

Major preharvest factors that influence the quality of grape juice are climate, cultivar, and cultural practices. Each of these factors has its own influence, but complex interactions should be considered. In a warm sunny season, grapes are generally higher in sugar and lower in acid and astringency than in a cool, cloudy season (Pederson 1980). The temperature, light, wind, rainfall, cloud, fog, and their distribution throughout the season are also important factors influencing the nutritional quality of fruits. Light intensity significantly affects vitamin concentrations, and temperature influences transpiration rate, which affects mineral uptake and metabolism (Kader and Barrett 2005). Some important cultural practices are soil type, irrigation, fertilization, pruning, thinning, and pest control. These factors have to be considered together because different conditions require different vineyard management systems. Loose soils with moderate fertility and excellent drainage characteristics are best (Morris and Striegler 2005). Furthermore, maintaining an adequate and balanced mineral nutrition is essential to produce high fruit yield and quality.

B. Harvesting

Maturity is one of the primary factors affecting juice quality. As the grape matures, acidity decreases, and pH and sugar increase. Color, flavor, and aroma also reach a peak during the ripening process. The ripening process stops as soon as the grape is harvested. Therefore, harvest date is a crucial determiner of juice quality. In the grape industry, the percent soluble solids is normally used as the maturity index. The Concord juice industry usually uses 15 percent soluble solids as the minimum level of acceptable quality and pays a premium for grapes based on each increase in percent soluble solids up to 18 percent (Morris and Striegler 2005).

Most grapes used for juice are mechanically harvested which reduced harvesting cost substantially. Quality of machine-harvested grapes can be influenced by type of machine, cultivar, production system, harvest temperature, and postharvest handling system (Morris and Striegler 2005). If not handled properly, mechanically harvested fruits presented a problem of browning and off-flavor development such as grassy or hay-like flavor (Bourne and others 1963; Friedman 1969) due to an enzymatic oxidation. However, Moyer and others (1969) suggested that mechanical harvesting can result in better quality fruit than hand harvesting due to speed of operation.

Color is one of the most important qualities for grape juice. Red color in red grape juice is largely the result of anthocyanin pigments while white grape juice contains minor pigments such as chlorophyll and carotenes (Morris and Striegler 2005). To prevent browning and enzymatic activity, sulfur dioxide (SO₂) is added to machine-harvested grapes. Morris and others (1979) reported that the addition of 80-

160 ppm SO₂ immediately after harvest slowed postharvest deterioration. Moreover, SO₂ also acts as an antimicrobial to prevent microbial spoilage and helps delay alcohol accumulation (Morris and Striegler 2005). However, a growing public awareness of sulfite sensitivity has created the need for its reduction or replacement.

C. Extraction

Disintegration

A stemmer-crusher is used in grape juice processing to remove residual stems, leaves, and petioles from grapes and to perform initial crush (McLellan and Padilla-Zakour 2005). Grapes are crushed prior to the pressing to break down the cell tissue, enhance ease of pressing, and allow the higher yield.

Hot Break and Cold Press

The thermal process is necessary for red grape juice processing to maximize juice yield and color-flavor extraction. This stage is called hot break or hot press depending on the temperature used. Hot break employs higher temperatures (80°C) than hot press (50-60°C). The process is aimed to extract color from skins into the juice and improve phenol and anthocyanin extraction. It also increases the inactivation of polyphenol oxidase (PPO) and hence reduces enzymatic browning. Excessive temperatures (> 65°C) must be avoided to preserve juice quality (Morris and Striegler 2005). Usually, press aids such as kraft (wood pulp) paper, rice hulls, and bleached kraft-fiber sheets or rolled stock are added into juice after hot break and mixed with a slow-moving agitator to facilitate the pressing. Ideally, press aids should neither develop off-flavors nor remove fruit's flavor (McLellan and Padilla-Zakour

2005). If juice is extracted by decanting or centrifugation, there is no need for press aids.

Unlike red grape juice, white grape juices are produced by cold pressing to maintain the light color even though the juice yield could be 20% lower compared to the hot press method (Pederson 1980; Morris and Striegler 2005). This differs from the hot break method, in that the mash heating step is omitted which can lead to oxidation and browning of juices. Therefore, antioxidant compounds such as sulfite or ascorbic acid are used to inhibit or delay undesirable oxidative flavor and color changes. Enzymes are still added to the cold-press juice to facilitate the pressing and clarification process.

Pressing

The mash is pressed in an extractor to separate the juice from the mash. Several types of extractors are available, namely rack and frame hydraulic press, horizontal piston press, bladder press, belt press, screw press, and decanter centrifuge (McLellan and Padilla-Zakour 2005). The selection of equipment should depend on the desired outcome such as type of operation (batch or continuous), batch size, and availability of labor (Bump 1989). Additional juice may be obtained by breaking up the press cake, addition of hot water, and repressing.

The hydraulic rack and frame press is commonly used for batch press systems in small juice operations. The process delivers good yield but is labor intensive. On the other hand, the horizontal piston press is a highly automated pressing system used in batch mode. Another system that is commonly used in the grape industry is a bladder press which is a pneumatic-based system. The continuous belt

press and screw press are also effective for grape processing. For decanter, juice separation can be performed by sedimentation through increased gravity. The centrifugal force is used to accelerate the settling of higher density insoluble particles in juices.

D. Cold Stabilization

One of the specific processes in grape juice processing is the removal of excess tartaric acid to avoid the undesirable precipitation in the final product (Konja and Lovric 1993). This process is called cold stabilization. Tartaric acid is the major acid in grape juice and is presented as free tartaric acid or as salts such as potassium bitartrate or sodium bitartrate. High acid content with the acidity above 0.85%, results in juice that is too tart (Pederson 1980) and cold stabilization can prevent this. The process is carried out by flash heating filtered juice to 80-85°C in a tubular or plate type heat exchanger and cooled in another heat exchanger to 2°C (Morris and Striegler 2005). The juice is then transferred into a tank and stored at low temperature, close to the freezing point. After the tartrate has settled, the juice is racked off for further processing. The sediment can be filtered for optimal recovery of juice.

E. Clarification

Juices can develop brown color after processing; thus, clarification and filtration are necessary to produce commercial clear grape juice. Clarification can be performed by 3 general ways: biochemical (enzymatic treatment), chemical (fining), and physical (mechanical separation) processes (Binnig and Possmann 1993).

Enzymatic Treatment

Pectinase enzymes break semi-stable emulsion of colloidal plant carbohydrates which support insoluble cloud material of freshly processed juices. As a result, they aggregate and drop down to the bottom of the tank. This reduces juice viscosity and changes the opacity of cloudy juice to clear (McLellan and Padilla-Zakour 2005).

Fining Process

Methods for nonenzymatic clarification are heating or addition of fining agents to improve its appearance (turbidity and color). Fining agents react with specific component either chemically or physically, to form new components that can be separated from juices by either precipitation or filtration. Bentonite and silica gel, both of which are negatively charged, can interact with positively charged protein molecules and precipitate out (Margalit 2004). Tannin, a polymer of phenolic molecules, is formed during storage and can precipitate out or cause browning in juice. To reduce the amount of phenols and tannins, proteins such as gelatin, isinglass, casein, and egg white are added to juices to form protein-tannin bonds and precipitate out. Polyvinylpolypyrrolidone (PVPP) interacts with low molecular phenols such as monomers and dimers. In white wine, PVPP at 100-700 ppm is used to reduce brown color (Margalit 2004). The advantage of PVPP is that it does not affect the wine aroma unlike many other fining agents.

Mechanical Separation

a) Diatomaceous Earth (DE) Filtration

The traditional method for filtration is the use of filter aids. However, there are some drawbacks of using filter aids due to the safety restrictions in handling the material, cost of waste disposal, and the difficulty of operation. The operation involves three steps in which a precoat of filter aid is built up on a filtration element, and the filtration is conducted using a continuous addition of filter aid to the juice, called body feed. Finally, the built-up cake is removed, and the entire cycle is started again. The DE filtration is typically used for final filtration (polish filtration). The system utilizes suspension of DE in juices as a means of renewing the filter surface in a plate and frame filter. This pressure filtration system is highly efficient and very effective. The effectiveness depends on the grade and amount of DE. Several types of filtration equipment are available namely filter press, cylindrical element filter, vertical leaf filter, rotating leaf filter, and horizontal rotating leaf filter (McLellan and Padilla-Zakour 2005). Selection of equipment depends on type and cost of operation, batch size, and labor availability.

b) Cross-Flow Filtration

Membrane filtration is a pressure-driven technology with pore sizes ranging from 100 molecular weight cut off (MWCO) to 5 μm . For juice clarification, ultrafiltration (UF) and microfiltration (MF) are commonly used, representing membranes with pore sizes from 10 kDa MWCO to 0.6 μm . The advantages of membrane filtration over traditional clarification methods include reduced processing time and enzyme usage, increased juice yield, elimination of filter aid and filter

presses, and better product quality (Cheryan 1998). Tubular polymeric membrane and hollow fiber membranes are currently used in juice industry (McLellan and Padilla-Zakour 2005). Two critical factors to be considered for choosing membranes are product quality and filtration rates or flux ($l/m^2/h$). Flux depends on four primary variables: transmembrane pressure (TMP), cross-flow velocity (circulation rate, m/s), temperature of feed stream, and concentration (Girard and Fukumoto 2000). Pectin is the major foulant of membranes; thus, depectinization improves flux. In addition, higher temperature and flow rate and lower feed concentration result in higher flux. The typical flux and pressure for UF and MF are 30-300 $l/m^2/h$ and 345-1380 kPa and 100-300 $l/m^2/h$ and 20-345 kPa, respectively (Paulson and others 1984; Renner and El-Salam 1991).

Membranes can be categorized by their materials: polymeric and inorganic. Polymeric UF membranes, with pore size ranging from 500 to 750 kDa MWCO, are available in the market. Girard and Fukumoto (1999) tested different type of polymeric membranes and reported that 0.2 μm or 10 kDa membranes had higher fouling layer resistance than 30 and 100 kDa membranes and apple juices filtered through 20, 100, and 200 kDa membranes had similar properties. In addition, polyvinylidene fluoride and polysulfone membranes had higher flux than polyethersulfone and cellulose membranes. The flux of polymeric membranes for depectinized apple juice improved as the membrane MWCO increased from 9 to 200 kDa (Girard and Fukumoto 1999). Inorganic or ceramic membranes offer advantages with the resistance to abrasion and chemical tolerance. They can be operated at high temperatures and pressures, are autoclavable, and have longer life than polymeric

membranes. They are more common in MF system with pore sizes of 0.1-0.6 μm . However, it has higher cost per membrane area compared to polymeric membranes. Fukumoto and others (1998) reported the chemical, physical, and sensory properties of filtered apple juices from 0.2 and 0.02 μm ceramic membranes were similar and changes during storage were comparable; however, the 0.02 μm membrane resulted in higher steady state flux and less fouling under optimal conditions (8 m/s, 414 kPa, and 50°C).

c) Decanters and Finishers

Decanters and finishers can be used as a filter or pre-filter to partially clarify a high-solids stream. Both equipments operate on the same principle with a spinning central cone, drum, and set of paddles pushing the juice through a screen. The unit is typically mounted horizontally. Total suspended solids may be reduced to 1% or less.

d) Centrifugation

A centrifuge places the juice under high gravimetric force induced by centrifugal action. It is effective to produce opaque juice but free of visible solids. Centrifuges with a high force of gravity are capable of producing clear juice. Operation of a centrifuge has to minimize excess oxygen in the product such as using an inert gas.

F. Bottling

Pasteurization and Hot fill

Hot-filling or pasteurization is used to produce shelf-stable juices in which the high-quality retention can range from 9 to 12 months. Juices are heated using a

heat exchanger such that the temperature after juice filling the container reaches 88-95°C. A holding time of at least 3 min is normally used before juices are cooled down (McLellan and Padilla-Zakour 2005). This whole process is called hot filling and it is adequate for highly acidic beverages such as apple, cherry, and grape juices. The maximum pH allowed for the hot-fill process is normally 4.

Aseptic Processing

In aseptic juice processing, juices and containers which are sterilized separately are brought together and hermetically sealed to produce a commercially sterile products which is free of microorganisms (both vegetative cells and spores) capable of reproducing in juices at normal non-refrigerated conditions of storage and distribution (GMA 2007). Juices are typically gone through thermal process in the hold tube where flow rate, residence time, and temperature are critical factors. Aseptic packaging machines create and maintain an aseptic zone (sterile environment) in which sterilized containers are filled and sealed. Sterilization agents such as heat, chemicals, high energy radiation, or a combination of these can be used to sterilize packages or machine surfaces (GMA 2007).

Non-thermal Processing

Non-thermal processing has the advantage of fresh-like characteristic with extended shelf-life compared to fresh juice. It uses less energy than thermal processes and minimizes loss of nutrients and flavors. One of non-thermal processes is sterile filtration using membrane filters. Sterile filtration is used extensively in the grape juice and wine industry for microbial control. All yeasts and molds and most bacteria are retained by 0.45 µm membranes; however, for production safety, membranes cut-offs

of 0.2 μm or less are recommended (Girard and Fukumoto 2000). Membrane filtration does not generally require additional thermal processing; therefore, limits thermal and oxidative degradation, and pH and ionic strength changes (Morris and Striegler 2005). However, some preservatives might be added to help extend the shelf-life. Previous study showed that ultrafiltration of juice through a 10,000 MWCO membrane, but not a 30,000, removed all PPO activity and reduced juice browning (Sims and others 1994). However, depending on the membrane pore size, PPO could be presented in juices and thus thermal processing is needed to produce commercially stable juices. In this case, membrane filtration acts as clarifying step and is used together with thermal processing.

Several other new technologies that are applicable to grape juice processing are high hydrostatic pressure, high-intensity-pulsed electric field, electron beam irradiation, pressurized liquid carbon dioxide, ultraviolet light, and chemical preservatives (Morris and Striegler 2005).

High hydrostatic pressure processing (HHP) or high pressure processing (HPP) is a non-thermal food preservation technique for microbial and enzyme inactivation with reduced effects on nutritional and quality parameters when compared to thermal treatments (Tiwari and others 2009). HPP treatment at ambient temperature is reported to have minimal effect on the anthocyanins content of various fruits and vegetables (Oey and others 2008).

High intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20–80 kV/cm) for short time periods (<1 s) to fluid foods placed between 2 electrodes (Señorans and others 2003). PEF has

been demonstrated to be effective against various pathogenic and spoilage microorganisms and enzymes without appreciable loss of flavour, colour and bioactive compounds such as anthocyanins (Yeom and others 2000; Hodgins and others 2002; Cserhalmi and others 2006; Elez-Martínez and others 2006). PEF treatment involves short treatment times to inactivate microorganisms at temperatures below those adversely affecting food qualities (Raso and Barbosa-Cánovas 2003). A pre-treatment of PEF is reported to increase the anthocyanin concentration in grape juice (Knorr 2003).

Irradiation (cold pasteurisation) of food is achieved by exposing the product to a source of ionizing energy. It is a physical means of food processing that involves exposing pre-packaged or bulk foodstuffs to gamma rays (Cobalt-60), X-rays, or electrons (Mahapatra and others 2005). Depending upon the radiation dose, foods may be pasteurised to reduce or eliminate foodborne pathogens. Inactivation of microorganisms by irradiation is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell (DeRuiter and Dwyer 2002). Application of gamma radiation to pomegranate juice (Alighourchi and others 2008), carrot and kale juice (Kim and others 2007) and UV radiation to orange, guava-and-pineapple juice (Keyser and others 2008) has been reported for the inactivation of microorganisms. Irradiation induces negligible or subtle losses of nutrients and sensory qualities in food compared to thermal processing as it does not substantially raise the temperature of food during processing (Wood and Bruhn 2000). Literature reveals that most of the reported applications of irradiation are limited to solid foods and there is scarcity of information regarding treatment of fruit juices (Tiwari 2009).

Dense phase CO₂ processing (DP-CO₂) or supercritical CO₂ processing is a continuous, non thermal processing system for liquid foods that utilises pressure (<90 MPa) in combination with carbon dioxide (CO₂) to destroy microorganisms as a means of food preservation (Del Pozo-Insfran and others 2006). DP-CO₂ is reported to have significant lethal effects on microorganisms in liquid foods (Park and others 2002) and to inactivate enzymes such as polyphenols oxidases (Del Pozo-Insfran and others 2007) and peroxidases (Gui and others 2006) which influence the stability of polyphenols during storage. Application of DP-CO₂ has been reported for various fruit juices such as apple cider (Gunes and others 2006); orange juice (Balaban 2003); grape juice (Gunes and others 2005) and mandarin juice (Yagiz and others 2005). These studies indicated minimal changes in organoleptic parameters.

Packaging

Glass is one of the dominant packaging used for shelf-stable juices. The primary advantages of glass are its chemical inertness, clarity, and heat resistance. Its heat resistance ensures that containers will not deform during hot filling; however, glass containers are subject to thermal shock and may shatter. In addition, glass is heavier than other packaging materials. The plastic (PET and HDPE) bottles layered with an oxygen barrier are becoming popular in juice industry (McLellan and Padilla-Zakour 2005). Because they are lighter in weight than glass, they can provide economic advantages in terms of lower shipping costs. Product-package interactions, however, are a major concern. Possible interactions are absorption of flavor from food or migration of components from plastic to food. The permeability of packaging

material to gases, water vapor, and volatiles also impacts the food quality and expected shelf-life.

Microbiology of Juices

A. Microorganisms in Fruit Products

The low pH of many fruits is the major factor that influences the composition of their microflora. Most yeasts and molds grow well under acid conditions and only few bacteria are sufficiently aciduric to be important; thus, fungi are often the predominant microorganisms in fruit products (Worobo and Splittstoesser 2005).

Fruits, as received at the processing plant, often are contaminated with large numbers of yeasts but none of the pathogenic species are common contaminants. Splittstoesser and Mattick (1981) reported that 43% of grapes contained 10^6 yeast per gram or higher and could be as many as 10^7 . Growth of spoilage yeast depends on product nature and yeast strain. Yeast growth in clear juices may produce slight haze and sediment. Yeast level of 10^5 cfu/ml or less can usually be detected with naked eye. Growth of fermentative type such as *Saccharomyces cerevisiae* may produce sufficient CO₂ to burst the container (Worobo and Splittstoesser 2005). While CO₂ and ethanol are the predominant metabolic products of yeasts, glycerol, acetaldehyde, pyruvic acid, α -ketoglutaric acid, and acetic acid are also formed.

Although molds are aerobic microorganisms, many are very efficient scavengers of oxygen; thus, processed fruits are susceptible to spoilage. When growth is permitted, colonies may develop in the headspace or as strands throughout a beverage. Growth of mold on processing equipment can result in off-flavor

development in wines and juices. The ascospores of some species are very heat-resistant and can survive the commercial pasteurization treatment for juices. Furthermore, heat resistant molds such as *Byssochlamys*, *Eurotium*, *Neosartorya*, and *Talaromyces* produce a number of mycotoxin when growth occurs (Worobo and Splittstoesser 2005). However, this does not present a serious public health problem because the spoiled food would be avoided by most consumers. The most common mycotoxin in processed fruits especially in apple is patulin. Patulin is resistant to thermal destruction between pH 3.5-5.5. According to the U.S. Food and Drug Administration (FDA) regulation, patulin level in apple juices has to be less than 50 ppb (FDA 2001).

The two common bacteria causing spoilage in juices, cider, and wines are lactic acid and acetic acid bacteria. The number of lactic acid bacteria on fruit is usually low but they can accumulate on processing equipment or during fermentation. Growth of lactic acid bacteria may result in formation of haze, gas, acid and other changes. Most lactic acid bacteria have the ability to decarboxylate malic acid to lactic acid. This malo-lactic fermentation is preferred in high-acid wines because of the acid reduction and production of desirable flavor. Juices, wines, and cider are susceptible to spoilage from acetic acid bacteria while held in the tanks prior to bottling. Some acetic acid bacteria can produce microfibrils composed of cellulose which lead to the floc formation in juices. Low numbers of viable bacteria spores can be recovered from fruit products because the low pH prevents spore germination or outgrowth. However, some rare bacterial spore-forming species are sufficiently aciduric to be a potential problem. Certain spore-forming bacilli can grow in wine that contained as much as

20% ethanol by volume. *Alicyclobacillus*, a new genus of thermophilic, acidophilic spore-forming bacteria, also poses problem in commercial pasteurized apple and grape juices (Splittstoesser and others 1994). This organism has a pH optimum between 2.5-5.5 and optimum temperature of 40-42°C. Most pathogenic bacteria originate from animal reservoirs and are not associated with fruit products. However, contamination can lead to foodborne illness. For example, outbreaks caused by contaminated juice with *Salmonella enteric* serovar Typhimurium or *Escherichia coli* O157:H7.

B. Fruit Juice Safety

Due to a number of outbreaks in fruit juices, FDA issued a rule titled “Hazard Analysis and Critical Control Point (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice” which is defined in Chapter 21, Part 120 of the Code of Federal Regulations (21 CFR Part 120). This regulation applies to all processors and importers of juices. The HACCP plan must be developed to minimize the risk of juice contamination with biological, chemical, or physical hazards for each processing establishment by a mean of knowledgeable individuals that includes person trained in juice HACCP. The law also requires the juice to be treated with a process that achieves at least a 100,000-fold decrease in number of pertinent pathogens likely to occur in the juice. This is known as the 5-log reduction performance standard. The pertinent pathogen that may occur is the most heat-resistant microorganism of public health concern such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria*, and *Cryptosporidium*. One pathogen specific to the grape juice industry is the mold that produces ochratoxin A (Morris and Striegler 2005). FDA does not mandate pasteurization of all juice and thus other pathogen reduction technologies, such as

aseptic system, ultraviolet light, high-pressure processing, irradiation, and chemical preservatives, are also used in juice industry. According to Juice HACCP Guidance for Industry (FDA 2004), the recommended time-temperatures for pasteurization of juices at pH of 4.0 or less are 71.1°C for 6 sec, 73.9°C for 2.8 sec, 76.7°C for 1.3 sec, 79.4°C for 0.6 sec, and 82.2°C for 0.3 sec.

For non-thermal treatments for juice, FDA approval of the means of treatment for the control of microorganisms is required if the treatment includes the use of a source of radiation (FDA 2004). Both UV radiation and pulsed light have been approved by FDA for the control of microorganisms, and the regulations specifying the conditions under which they may be safely used are at 21 CFR 179.39 (UV radiation) and 21 CFR 179.41 (pulsed light). Treatment technologies that do not involve the use of a source of radiation or a chemical agent, e.g., high pressure processing, are not likely to require FDA approval. However this should be verified with the process authority. If the treatment includes the use of a chemical antimicrobial agent, such as a sanitizer, to reduce pathogen levels on the surface of fruit, the chemical agent must be approved by FDA for that use (i.e., to control or reduce levels of microorganisms) under the agency's food additive regulations in 21 CFR Parts 170-199, or it must be generally recognized as safe (GRAS) for such use.

C. Antimicrobials

Various compounds are used to extend the shelf-life of fruit products and many are used together. These antimicrobials can be classified as traditional or naturally occurring (Davidson 2001). Growing concerns with chemical preservatives are due to concerns of potential toxicity and allergic reactions by consumers. To satisfy

consumer demands, reduction in conventional preservatives and more natural foods are needed.

Sulfur Dioxide and Sulfites

Sulfur dioxide (SO₂) is widely used throughout food industry due to its antimicrobial, antioxidant, and bleaching activity. It is used in grape and wine industry as an antioxidant, antimicrobial, clarifying agent, and to reduce browning and enzymatic activity. Its undissociated form is germicidal with its pK of 1.81 and thus it is more effective at lower pH (Worobo and Splittstoesser 2005). The bound forms of sulfur generally have reduced antimicrobial activity; therefore, the amounts of aldehydes, ketones, and other SO₂ binding compounds limit the effective use of the sulfite (Ough and Were 2005). SO₂ can loosely bind to anthocyanins and thus can reduce the color of red juices or wines. Yeasts are believed to be more resistant to SO₂ than molds and bacteria. However, certain asthmatic individuals are at risk for allergic-like reactions when consuming small amount of sulfites. Sulfites are considered as GRAS substrates by the FDA and they are allowed in fruit juices and concentrates, dehydrated fruits and vegetables, and wines. FDA also made labeling of any product containing 10 mg/l or more of sulfites mandatory. The SO₂ concentrations used as antimicrobial in juices and wines are 10-100 ppm and 100-300 ppm (Gould 2000). The maximum level of SO₂ allowed in wine is 350 mg/l and is regulated by Alcohol and Tobacco Tax and Trade Bureau (Ough and Were 2005).

Benzoic and Sorbic Acid

Benzoic and sorbic acids are traditionally used as preservatives in the juice industry but consumer acceptance has decreased. The advantages of these acids are ease of incorporation into products, low cost, lack of color, and low toxicity (Davidson 2001). They are active in their undissociated form which decreases with increasing pH. At pH 3.5, common pH for juices, 83% and 95% of benzoic acid and sorbic acid are undissociated (Worobo and Splittstoesser 2005). Both acids are more soluble in water in their salt forms such as sodium benzoate and potassium sorbate, and thus their preferred use in juices and beverages. Most fungi are inhibited by 0.05-0.1% of benzoate while the effective concentration of sorbates in most foods is in the range of 0.02-0.3%. Benzoic acid has a synergistic effect when using with sorbic acid and this synergism is pH dependent (Chipley 2005). The combination of ascorbic acid and benzoate in beverages could generate detectable levels of benzene (Chipley 2005). In the US, benzoic acid and sodium benzoate are GRAS preservatives and their permitted usage is up to 0.1% (FDA 2009). Additionally, sorbic acid and potassium sorbate are GRAS but they have a peppery or bitter taste when added more than 0.1% (Morris and Striegler 2005). Sorbic acid is effective against yeasts, molds, and some bacteria in acidic foods and enhances the heat activation and destruction of spores. Other food properties also influence the effectiveness of these preservatives. The ethanol content in wine affects the concentration of sorbic acid needed to prevent refermentation by yeasts. Some of the most resistant yeasts are *Zygosaccharomyces bailli*, *Candida parapsilosis*, and *Pichia membranaefaciens* (Deak and others 1992). Yeasts and

gluconobacters that possess resistance to sorbic acid are not uncommon on grapes (Splittstoesser and Churey 1992).

Dimethyl Dicarbonate (DMDC)

DMDC is a processing aid that has been approved for use as a food additive for microbial control in juices, wines, and other beverages. The legal limit for DMDC in juice and wine is 250 and 200 mg/l respectively, if the viable microbial load is less than 500/ml by good manufacturing practices prior to DMDC addition (Morris and Striegler 2005). It is effective against all yeast and does not have to be declared on the label since it is considered a processing aid. An optimum pH for DMDC is 3.0-4.0. The antimicrobial action takes place at bottling where DMDC is hydrolyzed to CO₂ and trace amounts of methanol. These byproducts yield no residual odors or flavors and have shown no threat of allergic response. Since DMDC hydrolyzes rapidly in water, the challenge is to destroy the contaminating microorganisms before hydrolysis is completed. As a result, it does not have a long-term protection against recontamination or later outgrowth of surviving organisms. Despite the more rapid hydrolysis at higher temperature, the effectiveness of DMDC is improved at increased temperatures, as opposed to sulfur dioxide and sorbic acid, which exhibit reduced antimicrobial activity at higher temperatures (Golden and others 2005). Moreover, the rate of hydrolysis is significantly decreased by increasing ethanol content and thus increasing the effectiveness of DMDC. An advantage of using DMDC is that no reactions occur with sugar or artificial sweeteners (Golden and others 2005). A study has shown that DMDC can be used in combination with minimal amounts of SO₂ to prevent yeast growth and visible fermentation from *Saccharomyces bayanus* in grape

juice and semi-sweet wine (Threlfall and Morris 2002). Another study (Terrell and others 1993) showed that DMDC was more effective than SO₂ and sorbic acid to prevent fermentative spoilage of grape juice. Furthermore, combination of DMDC with benzoate or sorbate increases effectiveness and offers enhanced protection (Golden and others 2005). DMDC is marketed and sold under the registered trademark Velcorin® (Lanxess AG Corp., Leverkusen, Germany).

Natamycin

Natamycin has been used as a natural preservative to prevent the spoilage from yeast or mold in certain foods and beverages. Due to its low solubility in water (approximately 40 ppm), it is commonly used as a surface treatment. Nevertheless, it is an effective preservative in unpasteurized and pasteurized fruit juices. Most molds and yeasts are inhibited at 0.5-6 ppm and 1.0-5.0 ppm. The suggested natamycin dosage in fruit juice is 2.5-10 ppm (Thomas and Delves-Broughton 2001). The advantage of natamycin is that it does not affect the flavor of fruit juices (Delves-Broughton and others 2005). The pH of a 2% suspension is 5-7.5 (Delves-Broughton and others 2005). Natamycin is available commercially under the trade names Natamax® (Danisco A/S Corp., Copenhagen, Denmark) and Delvocid® (DSM Food Specialties USA, Inc., Charlotte, NC) both of which contain approximately 50% natamycin blended with lactose. It is prepared by controlled fermentation in dextrose-based media by selected *Streptomyces* strains.

Antimicrobials from Bacteria

Bacteriocins are substances produced by bacteria that have inhibitory effect on other bacteria and thus can be used as natural preservatives. Nisin, a bacteriocin produced by lactic acid bacteria (LAB), *Lactococcus lactis*, is the only bacteriocin approved by FDA as preservatives in the US (Hoover and Chen 2005). The production of bacteriocins from LAB potentially shortens the regulatory process because most LAB have a GRAS status. Common LAB associated with foods include *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*. Besides bacteriocins, possible inhibitors produced by bacteria include lytic agents, bacteriophage, organic acid such as lactic acid and acetic acid, and other metabolic byproduct such as diacetyl and hydrogen peroxide. However, the concern for using bacteriocins is that it may trigger an allergic or toxicologic response. Therefore, use of bacteriocin-producing cultures in food fermentations or incorporation of food ingredients containing bacteriocin-containing growth extracts are alternative approaches that will continue to be used (Hoover and Chen 2005). For example, Danisco Corporation (Danisco A/S Corp., Copenhagen, Denmark) product, MicroGard™ 200, is inhibitory towards yeast and bacteria. It is used in the food industry and has regulatory approval in beverages as yeast inhibitor. MicroGard™ 200 is produced by the fermentation of dextrose by *Propionibacterium shermanii* or specific *Lactococci* to produce antimicrobial compounds such as diacetyl, lactic acid, propionic acid, acetic acid, and other unidentified compounds in the range of 700 Daltons (Al-Zoreky and others 1991; Staszewski and Jagus 2008).

Phenolic Compounds

Phenolic compounds are another type of natural antimicrobials and antioxidants found in plants. These compounds may be classified into the following groups: simple phenols and phenolic acids (e.g. vanillic, gallic, p-cresol); hydroxycinnamic acid derivatives (e.g. p-coumaric, caffeic, ferulic); flavonoids (e.g. catechins, proanthocyanins, anthocyanidins, flavonols and their glycosides); tannins (e.g. plant polymeric phenolics that can precipitate protein from aqueous solutions) (Vigil and others 2005). Their antimicrobial activity depends on the chemical structure, concentration, and extraction method. Some of these extracts have a broad spectrum of biological effects, whereas others may be specific toward certain groups of microorganisms such as Gram-positive bacteria, or only bacteria, yeast, or mold. Bonsi (2009) reported that grape seed extracts (250 ppm) were more effective against *Alicyclobacillus acidoterrestris* than pomace extract (500 ppm) and they also prevented the growth of *A. acidoterrestris* in apple juice. Recently, grape polyphenols have been shown to inhibit the virulence traits of *Streptococcus mutans*, the predominant microbial agent in the pathogenesis of dental caries (Thimothe and others 2007). Kabara (1991) reported that undissociated phenolic groups are more active as antimicrobials than dissociated forms. This is also shown in previous studies (Sykes and Hooper 1954; Juven and others 1994) in which a greater effect was observed as the pH was reduced. This could also be due to the increased solubility and stability of these compounds at low pH. Food components can also affect their antimicrobial activities. Interactions among phenolic groups and proteins, lipids, and aldehydes could partially reduce the antimicrobial effect. Therefore, the level needed to cause an

antimicrobial effect in food products would be higher and this could change the sensory characteristics of product making it unacceptable. As a result, the use of these compounds in combination with other antimicrobials or other environmental stress factors in the frame of hurdle technology can enhance antimicrobial properties and make it possible to develop products that consumers demand (Vigil and others 2005).

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CHAPTER 2

EFFECT OF HARVEST METHOD AND PROCESSING CONDITIONS ON NIAGARA GRAPE JUICE QUALITY

ABSTRACT

Niagara grapes are used for white grape juice production and its juice quality depends on numerous harvesting/processing techniques. We evaluated harvest and processing methods in relation to Niagara grape juice quality during 24-week storage at 18°C. Harvest method (handpick vs mechanical), harvest date (early vs late), chemical addition [80 ppm potassium metabisulfite vs 500 ppm ascorbic acid (AA)], use of aeration and chemical additions [polyvinyl polypyrrolidone (PVPP), acetaldehyde], use of hot-break prior pressing (76.7, 79.4, 82.2°C), hot-pack temperature (82.2, 87.8, 93.3°C), and filter material [diatomaceous earth (DE) vs 500 kDa polymeric membrane] were evaluated. Juices were analyzed for color, turbidity, total phenolics, antioxidant capacity using ORAC assay, and phenolic profile using HPLC assay at 0, 12 and 24 weeks. Sensory evaluations were conducted using triangle and ranking tests.

Late harvest juices had 1.6-3.0 times higher phenolic content and antioxidant capacity and 27% lower turbidity than early harvest juices. AA juice quality was comparable to sulfited juices and this was confirmed by sensory results. Hot-break produced juices with significantly higher phenolic content and antioxidant capacity (up to 50% higher) and 21-28% lower turbidity and brown color than traditional cold-press juices; however, hot-break juices darkened more rapidly over the 24-week storage period than cold-press juices. Harvesting method, pasteurization and hot-break

temperature did not affect juice quality as confirmed by sensory results. Membrane filtration produced juices with better quality, lower turbidity and brown color, and thus more stable over time. The chemical additions, PVPP and acetaldehyde, could partially remove browning precursors but their effectiveness in browning prevention was significantly lower than addition of sulfite to juice.

Introduction

NYS is the third largest grape producing state in the country. The juice industry uses 65.6% of all processed grapes. Niagara, a hybrid of native species (*Vitis labrusca*), is the leading variety for white grape juice in the US due to its unique aroma and flavor, and it is the major white cultivar in New York State (NYS); hence making NYS the 2nd largest Niagara producing state (NASS 2010).

Light color, clarity, and fruit flavor are important for consumer acceptability for Niagara grape juices. The juice and juice drink market in the U.S. is saturated and the key trend market driving the market is health and wellness (Mintel 2010). Consumers are looking for high nutritional quality, all natural, no preservatives or no artificial color added products. Attributes that contribute to personal health are considered to be important in 100% of the juice market. Polyphenolic content and antioxidants in juices are associated with many health benefits (Dillard and German 2000; Kaur and Kapoor 2001); however, these compounds could be reduced by enzymatic degradation, heat, light, and oxygen during processing and storage.

White grape juices are produced by a cold-press method to maintain the light color (Pederson 1954; Morris and Striegler 2005). The oxidation and browning can

occur during the process; therefore, antioxidant compounds are needed to inhibit or delay undesirable oxidative changes. Traditionally, sulfite has been used to prevent browning in juices. The key browning reaction that occurs during juice processing is enzymatic by the action of the polyphenol oxidase (PPO). PPO catalyzes the oxidation of polyphenols to quinones which can undergo further reactions resulting in dark colored substances, melanin. Previous studies (Wisseemann and Lee 1981; Lee and Jaworski 1987) showed that grape PPO had substrate specificity and high affinity toward the o-diphenols and flavanols. Sulfite lessens the reaction by acting as the reducing agent, converting quinones back to polyphenols. Furthermore, sulfite helps preventing the reaction by destabilizing disulfide bonds that maintain the enzyme in their active forms. However, growing public awareness of sulfite sensitivity has created the need for its reduction or replacement.

Ascorbic acid (AA) is a natural antioxidant that has been used in food industry for many applications. Acting as the reducing agent, AA can also convert quinones back to polyphenol and could potentially be used as sulfite substitute to prevent browning in juices. In the red grape juice industry such as the Concord grape industry, hot-break or hot-press is used during processing instead of cold-pressing, a standard procedure in white grape juices. The hot-break process employs higher temperature (>75°C) to heat the grape mash prior to pressing in order to extract color from skins into juices, to improve phenols and anthocyanins extraction and to maximize juice yield. In addition, hot-break is reported to deactivate the polyphenol oxidase (PPO) (Montgomery and others 1982) and hence reduces enzymatic browning early in the process.

Another technique to prevent browning in juices is the use of fining agents, which have been used in wine and juices industries as clarifying agents to remove polyphenols or proteins, both haze precursors. Turbidity may develop from unstable proteins reacting with polyphenols, forming particles of 0.3-1.0 μm diameter and particles greater than 0.5 μm may settle out and form precipitates (Van Buren 1989; Girard and Fukumoto 2000). Since some of the phenols are substrates for enzymatic browning, their removal with fining agents could potentially help lower the browning reaction during processing. Polyvinyl polypyrrolidone (PVPP) can form the hydrogen bond between its carbonyl group with the hydroxyl group in phenolic compounds. PVPP has an affinity for low-molecular-weight phenolics such as catechin which are precursors to browning. Acetaldehyde, on the other hand, can also condense with phenols (flavanol or tannin) to form larger molecules (Timberlake and Bridle 1976; Saucier and others 1997a, 1997b, 1997c; Es-Safi and others 1999a, 1999b) which could then be filtered out.

Filtration is an important step to produce clarified juices. Standard diatomaceous earth (DE) filtration, traditionally used in juice processing for final filtration (polish filtration), is highly efficient and very effective (McLellan and Padilla-Zakour 2005). However, there are several drawbacks due to safety restrictions in handling the material, cost of storage, delivery, and waste disposal, and operation challenges (Starbard 2008). Cross-flow microfiltration and ultrafiltration, both pressure-driven membrane technologies, are commonly used in the beverage industry primarily for biological stabilization and sterilization, and to increase product yield and/or quality (Zydney 1996). The advantages of CFF over traditional clarification

methods include reduced processing time and enzyme usage, increased juice yield, elimination of filter aid and filter presses utilizing a single unit operation that is easy to clean and operate (Zydney 1996; Cheryan 1998).

UF systems, representing membranes with pore sized from 1000-500,000 Da MWCO or about 0.001-0.02 μm (10-200 $^{\circ}\text{A}$), can be used to improve juice stability by removing large polyphenolic compounds and haze-causing proteins; thus, reducing browning, oxidation, and haze formation while minimizing flavor loss (Kosikowski 1986; Cheryan 1998). A previous study (Dietrich and others 1990) showed stabilization of apple juice against turbidity and browning using enzymatic oxidation of polyphenols with phenol oxidase (laccase) and simultaneous aeration and subsequent ultrafiltration (membrane cut-off 50,000). UF systems have been used for juice clarification in the production of clear apple, grape, pear, cranberry, pineapple, and citrus juices (Zydney 1996; Cheryan 1998). UF filtered juices are of much higher clarity (lower turbidity) than that of juices produced by conventional filtration (Porter 1990; Alvarez and others 1996). Overall yields in UF processes are typically 5-8% higher than in conventional processes (Cheryan 1998). Polymeric UF membranes, with pore size ranging from 500 to 750 kDa MWCO, are available in the market.

This project aimed to find alternative processing methods that deliver light color Niagara juices without using sulfite in response to an increasing consumer preference towards healthy products. We hypothesized that ascorbic acid could help prevent browning due to its antioxidant capacity and would produce juices with the same or better quality. Other techniques were also evaluated along with ascorbic acid addition to determine the best practice for producing high quality Niagara juices

without sulfite addition. These techniques were hot-break, filtration (polymeric membrane crossflow filter against traditional DE filter), aeration, and addition of polyvinyl polypyrrolidone (PVPP), and acetaldehyde.

Materials and Methods

Fruit Samples

Niagara grapes were either hand harvested or mechanically harvested (at maturity as determined by a horticulture specialist) from the Neil Simmon's farm, Penn Yan, NY, during the harvesting season (fall 2008 and 2009). Hand harvested grapes were processed immediately while mechanically harvested grapes were stored at 2°C for no more than 7 days before juice processing.

Chemicals

Folin-Ciocalteu reagent, gallic acid, procyanidin B₁, procyanidin B₂, catechin, epicatechin, caffeic acid, p-coumaric acid, quercetin, quercetin-3-glucoside, and kaempferol were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Sodium fluorescein was obtained from Pfaltz & Bauer, Inc. (Waterbury, CT) and 97% Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Morris Plains, NJ). Potassium metabisulfite and ascorbic acid used were food grade. All other chemicals used were analytical grade. Water from Nanopure water purifier (Barnstead Thermolyne, Boston, MA) was used throughout the study.

Juice Processing

Niagara grapes were processed into clear juices following standard pilot plant procedures that simulate industrial processing (McLellan and Padilla Zakour 2005) as shown in Figure 2.1. Grapes were treated with either 80 ppm potassium metabisulfite or 500 ppm ascorbic acid (AA). These concentrations were based on previous trials would result in measurable amounts of free SO₂ and AA at bottling. Treated grapes were crushed with a crusher-destemmer (Mori Destemmer Crusher E20, TCW Equipment, Saint Helena, CA). Paper press aid (Georgianeer J, ITT Rayonier Inc., New York, NY) was added at 6.6 g/kg grapes. Crushed grapes were heated in a steam kettle (Design 20CD, Lee Metal Products Co. Inc., Philipsburg, PA) until the temperature reached 27°C before the pectinase enzyme, Rapidase® ADEX G, (DSM Food Specialties USA, Inc., Charlotte, NC) was added at 40 ml/ton of grapes. The must was held at room temperature for 35 min. Juice was pressed in a continuous screw press (Model JP4, Buffalo Hammermill Corp., Buffalo, NY), then pasteurized at 85°C using UHT/HTST Lab-25 HV tubular heat exchanger (Micro Thermics Inc., Raleigh, NC) with 1 min holding time, and cooled down to 27°C. A clarifying enzyme, Klerzyme® 201, (DSM Food Specialties USA, Inc., Charlotte, NC) was then added to the juice at the level of 200 ml/L. Cold stabilization was conducted by keeping the juice refrigerated at 2°C for 7 days. Following cold storage, juices was siphoned off of the bitartrate precipitant. Sulfite and AA treated juices were filtered with a Shriver plate and frame filter using a Celite® diatomite grade filter aid no. 505 (World Minerals Inc., Santa Barbara, CA). Clear juice was pasteurized at 85°C, hot-

packed into glass bottles with 3 min holding time, cooled to room temperature, and kept at 2°C until ready for analysis.

Harvest method and chemical addition study: Grapes were either handpicked or mechanically harvested divided into 3 chemical addition treatments: none, 80 ppm potassium metabisulfite, and 500 ppm ascorbic acid (AA). Treated grapes were then processed into juices using the procedure described above.

Harvest date study: Two batches of grapes were handpicked a week apart. Both early and late harvested grapes were treated with AA and processed as described above.

Hot-break study: AA treated and crushed grapes were packed into cans no. 300 (diameter x height: 7.62 x 11.27 cm) for 375 g of grape per can with 20% headspace which then were sealed and submerged into the boiling water in a steam kettle. Five thermocouples were randomly attached to the cans and the probes were located in the product at the middle point of the cans, the slowest heating region, to measure the internal temperature. The time-temperature was recorded and the lethality was automatically calculated using 93.3°F as reference temperature and z-value of 8.9°C (Padilla-Zakour 2009). All the cans were submerged in the boiling water in the steam kettle and manually agitated every 30 sec. After reaching the target temperature, the cans were hold at room temperature before they were cooled with the cooling water to 48.9°C. Three different temperature and holding time combinations – 82.2°C with no holding time, 79.4°C with 40 sec holding time, and 76.7°C with 4.50 min holding time – were used in this study to ensure an equivalent accumulating lethality of 0.1 min at 93.3°F (z-value of 8.9°C) after cooling to achieve commercial sterility

(Padilla-Zakour 2009). After heating, grape must was processed into juices using the same procedure described above. Two control samples, sulfite and AA addition, were produced using standard cold-press procedure as described above. Juices from this study were subjected to shelf-life study and thus were stored at 18°C until ready for analysis.

Filtration study: Two lots of grapes were hand-picked two weeks apart and each lot of grapes was divided into 5 batches. One batch of grapes was treated with 80 ppm potassium metabisulfite while untreated grapes were divided into 4 different treatments: control, aeration, 100 ppm polyvinyl polypyrrolidone (PVPP) addition, and 50 ppm acetaldehyde addition (Figure 2.2). The control and sulfite treatments were processed into juices as previously described. For aeration treatment, air was incorporated into pressed juice using a gas diffusion tube with air pressure at 70 kPa for 30 min. PVPP was added to another batch of juice after the first pasteurization and prior to the addition of Klerzyme®, while acetaldehyde was added after the addition of Klerzyme®.

After cold stabilization, juices from each treatment were divided into 2 batches for final filtration using either diatomaceous earth (DE) filtration or ultrafiltration (UF) with a 500 kDa MWCO hollow fiber polysulfone membrane (Model UFP 500 C 3MA, Membrane Separations Group, A/G Technology Corporation, Needham, MA). Juice was circulated for 10 min before clear juice was collected. Membrane was thoroughly cleaned between each batch of juice by flushing with distilled water twice for 5 min, recycling 0.5 N NaOH (pH 11) with 200 ppm free chlorine solution at 50°C for 1 hr, and flushing with distilled water for 30 min. For DE filtration, juices were filtered

with a Shriver Plate and Frame Filter using Celite® Diatomite Grades Filter Aid no 505. All filtered juices were pasteurized at 85°C, hot-packed into glass bottles with 3 min holding time, cooled to room temperature, and kept at 18°C until ready for analysis.

Hot-pack study: AA treated grapes were used and juices were processed as described above. Instead of pasteurizing juices at 85°C, the final pasteurization temperature, hot-pack temperatures, and holding time were varied to 82.2°C for 2 min, 87.8°C for 25 sec, and 93.3°C for 6 sec in this study.

Quality Evaluation

Juices were analyzed for pH, titratable acidity (TA, as % tartronic acid), soluble solids, turbidity, color, brown color, total phenolics, antioxidant capacity, and phenolic profile. Only juices from hot-break and filtration studies were selected for the shelf-life study at 0, 12 and 24 weeks.

pH was measured with a pH meter model Orion 3 Star Series pH Benchtop (Thermo Electron Corp., Beverly, MA) and a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) was used to measure soluble solids in Brix scale. Turbidity was measured with a Hach 2100P Turbidimeter (Hach Co, Loveland, CO) and reported in Nephelometric Turbidity Units (NTU). The Hunter L, a, and b color components were measured in a 2 cm glass cuvette with a HunterLab Ultra Scan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) in transmittance mode (TTRAN mode). Brown color was measured as the absorbance at 430 nm using a Barnstead Turner SP830 Spectrophotometer (1-cm cuvette, Barnstead International, Dubuque, IA) as a browning indicator.

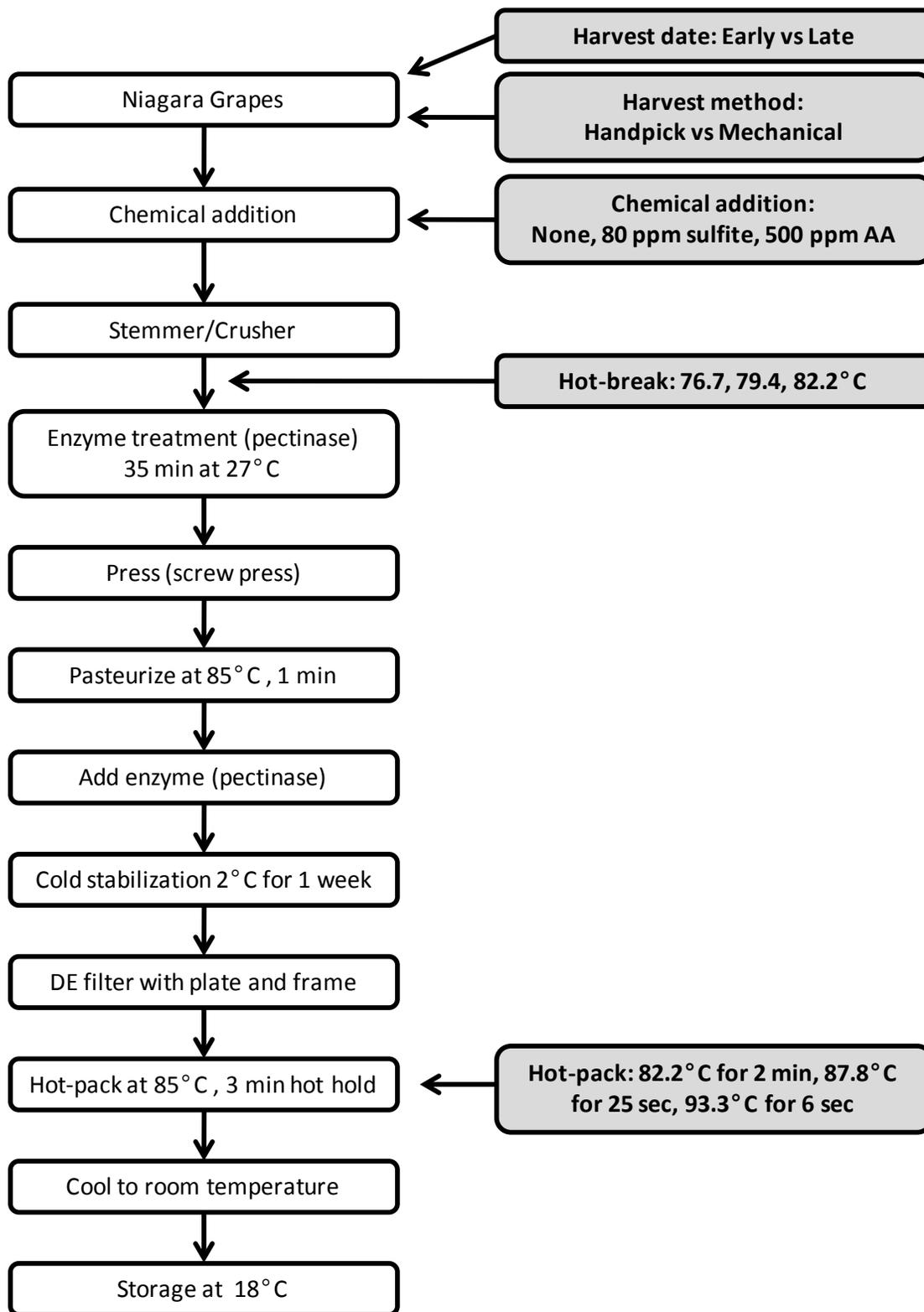


Figure 2.1. Diagram of Niagara grape juice processing showing experimental conditions

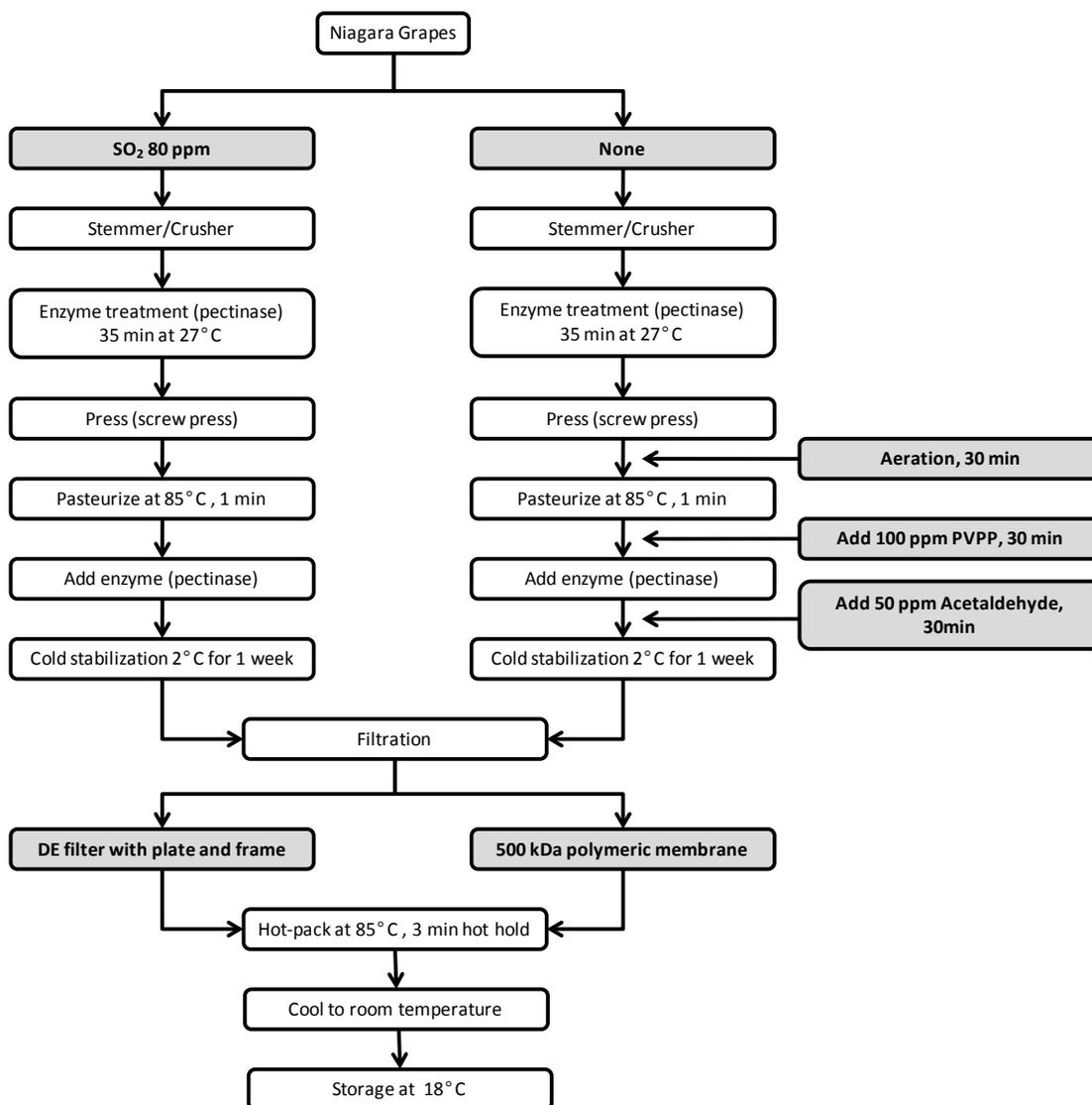


Figure 2.2. Diagram of Niagara grape juice production for filtration study

Total phenolic content was determined by the colorimetric analysis described by Singleton and Rossi (1965) using the protocol for Folin Ciocalteu (FC) reagent and expressed as mg gallic acid equivalent (GAE)/100 g of juice. Since ascorbic acid could interfere with the total phenolic assay, ascorbic acid content in all juices was measured by HPLC (described later) and used for phenolic content correction. Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) assay (Huang and others 2002; Held 2005) and the ORAC value was expressed as μM Trolox Equivalent Antioxidant Capacity (TEAC)/g of juice.

Phenol profile and ascorbic acid content were determined followed the HPLC procedures described by Bonsi (2009) using a reversed phase HPLC system (Hewlett-Packard model 1100; Palo Alto, CA) equipped with a photodiode array detector. A C18 reversed-phase Symmetry Analytical column (5 μm x 250 mm x 4.6 mm; Water Corp. Milford, MA) was used with a Symmetry Sentry guard column (Water Corp. Milford, MA) of the same packing material as the analytical column. A linear solvent gradient of binary mobile phases was used with 0.1% phosphoric acid in HPLC grade water and 0.1% phosphoric acid in HPLC grade acetonitrile. The detector was set to 280, 320, 370 nm for phenolic acid and flavanols, hydroxycinnamic acids, and flavonols, respectively.

Individual phenolic compounds were identified by comparison of UV-Visible spectra and retention time, as well as spiking samples with standards, and were quantified based on generated standard curves for each compound. Standards tested included phenolic acid: gallic acid; flavanols: procyanidin B₁, procyanidin B₂, catechin, and epicatechin; hydroxycinnamic acids: caffeic acid and p-coumaric acid;

flavonols: quercetin and kaempferol. Due to the unavailability of authentic commercial standard of caftaric acid, cis-coutaric acid, and trans-coutaric acid, its identification was accomplished with the comparison of previous report (Lee and Jaworski 1987) together with UV-Visible spectra comparison. The content of caftaric acid was expressed as mg caffeic acid equivalent/100 g while the content of cis-coutaric acid and trans-coutaric acid was expressed as mg p-coumaric acid equivalent/100 g. Furthermore, the identification of quercetin derivative 1, and quercetin derivative 2 were made on the basis of the retention time and the characteristic UV-Visible spectra and the contents of both compounds were expressed in mg quercetin equivalent/100 g. Total flavanol, hydroxycinnamic acid, and flavonol content were calculated based on 280, 320, 370 nm wavelength, respectively, and expressed as mg catechin equivalent/100 g, mg p-coumaric acid equivalent/100 g, mg quercetin equivalent/100 g, respectively.

Sensory Evaluation

Sensory evaluations of juices were conducted using triangle and preference ranking tests (Lawless and Heymann 1999). Juices were served at room temperature in the random order to twenty-four experienced panelists. Triangle test was used to assessed differences for harvest method (handpick vs mechanical), chemical addition (sulfite vs ascorbic acid), hot-pack temperature (82.2 vs 93.3°C), and hot-break temperature (76.7 vs 82.2°C) studies. The number of correct judgment from triangle test was compared to the minimum numbers of correct judgments with $p \leq 0.05$ and power of the test of 95% (Lawless and Heymann 1999). The paired preference test was used to compare the preference of AA treated and sulfited juices. Results from

paired preference test were compared to the critical value at $p \leq 0.05$ (Lawless and Heymann 1999a).

Statistical Analysis

Two batches of juice were prepared for each processing treatment and two analytical replicates were performed for each measurement. Results were reported in mean \pm standard deviation for each processing treatment at each storage time. Data were subjected to analysis of variance (ANOVA) and means were compared with Tukey's Significant Difference test at 95% confidence interval using JMP® 7.0 statistical software (SAS institute Inc., Cary, NC).

Results and Discussion

Harvest Method and Antioxidant Addition Study

Quality attributes of juices from harvest method and chemical addition studies are presented in Table 2.1. The pH, TA, and Brix of all juices were comparable and the average values were 3.15, 0.64, and 14.9, respectively. Juices made from handpicked grapes were not significantly different in any quality attributes namely turbidity, brown color, Hunter color, total phenol content, and antioxidant capacity compared to those from mechanically harvested grapes. Phenolic profile measured by HPLC analysis of both juices was in agreement with the total phenolic data from FC reagent assay (Table 2.2). There was no significant difference in phenolic profile between juices made from handpicked or mechanically harvested grapes. Sensory evaluation based on two sets of triangle test (one with sulfited juices and another one

with AA treated juices) also showed that juices from two different harvest methods were not significantly different.

Furthermore, both sulfite and AA addition produced juices with comparable turbidity, brown color, Hunter color, and antioxidant capacity. In addition, compared to juices without any chemical addition, both sulfite and AA additions significantly reduced juice turbidity and brown color, and increased Hunter L color or the lightness of the juices, confirming their effectiveness in improving juice quality. AA, a natural antioxidant, and sulfite improved juice quality by acting as reducing agents to convert quinones, substances from enzymatic browning reaction, back to polyphenols. However, AA treated juices had significantly lower total phenolic content compared to sulfited juices. This contradicted the phenol profile data from HPLC analysis. Flavanols in AA treated juices were significantly higher than those in sulfited juices; however, both phenolic acids and flavonols were comparable. The individual flavanols identified were procyanidin B₁, procyanidin B₂, catechin, and epicatechin while hydroxycinnamic acids including caftaric acid, cis-coutaric acid, trans-coutaric acid, and p-coumaric acid were the only phenolic acids identified at 320 nm. The individual flavanols and hydroxycinnamic acids identified were consistent with the result of Lee and Jaworski 1987. The compounds identified at 370 nm were quercetin derivatives. Triangle test of juices made from handpicked grapes showed that sulfited and AA treated juices were not significantly different. This result was also in agreement with the pair preference test in which both juices were preferred equally.

Table 2.1. Quality attributes of Niagara grape juices from harvest method and antioxidant addition studies

Harvest method	Chemical addition	Turbidity (NTU)	Brown Color (Abs 430 nm)	Hunter L color	Phenolic Content (mg GAE/100 g)	ORAC Value ($\mu\text{mol TE/g}$)
Hand-pick	No added	104.50 \pm 0.71 a	0.512 \pm 0.062 a	74.5 \pm 2.7 b	60.72 \pm 6.60 a	12.19 \pm 0.52 a
	Sulfite	11.55 \pm 1.06 c	0.159 \pm 0.008 b	86.2 \pm 0.5 a	54.56 \pm 5.49 a	9.75 \pm 1.62 bc
	Ascorbic acid	10.50 \pm 0.57 c	0.169 \pm 0.018 b	88.0 \pm 0.5 a	33.24 \pm 1.46 b	11.41 \pm 1.21 ab
Mechanical	No added	74.95 \pm 9.55 b	0.439 \pm 0.011 a	74.6 \pm 0.1 b	50.26 \pm 2.16 a	8.66 \pm 0.64 c
	Sulfite	9.95 \pm 1.77 c	0.089 \pm 0.007 b	89.3 \pm 0.3 a	58.93 \pm 4.24 a	9.85 \pm 0.82 bc
	Ascorbic acid	9.45 \pm 0.35 c	0.162 \pm 0.000 b	88.1 \pm 0.1 a	47.57 \pm 3.29 ab	11.14 \pm 0.47 ab

For each column, values followed by different letters are significantly different at $p \leq 0.05$

Table 2.2. Phenolic profile of Niagara grape juices from harvest method and antioxidant addition studies

Harvest method	Chemical addition	Flavanols (mg catechin equivalent/100 g)	Phenolic acid (mg p-coumaric equivalent/100 g)	Flavonols (mg quercetin equivalent/100 g)
Hand-pick	None added	92.14 \pm 6.16 bc	9.91 \pm 0.54 bc	2.36 \pm 0.02 a
	Sulfite	91.26 \pm 4.53 cd	11.02 \pm 0.97 ab	2.62 \pm 0.50 a
	Ascorbic acid	110.64 \pm 10.23 a	12.65 \pm 1.09 a	2.62 \pm 0.62 a
Mechanical	None added	76.06 \pm 8.57 d	8.36 \pm 0.61 c	1.82 \pm 0.11 a
	Sulfite	87.23 \pm 4.25 cd	10.88 \pm 1.29 ab	2.53 \pm 0.13 a
	Ascorbic acid	107.75 \pm 1.10 ab	12.49 \pm 0.18 a	2.88 \pm 0.12 a

For each column, values followed by different letters are significantly different at $p \leq 0.05$

Harvest Date Study

AA treated juices were used to compare juices from different harvest dates. Juices from early harvested grapes had lower pH and soluble solids but higher TA than those from grapes harvested a week later, as expected. The pH, TA, and Brix values of early harvest juices were 3.11, 0.66, and 14.81 while those of late harvest juices were 3.29, 0.58, and 16.74, respectively. Late harvest juices had 27% lower turbidity than early harvest juices, and their phenolic content and antioxidant capacity were 1.6-3.0 times higher than early harvest juices (Table 2.3). Results coincided with the phenolic profile from the HPLC assay (Table 2.4). Flavanols and phenolic acids in late harvest juices were 20-37% higher than those from early harvest juices; however, the flavonol content was not significantly different. This indicated that juice made with grapes with higher soluble solids had better quality. Our results support the widely used grape juice industry standard in which 15% soluble solids is used as the lower level of acceptable quality and a premium is paid for grapes based on increase in soluble solids up to 18%. A previous study (DeGolier 1978) showed that the ideal flavor, acid, and color levels in Concord grapes occurred when soluble solids was between 16-17% and the quality decreased when soluble solids increased above 18%.

Hot-break Study

The different time-temperature combinations tested in hot-break studies did not significantly affect any juice quality attributes; thus, values reported in Table 2.2 and Table 2.3 were the average of the 3 tested regimes. These were also in agreement with sensory results. Triangle test conducted between 2 different hot-break conditions, 76.7°C and 82.2°C, showed that both juices were not significantly different.

Table 2.3. Quality attributes of Niagara grape juices from harvest date, hot-pack, and hot-break studies

Study	Treatment	Turbidity (NTU)	Brown Color (Abs 430 nm)	Hunter L color	Phenolic Content (mg GAE/100 g)	ORAC Value ($\mu\text{mol TE/g}$)
Harvest date	Early	10.50 \pm 0.57 a	0.169 \pm 0.018 a	88.0 \pm 0.5 a	33.24 \pm 1.46 b	11.41 \pm 1.21 b
	Late	7.70 \pm 0.14 b	0.171 \pm 0.001 a	88.2 \pm 0.1 a	96.30 \pm 0.59 a	17.81 \pm 0.76 a
Hot-pack	Average of 3 treatments	7.18 \pm 0.27	0.178 \pm 0.006	87.7 \pm 0.4	85.15 \pm 3.85	17.35 \pm 0.58
Hot-break	Cold-press, sulfite	8.90 \pm 1.56 a	0.156 \pm 0.008 b	87.0 \pm 0.2 b	82.53 \pm 0.58 c	13.11 \pm 1.10 c
	Cold-press, ascorbic acid	7.70 \pm 0.14 ab	0.171 \pm 0.001 a	88.2 \pm 0.1 a	96.30 \pm 0.59 b	17.81 \pm 0.76 b
	Hot-break, ascorbic acid	6.43 \pm 0.41 b	0.135 \pm 0.002 c	87.7 \pm 0.4 a	126.2 \pm 10.7 a	19.42 \pm 0.64 a

For each study in each column, values followed by different letters are significantly different at $p \leq 0.05$

Table 2.4. Phenolic profile of Niagara grape juices from harvest date, hot-pack, and hot-break studies

Study	Treatment	Flavanols (mg catechin equivalent/100 g)	Phenolic acid (mg p-coumaric equivalent/100 g)	Flavonols (mg quercetin equivalent/100 g)
Harvest date	Early	110.6 ± 10.2 b	12.65 ± 1.09 b	2.62 ± 0.62 a
	Late	151.8 ± 5.1 a	15.18 ± 0.68 a	3.47 ± 0.10 a
Hot-pack	Average of 3 treatments	141.9 ± 13.4	14.91 ± 1.18	3.60 ± 0.18
Hot-break	Cold-press, sulfite	122.6 ± 1.6 b	15.34 ± 0.44 a	2.89 ± 0.24 b
	Cold-press, ascorbic acid	151.8 ± 5.1 ab	15.18 ± 0.68 a	3.47 ± 0.10 b
	Hot-break, ascorbic acid	195.7 ± 29.8 a	18.39 ± 3.70 a	5.41 ± 0.83 a

For each study in each column, values followed by different letters are significantly different at $p \leq 0.05$

Compared with the traditional cold-press juices, juices from hot-break processing had 21-28% lower turbidity and brown color (Table 2.2). The heat treatment from hot-break step helped inactivate enzymes responsible for browning reactions, polyphenoloxidases (PPO). In addition, the high temperature from hot-break also helped extract more polyphenols from grape skins into the juice, resulting in a significantly higher phenolic content and antioxidant capacity than cold press juices. These results indicate that applying the heat treatment together with the AA addition at the early stage of processing could potentially improve juice quality. It was worth noting that cold-press juices from AA treated grapes had significantly higher phenolic content as well as antioxidant capacity (17-40% higher) than those from sulfite addition. This result differed from our previous finding in the chemical addition study and could be due to the difference in raw materials such as maturity, geographic location, and environmental factors (Kalt and others 1999) since different batches of grapes were used in these 2 studies.

The same trend in phenolic content was observed in the phenolic profile data from the HPLC assay (Table 2.4). There was no significant difference between juices produced at different hot-break temperatures. HPLC data confirmed that juices produced from the hot-break process were high in polyphenols. Flavanols and flavonols in hot-break juices were higher than those from cold-press juices but the phenolic acid content was not significantly different.

The changes in quality attributes over 24 weeks of Niagara juices from hot-break study are shown in Figure 2.3. Turbidity and brown color increased significantly while the Hunter L value decreased over 24 weeks. Both phenolic content and

antioxidant capacity remained stable during the shelf-life study. Hot-break juices had a darker color (higher brown color and lower L value) and the changes of these attributes over 24 weeks were more pronounced than in cold-press juices. The increase in brown color and thus lower Hunter L color could be due to AA degradation in which L-ascorbic acid is converted to brown pigments, furfural, via dehydro-ascorbic acid (Kurata and others 1967, Johnson and others 1995). The remaining AA in juices was measured by HPLC analysis. AA in hot-break juices decreased significantly from 22.9 to 12.9 mg AA/100g after 24-week storage. However, the AA content of AA treated juice from cold press process was stable over time ranging from 29.1-29.5 mg AA/100g.

HPLC analysis showed that flavanols namely procyanidin B₁, procyanidin B₂, catechin, and epicatechin increased significantly over 24-week storage while the phenolic acid and flavonols were stable over time (Figure 2.4). The increase in these flavanols could be due to the degradation of large molecular polyphenols and could partially explain an increase in brown color along with AA degradation over time in all juices. Hot-break juices were significantly higher in flavanols, hydroxycinnamic acids, and flavonols compared to cold-press juices. Furthermore, cold-pressed AA treated juices were significantly higher in flavanols than cold-pressed sulfited juices, while hydroxycinnamic acids and flavonols were comparable. This was in agreement with the result from the chemical addition study.

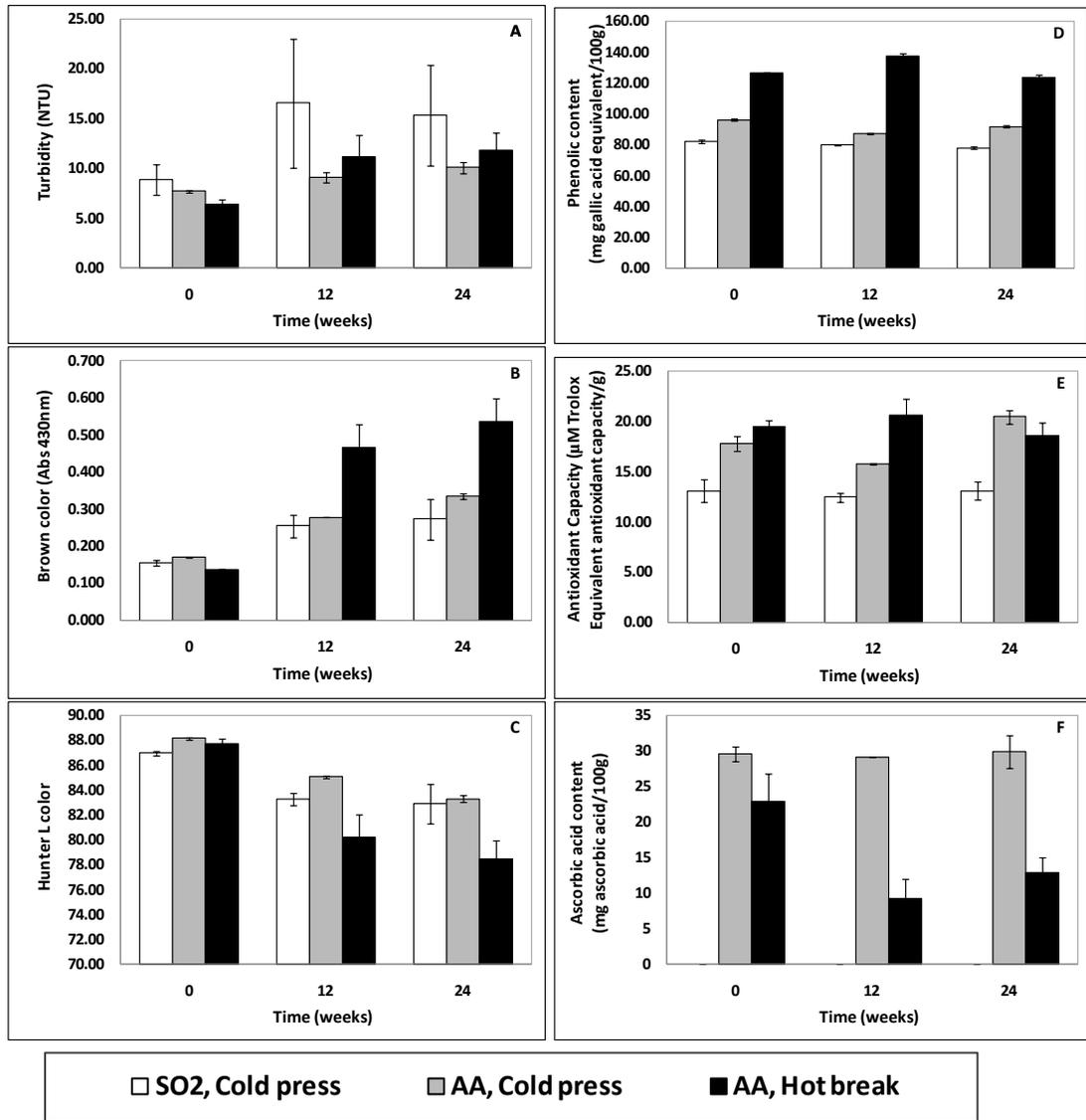


Figure 2.3. Changes in quality attributes, turbidity (A), brown color (B), Hunter L color (C), phenolic content (D), antioxidant capacity (E), ascorbic acid content (F), of Niagara juices from the hot-break study over 24-week storage at 18°C.

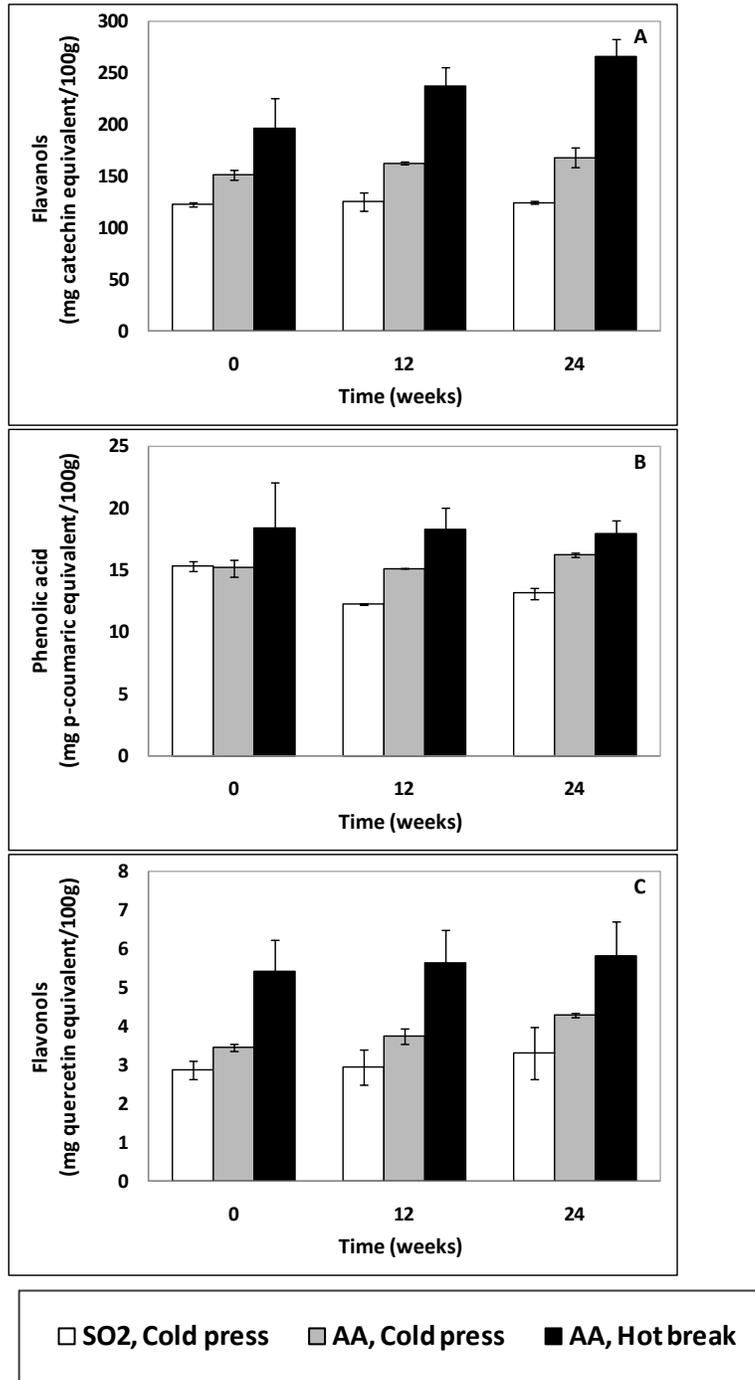


Figure 2.4. The changes in flavanols (A), phenolic acid (B), and flavonols (C) in Niagara juices from hot-break study over 24-week storage at 18°C.

Filtration Study

The average pH, TA, and Brix of early harvest juices were 2.92, 1.05, and 12.7 while these values in late harvest juices were 3.02, 0.81, and 16.01, respectively. The changes in pH, TA, and Brix were more pronounced compared to the harvest date study. This was because the time difference in harvest of early and late harvest grapes used in this study were 2 weeks while those in harvest date study were 1 week. The difference in juice quality attributes was consistent with the results from harvest date study in which early harvest juices were significantly higher in turbidity, brown color, Hunter L color but significantly lower in phenolic content and ORAC values (Figure 2.5, Figure 2.6).

Juices filtered with 500 kDa MWCO polymeric membrane had significantly lower turbidity and brown color but higher Hunter L color than juices from DE filtration indicating that the membrane used was more effective in removing brown substances as well as haze precursors such as tannins and proteins (Figure 2.5). However, phenolic content and antioxidant capacity were not significantly different. Turbidity of membrane filtered juices ranging from 0.77-2.81 NTU was in the range generally required to produce stable clarified juices (3 NTU or less) while the turbidity of DE filtered juices was high enough (10 NTU or more) to have a turbidity problem (Van Buren, 1989).

Furthermore, juices from different treatments with the same filtration type were not significantly different in turbidity, phenolic content, and antioxidant capacity. The inverse correlation between brown color and Hunter L color was observed.

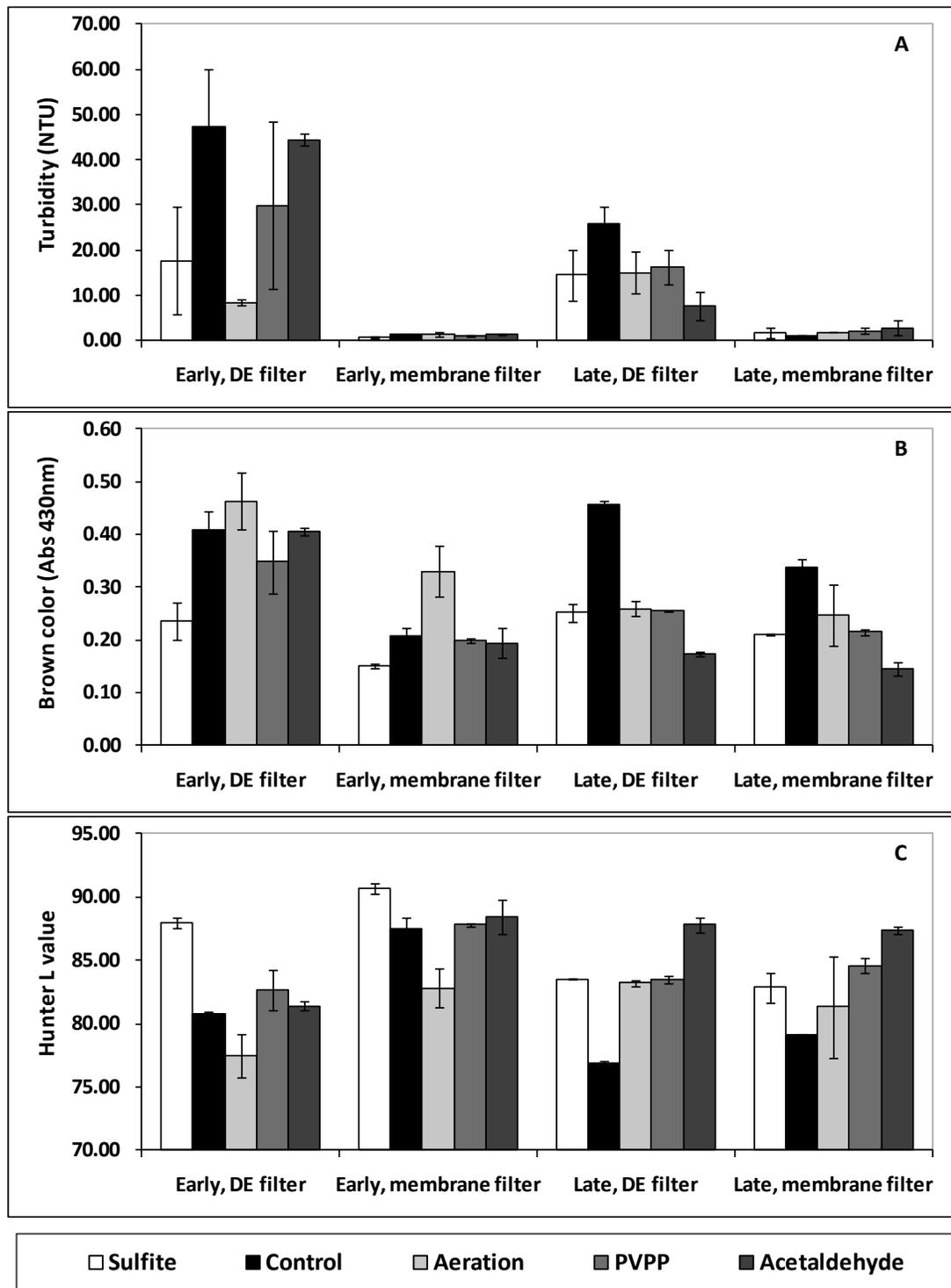


Figure 2.5. Turbidity (A), brown color (B), and Hunter L color (C) of Niagara juices from filtration study.

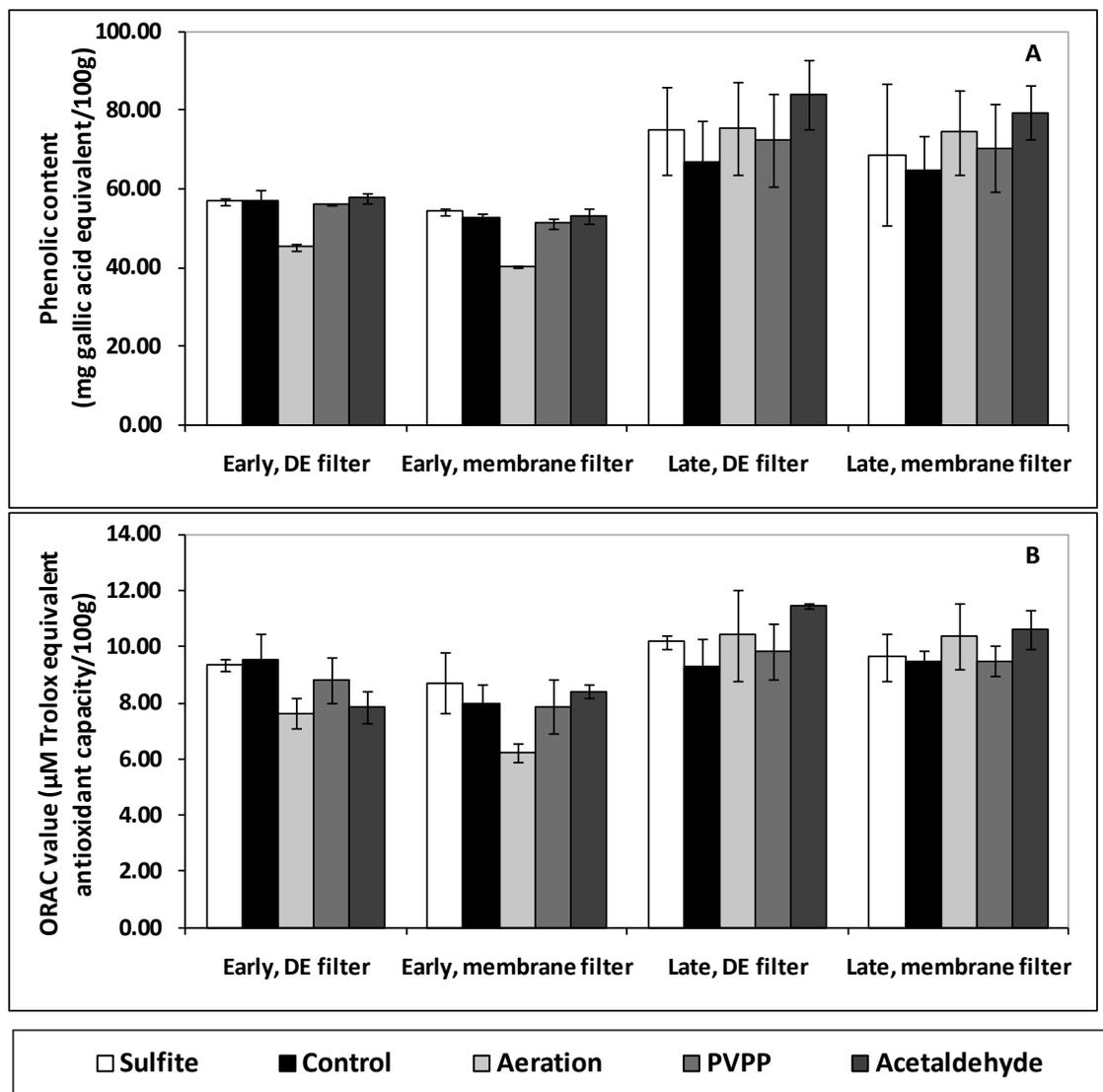


Figure 2.6. Total phenolic content (A) and antioxidant capacity (B) of Niagara juices from filtration study

Sulfited juices had the lowest brown color and the highest Hunter L color while aerated juices had the highest brown color and the lowest Hunter L color. The brown color from other treatments namely control, PVPP, and acetaldehyde was the same. This indicated that the chemical additives, PVPP and acetaldehyde, at the level used could not effectively remove phenols involved in browning reactions. The high brown color in aerated juices indicated that aeration helped promote the enzymatic browning reaction from PPO. PPO catalyzed the oxidation reaction of polyphenols in juices to quinones that further undergone reactions resulting in melanins, dark colored substances. However, the filter used in our study could not take out these brown substances resulting in dark colored juices. Dietrich and others (1990) also reported that more than 50% of polyphenols in apple juices was oxidized by phenol oxidase (laccase) and simultaneous aeration resulted in dark brown colored juices; however, contradicting to our result, filtration of oxidized juices with 50,000 MWCO membranes produced stable apple juice with appealing color with no negative effects on sensory quality. The difference in the result could be due to the difference in membrane pore size, suggesting that membranes with smaller pore size should be further investigated with the aeration treatment.

Late harvest juices were subjected to a shelf-life study and the changes in juice characteristics at 12-week and 24-week storage are presented in Figure 2.7 and Figure 2.8. The changes were more pronounced during the beginning to 12-weeks storage than from 12-week to 24-week storage.

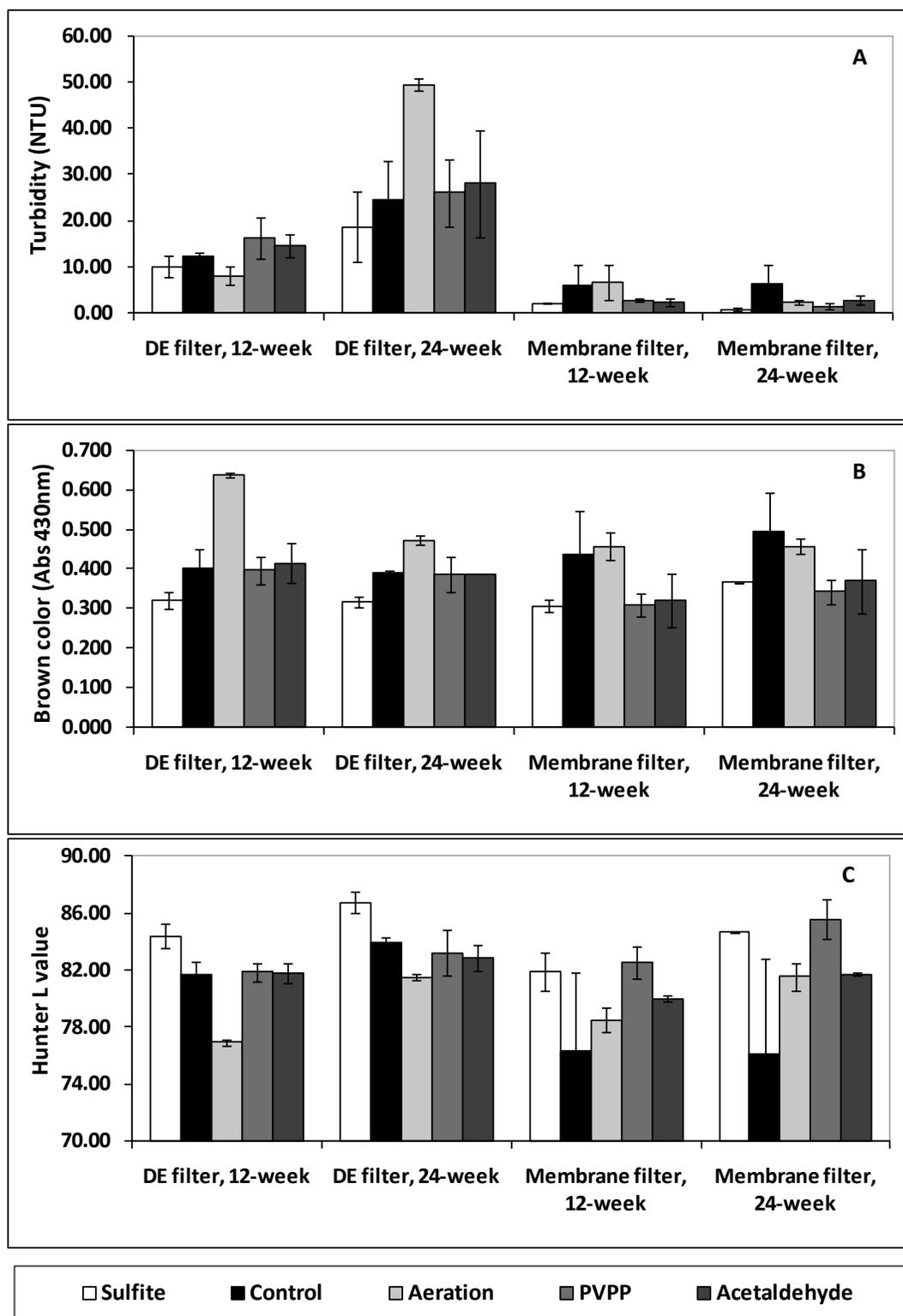


Figure 2.7. Turbidity (A), brown color (B), and Hunter L color (C) of Niagara juices from the filtration study at 12 and 24 week storage at 18°C.

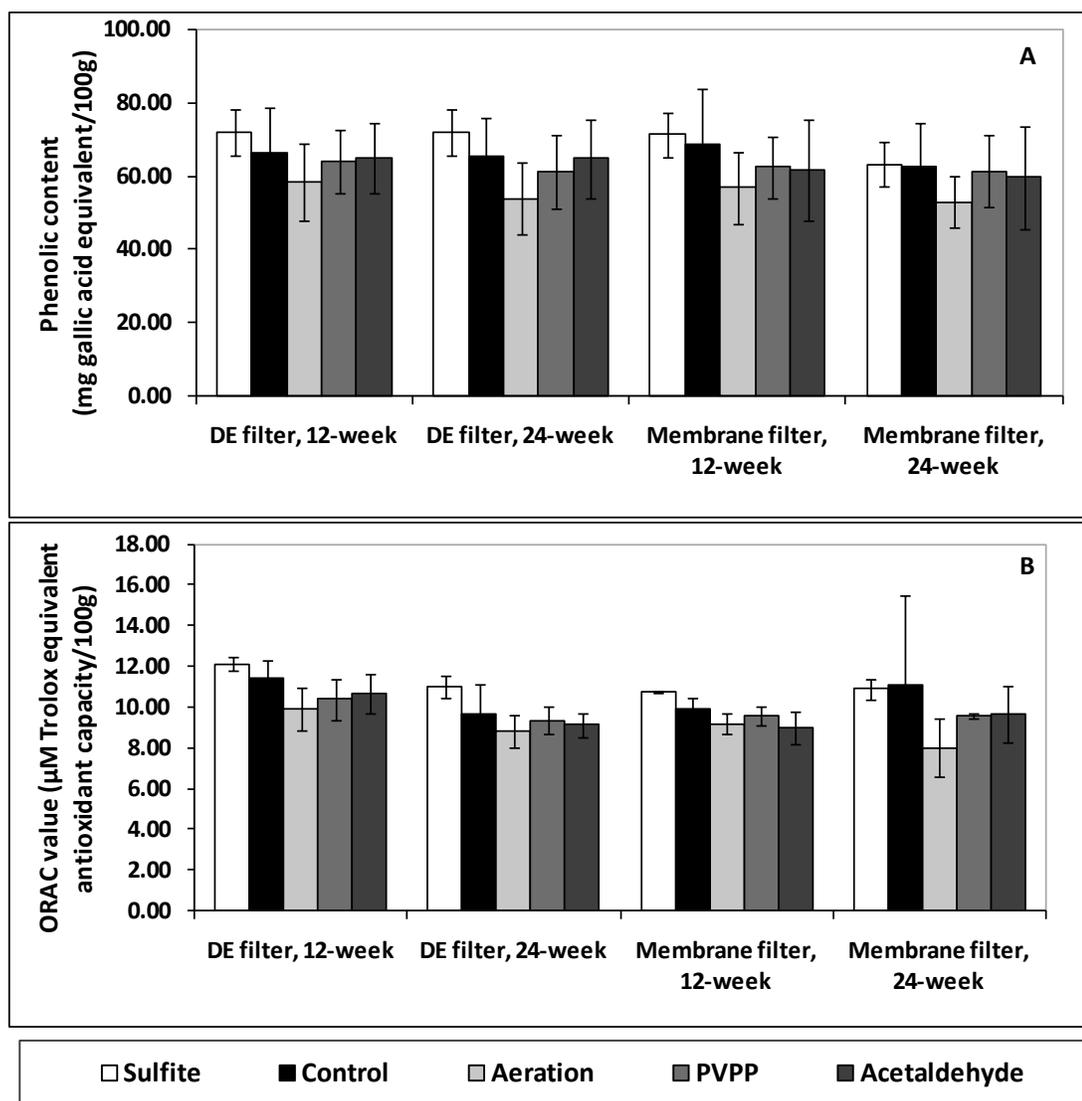


Figure 2.8. Total phenolic content (A) and antioxidant capacity (B) of Niagara juices from the filtration study at 12 and 24 week storage at 18°C.

Brown color increased significantly after 12-week storage while phenolic content decreased significantly after 12 weeks of storage but both values were stable after that. Antioxidant capacity and Hunter L color, on the other hand, were stable throughout the 24-week storage. This suggests that degraded phenolics still had antioxidant activities and might have higher antioxidant capacity compared to their original compounds (Kalbasi and Cisneros-Zevallos 2007). Turbidity of DE-filtered juices increased significantly over time while turbidity of membrane filtered juices was stable over time. This could be explained by the high initial turbidity in DE filtered juices as mentioned previously. The increasing turbidity after storage could be a result of protein/tannin hazes from protein-phenol aggregation or phenol oxidation and polymerization (Beveridge 1997).

DE filtered juices had significantly higher brown color and turbidity but lower Hunter L color than membrane filtered juices throughout the shelf-life study. Furthermore, both filtration and chemical treatments did not significantly affect phenolic content and antioxidant capacity in all juices during storage. Sulfited juices still had the lowest brown color and the highest Hunter L color while aerated juices had the highest brown color and the lowest Hunter L color. However, brown color from PVPP and acetaldehyde treatments was significantly lower than that of control samples. This implied that some of the browning precursors were removed by these chemical additions and thus, juices were less brown than the controls.

Hot-pack Study

The different timextemperature regimes tested in hot-pack studies did not significantly affect any juice quality attributes; thus, values reported in Table 2.2 and

Table 2.3 were the average of the 3 tested timextemperature conditions. These were also in agreement with sensory results. Triangle test conducted between 2 different hot-pack temperatures, 82.2°C and 93.3°C, showed that both juices were not significantly different.

Conclusion

The different harvest method, handpicked and mechanical, did not significantly affect juices quality when antioxidants such as sulfite or ascorbic acid were added early in the process, during fruit crushing. Furthermore, ascorbic acid could potentially be used as sulfite substitute if added early in the process since the quality of ascorbic acid treated juices was comparable to sulfited juices and both were equally preferred and accepted. Hot break and final pasteurization regimes in hot-packing did not affect juice chemical, physical and sensory attributes. Even though the hot-break process produced juices with lower turbidity and brown color, and higher phenolic compounds and antioxidant capacity than cold-press juices, they darkened more rapidly over time, indicating that they were less stable over storage at 18°C. Juices filtered with a 500 kDa MWCO polymeric membrane had lower turbidity and brown color values than DE filtered juices resulting in better quality juices. Other treatments studied such as aeration and addition of PVPP and acetaldehyde were not viable options as they rendered juices with inferior quality.

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CHAPTER 3

CROSSFLOW FILTRATION AND PACKAGING MATERIAL EFFECT ON QUALITY OF SHELF STABLE NIAGARA GRAPE JUICE

ABSTRACT

We evaluated ceramic and polymeric cross-flow filters (CFF) against traditional diatomaceous earth (DE) filtration to assess improvements in overall quality of Niagara grape juice. Polymeric (0.45- μm pore size) and ceramic membranes (0.2-, 0.01- μm) were evaluated. Juices were filtered with optimized pressure at 25°C. Prior to juice processing, grapes were treated with either 80 ppm potassium metabisulfite or 500 ppm ascorbic acid (AA). Samples were analyzed for brown color (Abs 430 nm), Hunter L, a, b color, turbidity, phenolics, antioxidant capacity (ORAC assay), and phenolic profile using HPLC analysis. Sensory evaluations were also conducted.

Sulfited juices had significantly higher turbidity than AA treated juices but lower total phenols, antioxidant capacity, and phenolic compounds throughout the 24-week shelf life study. Among AA treated juices, filtration type did not affect juice color (both brown color and Hunter color) and turbidity; however CFF juices resulted in lower total phenols and antioxidant capacity compared to DE filtered juices. Brown color increased significantly over 24 weeks and juices from PET bottles were darker than from glass bottles. Turbidity, total phenols, and antioxidant capacity were stable over storage while phenolic compounds (acids, monomers and dimers) identified by HPLC analysis increased significantly which could be due to the degradation of larger polyphenols.

Acceptance test showed that all juices were not rated differently for color, flavor, and overall acceptability. In addition, all juices were not significantly different based on preference test. Therefore, AA could potentially be used as sulfite substitute, and 4 different filters produced juices that were not significantly different according to preference and acceptance tests. Using ceramic CFF instead of DE filtration produced juice with equal or better quality and represented more sustainable operations.

Introduction

NYS is the third largest grape producing state in the country. Of all processed grape in NYS, 65.6% is dedicated to the juice industry. Niagara, a hybrid of native species (*Vitis labrusca*), is the leading variety for white grape juice in the US due to its unique aroma and flavor, and it is the major cultivar in New York State (NYS); hence making NYS the 2nd largest producing state (NASS 2010).

Color, clarity, and health benefits from polyphenols in juice are important for consumer acceptability. Juice components can react over time producing haze that may settle out and precipitate; thus, filtration is necessary to produce commercially clear products. Standard diatomaceous earth (DE) filtration, traditionally used in juice processing for final filtration (polish filtration), is highly efficient and very effective (McLellan and Padilla-Zakour 2005). However, there are several drawbacks due to safety restrictions in handling the material, cost of storage, delivery and waste disposal, and operational challenges (Starbard 2008). Product quality relies on the grade and amount of DE, causing its quality to be very variable. In addition, the

system could plug up easily and need to be disassembled for cleaning, resulting in significant losses of valuable juice and increased operational costs (Starbard 2008).

Cross-flow filtration (CFF), a pressure-driven membrane technology that includes microfiltration (MF) and ultrafiltration (UF) is commonly used in the beverage industry primarily for biological stabilization and sterilization, and to increase product yield and/or quality (Zydney 1996). The advantages of CFF over traditional clarification methods include reduced processing time and enzyme usage, increased juice yield, elimination of filter aid and filter presses with a reusable single unit operation that is easy to clean and operate (Zydney 1996; Cheryan 1998). Cross-flow membranes can act as microbiological filters to reduce or eliminate spoilage microorganisms, depending on the pore size of the membrane, and can be used as cold-sterilizing microfilters, possibly eliminating the need for heat pasteurization (Zeman 1996; Zydney 1996). With regard to MF, the 0.22 μm membrane represents the current industrial standard for a cold-sterilizing microfilter in beverages (Zeman 1996; Zydney 1996). Juice CFF also minimized volatilization or destruction of flavor-producing compounds during heat treatment and thus, improved quality of final products (Cheryan 1998).

UF systems have been used for juice clarification in the production of clear apple, grape, pear, cranberry, pineapple, and citrus juices (Zydney 1996; Cheryan 1998). UF filtered juices are of much higher clarity (lower turbidity) than that of juice produced by conventional filtration (Porter 1990; Alvarez and others 1996). Overall yields in UF processes are typically 5-8% higher than in conventional processes (Cheryan 1998). Zydney (1996) compared the economics of UF and conventional

filtration for juice processing at the rate of 250,000 L/day with operation for 200 days/yr and reported that overall cost saving with UF process are estimated to be \$275,000/yr, the amount comparable to the capital investment required for installation of a new UF system. In addition, the increased yield for a UF process (approx. 5%) can contribute another \$750,000 in annual revenue. DE and labor costs are major contributors to the overall cost of conventional processes.

Membranes can be categorized by their construction materials: polymeric and inorganic. Polymeric UF membranes, with pore size ranging from 500 to 750 kDa MWCO, are available in the market while inorganic or ceramic membranes are more common in MF systems with pore sizes of 0.1-0.6 μm . Ceramic membranes are extremely versatile and offer advantages with resistance to abrasion and chemical tolerance. They can be sterilized and operated at high temperatures and pressures, are autoclavable, have backflushing capability and operate over a wide pH range with longer life than polymeric membranes (Padilla-Zakour and McLellan 1993; Cheryan 1998; Starbard 2008). They are better for maintaining flavor and color, as they are chemically inert. Ceramic CFF is reusable and has virtually unlimited life, representing a more sustainable system. The main disadvantage of the CFF is the high initial investment in the unit leading to the higher cost per membrane area compared to polymeric membrane (Cheryan 1998; Starbard 2008). A previous study in wine filtration showed that even though the capital cost of ceramic MF units are double that of DE filtration system, the savings in filters alone justified the ceramic systems (Short 1995).

This project aimed to improve quality of Niagara grape juices using CFF. We evaluated the efficiency of ceramic and polymeric CFF against traditional DE filtration to improve overall quality of Niagara grape juices.

Materials and Methods

Fruit Samples

Niagara grapes were hand harvested (at maturity as determined by a horticulture specialist) from the Neil Simmon's farm, Penn Yan, NY, during the harvesting season (fall, 2008) and then stored at 2°C, for no more than 7 days, prior to processing.

Chemicals

Folin-Ciocalteu reagent, gallic acid, procyanidin B₁, procyanidin B₂, catechin, epicatechin, caffeic acid, p-coumaric acid, quercetin, quercetin-3-glucoside, and kaempferol were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Sodium fluorescein was obtained from Pfaltz & Bauer, Inc. (Waterbury, CT) and 97% Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Morris Plains, NJ). Potassium metabisulfite and ascorbic acid used were food grade. All other chemicals used were analytical grade. Water from Nanopure water purifier (Barnstead Thermolyne, Boston, MA) was used throughout the study.

Juice Processing

Niagara grapes were processed into clear juices following standard pilot plant procedures that simulate industrial processing (McLellan and Padilla-Zakour 2005) as shown in Figure 3.1. Grapes treated with 80 ppm potassium metabisulfite were used as

the control while the rest of the grapes were treated with 500 ppm ascorbic acid (AA). These concentrations were based on previous trials would result in measurable amounts of free SO₂ and AA at bottling. Treated grapes were crushed with crusher-destemmer (Mori Destemmer-Crusher E20, TCW Equipment, Saint Helena, CA). The paper press aid (Georgianer J, ITT Rayonier Inc., New York, NY) was added at 6.6 g/kg grapes. Crushed grapes were heated in a steam kettle (Design 20CD, Lee Metal Products Co. Inc., Philipsburg, PA) until the temperature reached 27°C before the pectinase enzyme, Rapidase® ADEX-G, (DSM Food Specialties USA, Inc., Charlotte, NC) was added at 40 ml/ton of grapes. The must was held at room temperature for 35 min. Juice was pressed in a continuous screw press (Model JP4, Buffalo Hammermill Corp., Buffalo, NY), then pasteurized at 85°C using UHT/HTST Lab-25 HV heat exchanger (Micro Thermics Inc., Raleigh, NC) with 1 min holding time, and cooled down to 27°C. A clarifying enzyme, Klerzyme® 201, (DSM Food Specialties USA, Inc., Charlotte, NC) was then added to the juice at the level of 200 ml/L. Cold stabilization was conducted by keeping the juice refrigerated at 2°C for 7 days.

Following cold storage, juices were siphoned off of the bitartrates precipitant. Sulfite and AA treated juices were filtered by Shriver Plate and Frame Filter size 7 (T. Shriver & Co., Inc., Harrison, NJ) using a Celite® Diatomite Grades Filter Aid no. 505 (World Minerals Inc., Santa Barbara, CA). For CFF, a 0.45 µm hollow fiber polysulfone polymeric membrane with 0.12 m² surface area and 1.0 mm fiber inner diameter (Model CFP-4-E-5A, Membrane Separations Group, A/G Technology Corporation, Needham, MA) and 0.2- and 0.01-µm tubular ceramic membranes with 0.13 m² surface area and 2.0 mm x 2.0 mm square opening (Ceramic membrane cross-

flow liquid filtration system, HilCo Division, Hilliard Corp., Elmira, NY) were used to filter AA treated cold stabilized juices. The inlet and outlet pressure were 179.3 and 96.5 kPa for ceramic, and 103.4 and 34.5 kPa for polymeric membrane. Juice was circulated through membrane for 10 min before clear juice was collected. Juices were filtered at 25°C and filtered juices were pasteurized at 85°C with 3 min hold, hot packed into glass and 32 fl oz (946.35 ml) Heat Set Round Double Bell PET bottles (Amcor Rigid Plastics, Allentown, PA), and kept at 18°C until ready for analysis.

Membrane was thoroughly cleaned between each batch of juice and the water flow rate after cleaning was in the range of 85-100% of the original flow rate. Following the cleaning procedure, membrane was flushed twice with distilled water for 5 min, 0.5 N NaOH (pH 11 for polymeric and pH 12 for ceramic membrane) at 50°C for an hour, sodium hypochlorite (NaOCl) solution (200 ppm free chlorine) for an hour, and distilled water for half an hour. The additional step of flushing with nitric acid solution (pH 2) after flushing with chlorine solution was used for ceramic membranes.

Chemical and Physical Analysis

All juices were analyzed for pH, titratable acidity (TA, as % tartronic acid), Brix, turbidity, color, total phenolic content, phenolic profile, and antioxidant capacity. Shelf-life study of Niagara juices in glass and PET bottles was also conducted at 0, 12, 24 weeks of storage.

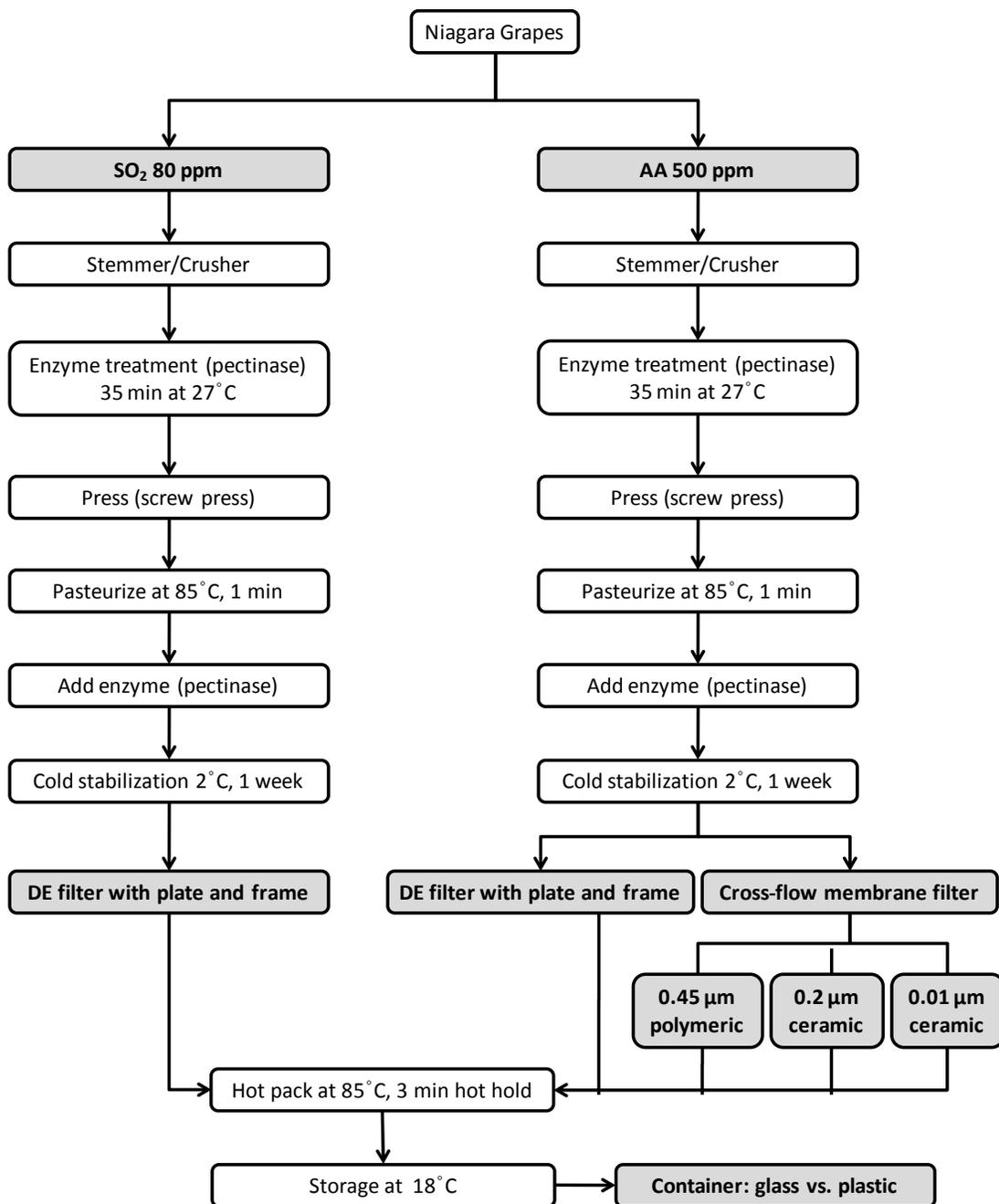


Figure 3.1. Processing diagram of Niagara grape juices

pH was measured with a pH meter model Orion 3 Star Series pH Benchtop (Thermo Electron Corp., Beverly, MA) and a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) was used to measure Brix levels. Turbidity was measured with a Hach 2100P Turbidimeter (Hach Co, Loveland, CO) and reported in nephelometric turbidity units (NTU). Brown color was measured as the absorbance at 430 nm with 1.0 cm cuvette (Fisher Scientific CO, Agawam, MA) using a Barnstead Turner SP830 Spectrophotometer (Barnstead International, Dubuque, IA). The Hunter L, a, and b color components were measured in 2 cm glass cuvettes with HunterLab Ultra Scan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) with a transmittance mode (TTRAN mode). Total phenolic content was determined using the protocol for Folin-Ciocalteu (FC) reagent in colorimetric analysis described by Singleton and Rossi (1965) and expressed as mg gallic acid equivalent (GAE)/100 g. Since ascorbic acid interfered the total phenolic assay, ascorbic acid content in all juices was measured using a HPLC method (described later) and used to correct phenolic content of all juices. Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) assay (Huang and others 2002; Held 2005) and the ORAC value was expressed as μM Trolox Equivalent Antioxidant Capacity (TEAC)/g.

Phenolic profile and ascorbic acid content were determined by High Performance Liquid Chromatography (HPLC) as described by Bonsi (2009) using a reversed phase HPLC system (Hewlett-Packard model 1100; Palo Alto, CA) equipped with a photodiode array detector. A C18 reversed-phase Symmetry Analytical column ($5\ \mu\text{m}$ x 250 mm x 4.6 mm; Water Corp. Milford, MA) was used with a Symmetry

Sentry guard column (Water Corp. Milford, MA) of the same packing material as analytical column. A linear solvent gradient was used with binary mobile phases of 0.1% phosphoric acid in HPLC grade water and 0.1% phosphoric acid in HPLC grade acetonitrile. The detector was set to 280, 320, 370 nm for phenolic acid and flavanols, hydroxycinnamic acids, and flavonols, respectively.

Individual phenolic compounds were identified by comparison of UV-Visible spectra and retention time, as well as spiking samples with standards, and were quantified based on generated standard curves for each compound. Standards tested included phenolic acid: gallic acid; flavanols: procyanidin B₁, procyanidin B₂, catechin, and epicatechin; hydroxycinnamic acids: caffeic acid and p-coumaric acid; flavonols: quercetin and kaempferol. Due to the unavailability of authentic commercial standard of caftaric acid, cis-coutaric acid, and trans-coutaric acid, its identification was accomplished with the comparison of previous report (Lee and Jaworski 1987) together with UV-Visible spectra comparison. The content of caftaric acid was expressed as mg caffeic acid equivalent/100 g while the content of cis-coutaric acid and trans-coutaric acid was expressed as mg p-coumaric acid equivalent/100 g. Furthermore, the identification of quercetin derivative 1, and quercetin derivative 2 were made on the basis of the retention time and the characteristic UV-Visible spectra and the contents of both compounds were expressed in mg quercetin equivalent/100 g. Total flavanol, hydroxycinnamic acid, and flavonol content were calculated based on 280, 320, 370 nm wavelength, respectively, and expressed as mg catechin equivalent/100 g, mg p-coumaric acid equivalent/100 g, mg quercetin equivalent/100 g, respectively.

Sensory Evaluation

Sensory evaluations of freshly prepared Niagara juices were conducted using preference ranking and acceptance tests (Lawless and Heymann 1999). The 7-point hedonic scale (Lawless and Heymann 1999) was used to assess the acceptability of juices in three attributes: color, flavor, and overall acceptability. Juices were randomly served at room temperature to 24 experienced panelists.

Statistical Analysis

Two batches of juice were prepared for each processing treatment and two analytical replicates were performed for each measurement. Results were reported in mean \pm standard deviation for each processing treatment at each storage time. Data were subjected to analysis of variance (ANOVA) and means were compared with Tukey-Kramer HSD at 95% confidence interval using the JMP® 7.0 statistical software package (SAS institute Inc., Cary, NC).

The Friedman test was used to analyze preference ranking data and mean comparison was made using the Least Significant Ranked Difference (LSRD) test at 95% confidence interval (Lawless and Heymann 1999). For acceptance test, means of each attribute were subjected to ANOVA and compared using the Tukey-Kramer HSD at 95% confidence interval using the JMP® 7.0 software package.

Results and Discussion

Effect of Processing on Niagara Grape Juice Quality

The pH, TA and Brix levels of filtered juices were not significantly different and the averages of these values were 3.28, 0.58, and 16.66 respectively. Residual ascorbic acid content measured by HPLC assay in all AA treated juices were comparable ranging from 29.5 – 37.7 mg AA/100 g while none was detected in sulfited juices as expected. Turbidity may develop from unstable proteins reacting with polyphenols, forming particles of 0.3-1.0 μm diameter and particles greater than 0.5 μm may settle out and form precipitates (Van Buren 1989; Girard and Fukumoto 2000). Turbidity values of DE-filtered sulfited and AA treated juices were not significantly different (Table 3.1), indicating that AA was as effective as SO_2 at levels used in this study in preventing juice turbidity. Among AA treated CFF juices, turbidity increased as the membrane pore size increased. Juices from 0.01 μm membrane had the lowest turbidity followed by 0.2 μm and 0.45 μm , respectively.

The Hunter L value (lightness) and b value of AA treated juices were significantly higher than sulfited juices while Hunter a value was significantly lower (Table 3.1). This indicated that AA treated juices were lighter and had more yellow and green color. According to L value and b values, ceramic membrane filtered juices were darker but more yellow than other AA treated juices. Furthermore, there was no significant difference in Hunter color between juices from 2 different ceramic membrane pore sizes, 0.2 and 0.01 μm . The absorbance at 430 nm was used as a browning indicator since an increase in the brown coloration would be detected at 400-440 nm wavelength (Zoecklein and others 1995). Sulfited juices had the lowest

brown color followed by AA treated juices from 0.45 μm polymeric membrane and DE filter, respectively, while ceramic membrane filtered juices had the highest brown color (Table 3.1).

Phenolic content of sulfited juices was significantly lower than DE filtered AA treated juices (16.7% lower) (Figure 3.2A). Among AA treated juices, DE filter produced juices with higher polyphenol content than CFF, indicating that CFF retained more phenolic compounds than DE. Phenolic content of juices from 0.2 μm ceramic and 0.45 μm polymeric membranes were comparable while that of the tightest pore size, 0.01 μm ceramic membrane, was the lowest as expected. The same trend in total phenolic content was observed in antioxidant capacity. Antioxidant capacities in all CFF juices were comparable but significantly lower than that of AA-treated DE filtered juices (Figure 3.2B). Sulfited juice had 13.3-32.5% lower ORAC values compared to AA treated juices.

Phenolic compounds identified by HPLC assay are shown in Table 3.2. Flavanols (procyanidin B₁, procyanidin B₂, catechin, and epicatechin), hydroxycinnamic acids (caftaric acid, cis-coutaric acid, trans-coutaric acid, and p-coumaric acid), and flavonols (quercetin, quercetin derivative 1, quercetin derivative 2, and kaempferol) were detected at 280 nm, 320 nm, and 370 nm, respectively. Procyanidin B₁, catechin, trans-coutaric acid, and quercetin derivative 1 contents were not significantly different in all juices. Furthermore, quercetin and kaempferol were not detected in all juices.

Table 3.1. Physical characteristics of Niagara grape juices treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes

Treatment	Turbidity (NTU)	Brown color (Abs 430 nm)	Hunter color L	Hunter color a	Hunter color b
SO₂, DE	8.90 ± 1.56 a	0.156 ± 0.008 c	86.95 ± 0.18 c	-2.27 ± 0.03 a	19.24 ± 0.72 d
AA, DE	7.70 ± 0.14 ab	0.171 ± 0.001 ab	88.15 ± 0.06 ab	-4.05 ± 0.05 b	22.89 ± 0.04 bc
AA, 0.2 µm ceramic	5.35 ± 0.21 bc	0.181 ± 0.001 a	87.82 ± 0.12 b	-3.91 ± 0.04 b	24.39 ± 0.87 ab
AA, 0.01 µm ceramic	4.00 ± 2.26 c	0.180 ± 0.006 a	87.78 ± 0.29 b	-3.95 ± 0.18 b	25.21 ± 0.70 a
AA, 0.45 µm polymeric	6.05 ± 0.49 abc	0.163 ± 0.003 bc	88.30 ± 0.04 a	-3.99 ± 0.12 b	22.68 ± 0.35 c

For each column, values followed by different letters are significantly different at $p \leq 0.05$

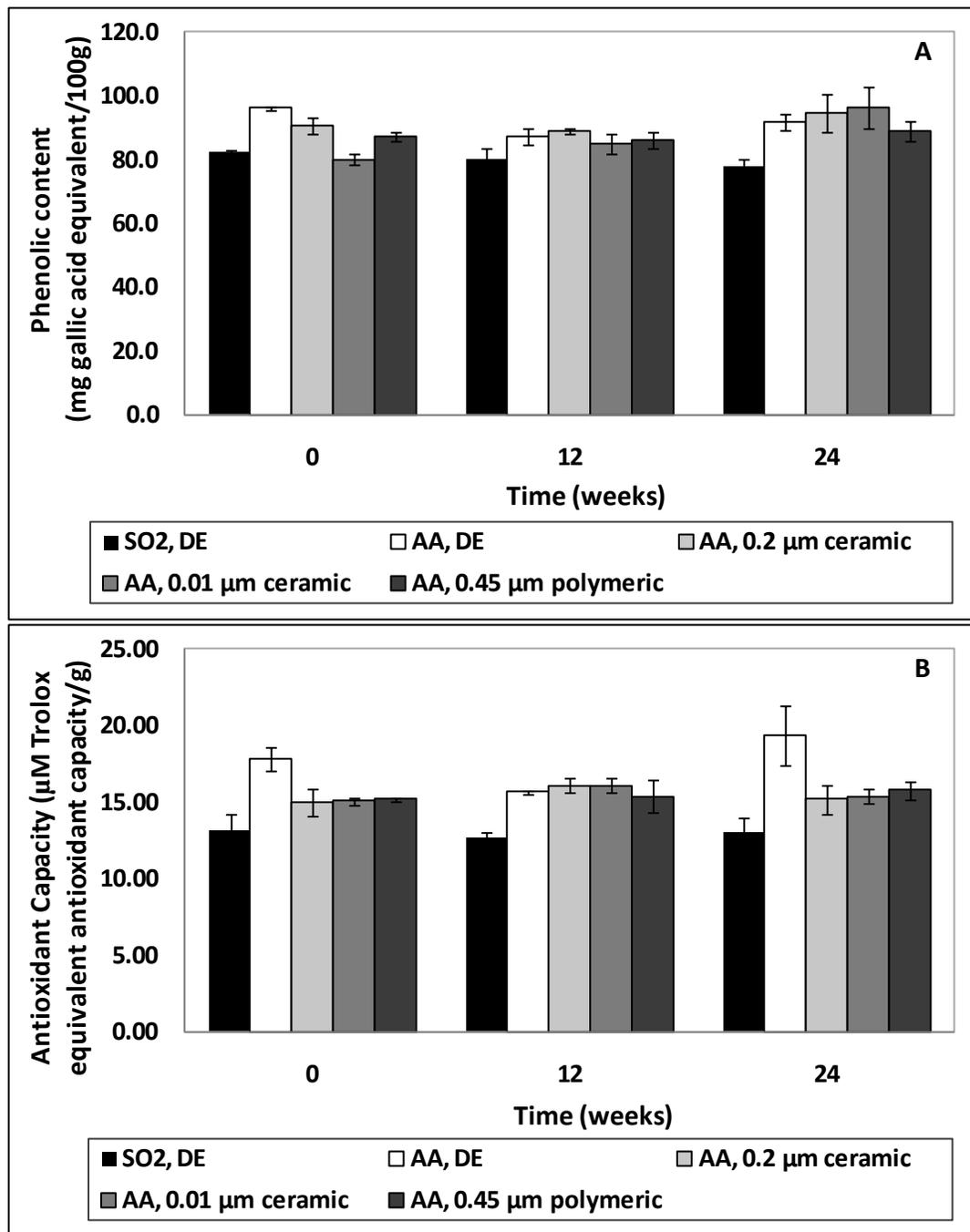


Figure 3.2. Changes of phenolic content (A) and antioxidant capacity (B) in Niagara grape juice treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes over 24-week storage at 18°C.

Table 3.2. Phenolic profile of Niagara grape juices from HPLC analysis treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes

Phenolic compounds		SO ₂ , DE	AA, DE	AA, 0.2 μm ceramic	AA, 0.01 μm ceramic	AA, 0.45 μm polymeric
Phenolic acid	Gallic acid	0.30 ± 0.02 b	0.43 ± 0.00 a	0.44 ± 0.01 a	0.44 ± 0.01 a	0.41 ± 0.00 a
Flavanols	Procyanidin B ₁	2.25 ± 0.45 a	1.61 ± 0.03 a	1.63 ± 0.03 a	2.09 ± 0.42 a	1.52 ± 0.07 a
	Procyanidin B ₂	1.19 ± 0.11 d	1.44 ± 0.06 c	1.45 ± 0.01 c	1.97 ± 0.08 b	2.44 ± 0.04 a
	Catechin	5.81 ± 0.00 a	7.51 ± 0.27 a	7.47 ± 0.26 a	6.40 ± 0.82 a	6.94 ± 0.99 a
	Epicatechin	0.13 ± 0.12 b	0.12 ± 0.01 b	0.61 ± 0.13 a	0.79 ± 0.43 a	1.06 ± 0.04 a
Hydroxycinnamic acids	Caftaric acid	8.23 ± 0.07 b	10.78 ± 0.11 a	10.51 ± 0.12 a	10.60 ± 0.58 a	10.69 ± 0.71 a
	cis-coutaric acid	0.83 ± 0.02 b	0.86 ± 0.01 b	1.00 ± 0.06 a	1.02 ± 0.07 a	1.00 ± 0.01 a
	trans-coutaric acid	2.27 ± 0.03 a	2.39 ± 0.02 a	2.37 ± 0.05 a	2.40 ± 0.09 a	2.38 ± 0.04 a
	p-coumaric acid	0.42 ± 0.02 b	0.41 ± 0.01 b	0.51 ± 0.03 a	0.52 ± 0.05 a	0.48 ± 0.00 ab
Flavonols	Quercetin	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Quercetin derivatives 1	0.58 ± 0.01 b	0.75 ± 0.05 a	0.67 ± 0.05 ab	0.69 ± 0.07 a	0.69 ± 0.01 a
	Quercetin derivatives 2	0.59 ± 0.01 a	0.50 ± 0.03 b	0.46 ± 0.01 bc	0.42 ± 0.05 c	0.51 ± 0.01 b
	Kaempferol	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

For each row, values followed by different letters are significantly different at $p \leq 0.05$

Sulfited juice was significantly lower in gallic acid and caftaric acid than AA treated juices and these contents in all AA treated juices were comparable. The only phenolic compound in sulfited juice that was significantly higher than that of AA treated juice was quercetin derivative 2. Epicatechin, cis-coutaric acid, and p-coumaric acid contents in AA treated CFF juices were comparable and significantly higher than those of AA treated DE filtered and sulfited juices.

Sensory results showed very promising results. Even though there are some differences in physical and chemical attributes, all juices were not rated differently for color, flavor, and overall acceptability in the acceptance test (Table 3.3). In addition, there was no significant difference in preference of all juices. Therefore, AA could potentially be used as a sulfite substitute and 4 different filtration produced juices that were not significantly preferred differently.

Shelf-life Study

Turbidity was stable over the 24-week storage period and this could be due to the initial low turbidity (less than 10 NTU). Van Buren (1989) reported that clear juice turbidity of 10 NTU or more was high enough to have a turbidity problem. Furthermore, phenolic content and antioxidant capacity were stable over the 24-week storage period (Figure 3.2). However, flavanol, hydroxycinnamic acid, and flavonol contents measured by HPLC increased significantly over the 24-week storage period (Figure 3.3). The difference of phenolic content from the Folin-Ciocalteu reagent assay and HPLC analysis suggests that changes observed with individual phenolic compounds do not have a significant effect on the antioxidant capacity of the juice.

Table 3.3. Acceptance and preference ranking test results of Niagara grape juices treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes

Treatment	Acceptance score			Preference ranking score
	Color	Flavor	Overall Acceptability	
SO ₂ , DE	5.00 ± 1.41 a	4.63 ± 1.95 a	4.92 ± 1.59 a	3.46 ± 1.72 a
AA, DE	5.88 ± 1.12 a	5.42 ± 1.56 a	5.38 ± 1.50 a	3.04 ± 1.60 a
AA, 0.2 µm ceramic	5.67 ± 1.24 a	5.25 ± 1.33 a	5.46 ± 1.22 a	2.75 ± 1.07 a
AA, 0.01 µm ceramic	5.71 ± 1.37 a	5.29 ± 1.20 a	5.46 ± 1.28 a	2.79 ± 1.32 a
AA, 0.45 µm polymeric	5.79 ± 1.25 a	5.54 ± 1.02 a	5.42 ± 1.10 a	2.88 ± 1.23 a

For each column, values followed by different letters are significantly different at $p \leq 0.05$

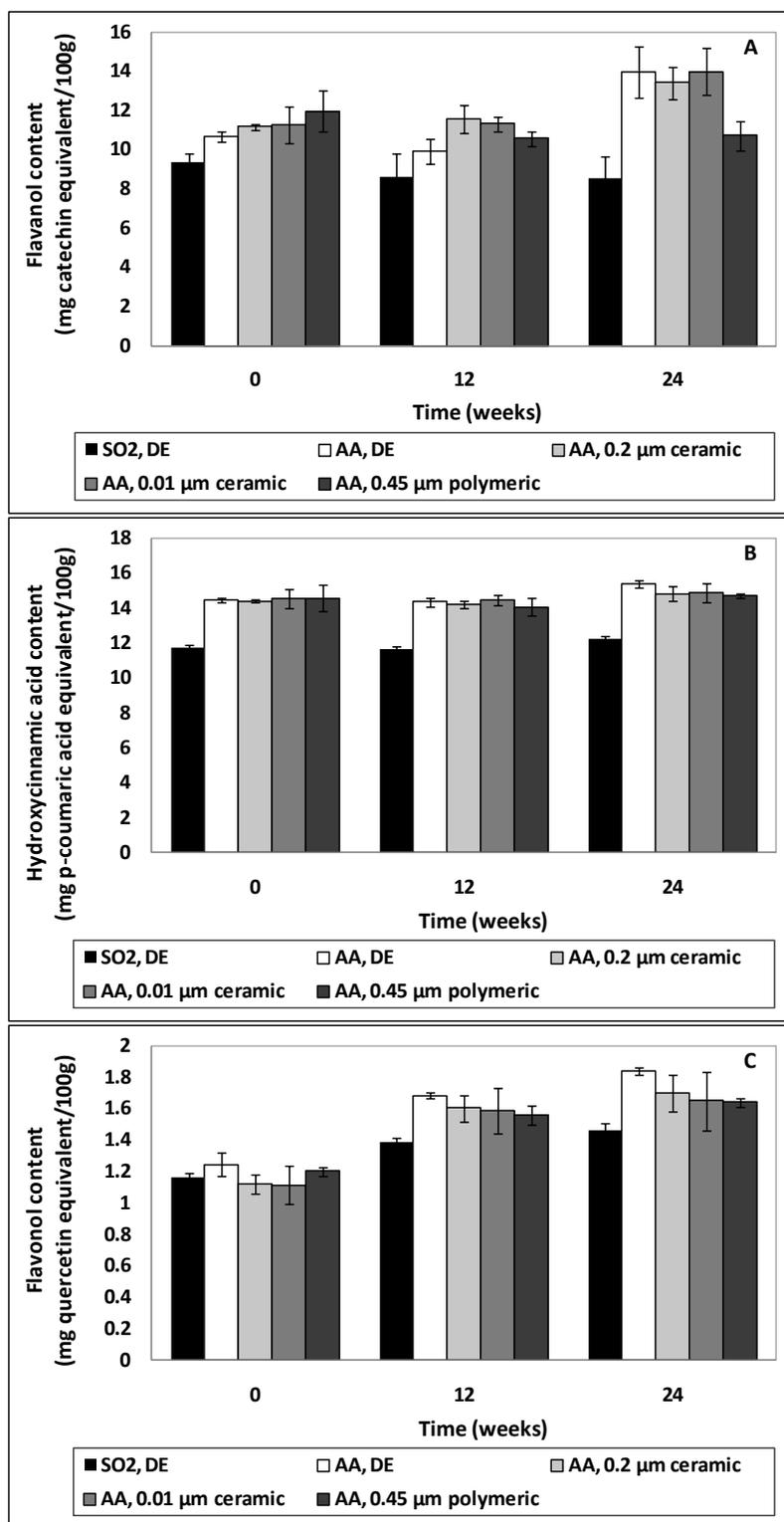


Figure 3.3. Changes of flavanols (A), hydroxycinnamic acid (B), and flavonols (C) in Niagara grape juice treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes over 24-week storage at 18°C

Gallic acid decreased over time while procyanidin B₁, catechin, caftaric acid, and trans-coutaric acid were stable over the shelf-life study. Procyanidin B₂, epicatechin, cis-coutaric acid, p-coumaric acid, and both quercetin derivatives increased significantly over the 24-week storage period. Furthermore, even though quercetin and kaempferol were not detected at 0-week storage, both compounds were detected after storage. The increase in these phenolic compounds could be due to the hydrolysis of large molecular polyphenols that were not identified by HPLC analysis. Lee and Talcott (2002) observed a decrease in total ellagitannins in hot press muscadine grapes juice and an increase in free ellagic acid after storage for up to 120 days at 20°C, indicating hydrolysis during storage. Musingo and others (2001) also reported an increase in ellagic acid in both white and red muscadine grape juices during storage at 25°C which was likely to come from hydrolysis of higher molecular weight compounds.

Juice color including brown color and Hunter color changed significantly over 24 weeks of storage. The inverse relation between brown color and Hunter L value was also observed. Brown color increased significantly while Hunter L value decreased significantly (Figure 3.4). This could be explained by an increase in flavanol, hydroxycinnamic acid, and flavonol, all of which could be substrates for chemical oxidation resulting in brown substance products. The chemical oxidation of phenolic compounds has a slower rate of reaction than enzymatic oxidation by PPO (Monagas and others 2005) and could take place during storage. Previous studies showed the chemical oxidation in model systems of gallic acid (Tulyathan and others 1989), caffeic acid (Fulcrand and others 1994; Cilliers and Singleton 1991), catechin

(Oszmianski and others 1996), and monomeric flavan-3-ols and dimeric procyanidins (Vivas de Gaulejac and others 2001).

An increase in brown color over time could also be due to an increase in brown substances from ascorbic acid degradation. Ascorbic acid content in all AA treated juices decreased significantly after the 24-week storage period but still was comparable, ranging from 18.0 – 27.5 mg AA/100g. During the AA degradation, L-ascorbic acid is converted to, furfural, via dehydro-ascorbic acid, which then undergo further reactions producing browning substances (Kurata and Sakurai 1967; Johnson and others 1995). Both Hunter a and b color values decreased significantly over time indicating that juices became greener and less yellow (data not shown).

When comparing the effect of container material (glass vs. PET), turbidity, phenolic content, and antioxidant capacity were not significantly affected by different packaging materials. According to HPLC analysis, flavanol, hydroxycinnamic acid, and flavonol contents as well as all individual phenolic compounds except gallic acid were not significantly affected by different packaging materials, glass and PET, and this was in agreement with phenolic content results from FC reagent assay. Glass containers retained significantly higher amounts of gallic acid than that of PET bottles.

However, container materials, glass and PET had significant effect on both brown color and Hunter color (Figure 3.4). According to Hunter a and b values, juices stored in glass containers had more green and less yellow color (lower Hunter a and b value) compared to those in PET containers. Furthermore, juices in PET bottles were significantly higher in brown color and darker than juices kept in glass bottles (Figure 3.4). This could be due to the higher oxygen transmission rate of PET compared to glass.

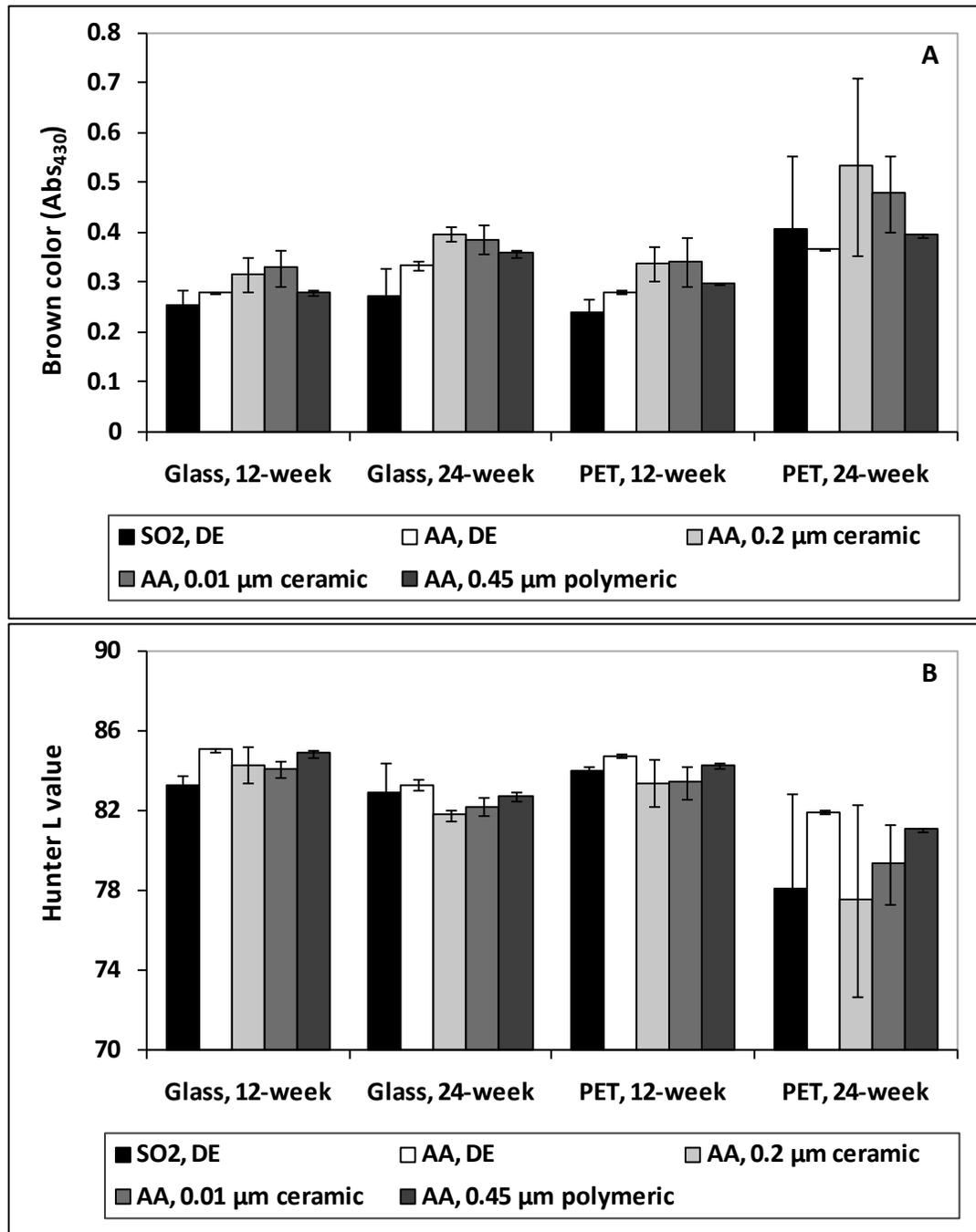


Figure 3.4. Changes of brown color (A) and Hunter L color value (B) in Niagara grape juice treated with sulfite (SO₂), ascorbic acid (AA) and filtered with diatomaceous earth (DE) or crossflow membranes over 24-week storage at 18°C

A chemical oxidation of phenolic compounds could take place during the storage. Previous studies (Guyot and others 1996; Oszmianski and others 1996; Vivas de Gaulejac and others 2001) showed that chemical oxidation of catechin lead to the same browning substances as enzymatic oxidation, although at different rates. As a result, even though the phenolic content and flavanol, hydroxycinnamic acid, and flavonol contents measured by HPLC analysis in juices from glass and PET containers were not significantly different, the higher oxygen in PET bottles as a result of higher oxygen transmission rate resulted in higher brown color and lower lightness (Hunter L value) compared to juices stored in glass bottles. Another source for the darker color in PET stored juices was the products from AA degradation as mentioned earlier. Our result showed that AA, as measured by HPLC analysis, in PET stored juices degraded at the higher rate and ranged 16.9 – 25.0 mg AA/100 g after 24-week storage as compared to 17.8 – 29.9 mg AA/100 g from glass bottle.

Compared among different treatments, turbidity of sulfited juices (21.7 NTU) was significantly higher than those of AA treated juices (5.0-9.7 NTU) after 24-weeks of storage, indicating that AA was more effective than SO₂ at levels used in this study in preventing juice turbidity. The higher turbidity in sulfited juices could be explained by the higher initial turbidity compared to AA treated juices (Table 3.1). Furthermore, turbidity was not affected by different types of filtration used in the study; as a result, turbidity of all AA treated juices was comparable.

The difference in juice qualities namely brown color, phenolic content, and antioxidant capacity among juices at the beginning of the storage was also observed after the 24-week storage in glass bottles. From all juices kept in glass bottles, sulfited

juices had the lowest brown color ($Abs_{430} = 0.273$) while both ceramic membrane filter juices had the highest ($Abs_{430} = 0.386-0.396$) (Figure 3.4A). However, there was no significant difference in brown color of all juices stored in PET bottles over the entire shelf-life study ($Abs_{430} = 0.366-0.532$). This same trend was observed in Hunter L-value in which the L-values of all juices stored in PET bottles were comparable (Figure 3.4B). The L-value of sulfited juices remained significantly lower than those of AA-treated DE filtered and polymeric membrane filtered juices after 24-week storage but was comparable to those of AA-treated ceramic membrane filtered.

Among the juices kept in glass bottles, sulfited juice was less green with the highest a-value while AA-treated DE filtered and polymeric membrane filtered juices were greener with the lowest a-value. The a-values of all AA treated CFF juices stored in glass bottles were comparable over the 24-week storage period. On the other hand, a-values of all juices stored in PET bottles were not significantly different. The b-values of all AA-treated juices kept in PET bottles were not significantly different. However, for juices stored in glass bottles, the b-values of both ceramic membrane filtered juices were significantly higher than those of AA treated DE filtered and polymeric membrane filtered. The b-values of all AA-treated juices kept in both glass and PET bottles were significantly higher than that of sulfited juices after storage, indicating that sulfited juice was less yellow than AA treated juices.

Phenolic content of sulfited juice was significantly lower than those of all AA treated juices over 24-week storage and phenolic contents of all AA treated were comparable (Figure 3.2A). The same trend was observed in antioxidant capacity (Figure 3.2B). Antioxidant capacity of sulfited juice was the lowest and that of AA-

treated DE filtered juice was the highest. The ORAC values for all AA-treated CFF juices were not significantly different throughout the shelf-life study (15.0-16.1 μM TEAC/g). The result of phenolic compounds measured from HPLC is also in agreement with phenolic content data measured by FC reagent assay. Sulfited juices had lowest flavanol, hydroxycinnamic acid, and flavonol contents throughout 24-week storage (Figure 3.3). Flavanol and hydroxycinnamic acid contents in all AA treated juices were comparable. Flavonols in CFF AA treated juices were comparable but significantly lower than DE filtered AA treated juices. According to HPLC analysis, sulfited juice was significantly lower in gallic acid, procyanidin B₂, catechin, caftaric acid, trans-coutaric acid, and kaempferol than AA treated juices and these contents in all AA treated juices were comparable. Furthermore, epicatechin, cis-coutaric acid, p-coumaric acid, quercetin contents in AA treated CFF juices were comparable and significantly higher than those of AA treated DE filtered and sulfited juices. Only procyanidin B₁ was not significantly different in all juices.

Conclusions

Ascorbic acid can be used as a sulfite substitute in Niagara grape juice processing to improve juice quality as the juice was lower in turbidity and higher in phenolic and antioxidant capacity than sulfited treated juices, but were not significantly different in preference and acceptability. Furthermore, there are less health related issues with ascorbic acid, as compared to sulfite. Filtration type, namely DE and crossflow using polymeric or ceramic membranes, did not affect juice color, turbidity, preference, and acceptability; however, DE filtration produced juices with

higher total phenols and antioxidant capacity than crossflow filters. All juice quality attributes were stable over 24 weeks except the brown color which increased significantly. Different packaging materials, glass and PET, did not affect juice quality significantly except for juice color, in which juices from PET bottles were darker than from juice stored in glass bottles.

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CHAPTER 4

EFFECTIVENESS OF ANTIMICROBIALS IN EXTENDING THE SHELF-LIFE OF ALL NATURAL COLD-FILLED NIAGARA GRAPE JUICES

ABSTRACT

This study evaluated the effectiveness of natural antimicrobials for shelf-life extension in cold-filled still and carbonated pasteurized Niagara grape juices, which have traditionally been preserved with chemical preservatives. Commercial juices were inoculated with a spoilage yeast cocktail comprised of *Dekkera*, *Kluveromyces*, *Brettanomyces*, and *Zygosaccharomyces* at 10^2 and 10^4 cfu/ml. The following agents were added to still juices: no preservative (negative control), 0.05% potassium sorbate + 0.05% sodium benzoate (positive control), 0.1% or 0.2% MicroGard™ 730, 250 ppm Velcorin™ [dimethyldicarbonate (DMDC)], 10 ppm or 20 ppm Natamax™, and 250 ppm Velcorin™ + 5 ppm or 10 ppm Natamax™. Carbonated juice was treated with negative control, positive control, and 250 ppm Velcorin™ + 10 ppm Natamax™. Microbial stability of the samples were assessed every 2 weeks over 6-months storage at 21°C, using yeast enumeration together with juice quality indexes measurements namely turbidity, pH, and °Brix. Juices were deemed spoiled when yeast counts exceeded 10^6 cfu/ml.

MicroGard™ 730 was not effective at levels tested. The most promising results were obtained with Velcorin™ and Natamax™ combination treatments in both still and carbonated juices. In these treatments, shelf-life extension on par with the positive control (153-161 days) was achieved, while also maintaining similar turbidity, pH, and

°Brix. Spoiled juices had lower pH and °Brix values and higher turbidity due to microbial activity of increased yeast growth.

Introduction

Modern consumer predilections are increasingly demanding foods of high-quality, free from preservatives, mildly processed, possessing a lengthy shelf life, palatable, and compliant with food legislation (Brul and Coote 1999). The application of preservative treatments for grape juice is particularly useful in cold-filled juice production. Whereas hot-filled juices are filled at 70-85°C during bottling for improved protection against microbes, this final heat treatment is not applied to cold-filled juices, rendering the need for preservatives much greater in the latter case (Ashurst 2005). Wild and preservative-resistant yeasts (*Brettanomyces* spp., *Schizosaccharomyces* spp., *Zygosaccharomyces* spp., etc.) are major microbial spoilage problems for cold-packed and carbonated grape juices. Growth of these spoilage yeasts often results in alcohol production and off flavors. In addition, gas production from microbial activities can cause glass bottles to explode due to the high internal pressures inside, potentially becoming a consumer hazard. Various compounds are used to extend the shelf-life of fruit products and many are used together. These antimicrobials can be classified as traditional or naturally-occurring (Davidson 2001). Traditional preservative systems that rely on sorbate and benzoate are ineffective to prevent the growth of *Zygosaccharomyces* spp. Furthermore, growing consumer concerns with chemical preservatives due to their potential toxicity and allergic reactions has also resulted in a decreased consumer acceptance.

Dimethyldicarbonate (DMDC), an approved food additive in 100% juice products (FDA 2001) added during bottling, is a potent, broad-acting antimicrobial compound that quickly degrades in water matrices. After inducing its antimicrobial protection, DMDC is hydrolyzed to carbon dioxide and trace amounts of methanol at levels which are not considered toxicologically significant. These byproducts yield no residual odors or flavors and have shown no threat of an allergic response (Morris and Striegler 2005). Due to methanol production, legal limits on the usage of DMDC have been set at 250 ppm in fruit juices (FDA 2001). The effective antimicrobial agent is the parent compound, DMDC, and therefore the primary protective effect occurs during the initial dosing (Golden and others 2005) prior to DMDC degradation. As a result, it does not have a long-term protection against recontamination or later outgrowth of surviving microorganisms. The action of DMDC is particularly active when the pH is low, and/or when the concentration of DMDC or product temperature is high (Ough and Ingraham 1961). There is a strong precedent indicating the effectiveness of DMDC in specifically inhibiting the spoilage of grape juice. Terrell and others (1993) investigated the impact of varying levels of DMDC, sulfur dioxide, and sorbic acid, separately as well as in combination, in inhibiting the growth of *Saccharomyces cerevisiae* strain Montrachet in Venus grape juice. The study investigated initial inoculum levels of 2, 200, and 20,000 colony forming units per ml (cfu/ml) stored at both 21°C and 31°C. It was observed that fermentation was inhibited for over a year when 0.8 mM DMDC (~108 ppm) was applied to the 2 cfu/ml and 200 cfu/ml treatments at 21°C and all inoculum levels at 31°C (Terrell and others 1993; Morris and others 1996). The efficacy of DMDC improved at increased temperatures,

as opposed to sulfur dioxide and sorbic acid which exhibit reduced antimicrobial activity at higher temperatures. DMDC is commercially available under the trade names Velcorin™ (Bayer Corp., Pittsburgh, PA).

Natamycin, an antimicrobial polyene macrolide produced from the controlled fermentation of dextrose by *Streptomyces natalensis*, has been used as a natural preservative in certain foods and beverages to prevent spoilage due to yeasts or molds, but not bacteria. Given its low solubility in water (40 µg/ml), natamycin is more commonly used as a surface treatment, but has nonetheless been reported as an effective preservative in both pasteurized and unpasteurized juices, most likely due to the very low minimum inhibitory concentration (MIC) required for effectiveness against most mold species (Thomas and Delves-Broughton 2001; Delves-Broughton and others 2005). It does not affect the flavor of fruit juices (Delves-Broughton and others 2005) when applied at the suggested dosage level of 2.5-10 ppm (Thomas and Delves-Broughton 2001). Thomas and Delves-Broughton (2001) observed that levels of 10-40 ppm delayed the growth of most of the yeast strains studied by over 63 days in apple, orange, and pineapple juice. Natamycin is available commercially under the trade names Natamax™ (Danisco A/S Corp., Copenhagen, Denmark) and Delvolid® (DSM Food Specialties USA, Inc., Charlotte, NC), both of which contain approximately 50% natamycin blended with lactose.

MicroGard™ 730 (Danisco A/S Corp., Copenhagen, Denmark) is inhibitory towards yeast and bacteria. It is used in the food industry and has regulatory approval in beverages as yeast inhibitor. MicroGard™ 730 is produced by the fermentation of dextrose by *Propionibacterium shermanii* or specific Lactococci to produce

antimicrobial compounds such as diacetyl, lactic acid, propionic acid, acetic acid, and other unidentified compounds in the range of 700 Daltons (Al-Zoreky and others 1991; Staszewski and Jagus 2008).

Due to the growing concerns with chemical preservatives, natural antimicrobials could be used instead of conventional preservatives to satisfy consumer demands. The effectiveness of three alternative antimicrobials, Velcorin™, MicroGard™ 730, and Natamax™, alone and in combination was tested against traditional chemical preservatives, benzoate and sorbate, in Niagara grape juices to assess a potential improvement in microbial stability and product quality.

Materials and Methods

Juice and Preservatives

Single strength commercial Niagara grape juice was purchased from a local store (Geneva, NY location, Wegmans Food Markets, Inc.; Rochester, NY) and used for all studies. Two grape juice products, still and carbonated (3 volumes of CO₂), were used as a test system for different antimicrobials. Potassium sorbate and sodium benzoate (Fisher Scientific, Pittsburgh, PA) were used together as traditional preservatives. Three natural preservatives were tested: (1) cultured dextrose in the form of MicroGard™ 730 (Danisco A/S Corp., Copenhagen, Denmark), (2) natamycin in the form of Natamax™ (Danisco A/S Corp., Copenhagen, Denmark), and (3) dimethyl dicarbonate in the form of Velcorin™ (Bayer Corp., Pittsburgh, PA).

Juice Preparation

Niagara grape juices were inoculated with a cocktail of *Zygosaccharomyces* spp., *Dekkera* sp., *Kluveromyces* sp., and *Brettanomyces* sp. (from Dr. Worobo's culture collection) at a level of either 100 or 10,000 cfu/ml, representing low and high contamination levels. The preservatives were then added and the juices were packed into 50 ml pre-sterilized polypropylene tubes and stored at 21°C for 24 weeks. Only Velcorin™ was added into pre-packed juices immediately before being capped.

Potassium sorbate and sodium benzoate, each at concentration of 0.05% (w/v), were used together as a positive control in both still and carbonated juices while the negative control contained no preservatives. In still juice, 0.1 and 0.2% (w/v) MicroGard™ 730, 250 ppm Velcorin™, and 10 and 20 ppm Natamax™ were tested. A combination of 250 ppm Velcorin™ with 5 or 10 ppm Natamax™ was tested in still juice while a combination of 250 ppm Velcorin™ with 10 ppm Natamax™ was tested in carbonated juice.

Quality Evaluation

Samples were tested biweekly for pH, soluble solids (Brix), turbidity, and microbial counts. The pH was measured with an Orion 3 Star Series benchtop model pH meter (Thermo Electron Corp., Beverly, MA) and a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) was used to measure Brix levels. Turbidity was measured with a Hach 2100P Turbidimeter (Hach Co, Loveland, CO) and reported in nephelometric turbidity units (NTU). Carbonated samples were sonicated in a Branson 2200 sonicator (Fisher Scientific, Agawam, MA) to remove the air bubbles prior to Brix and turbidity measurements. Free sulfur dioxide (SO₂) in starting

juice matrix was measured immediately upon bottle opening using FIAstar™ 5000 (FOSS Analytical A/S, Eden Prairie, MN) with method from FIAstar™ 5000 Application Note 5270 (FOSS 2008).

Total yeast counts were enumerated in duplicate via serial dilution on potato dextrose agar (PDA) acidified to pH 3.5 with 10% tartaric acid. All plates were incubated at 30°C for 72 hours prior enumeration. An upper limit of 10⁶ cfu/ml was used as an indicator for spoilage.

Means of all duplicate measurements were reported for each juice at each storage time.

Results and Discussion

Quality Evaluation

The Niagara grape juice used in this study was found to have 7.80 ± 0.00 ppm of free SO₂. This is most likely attributed to the addition of sulfites to the Niagara grapes upon mechanical harvesting to inhibit enzymatic browning. Initial pH, Brix, and turbidity values of the juices, demarcated by individual treatment and yeast inoculum level, are shown in Table 4.1 and Table 4.2. Spoiled juices resulted in a decrease in pH (pH 3.07-3.12) and Brix (6.64-13.76 °Brix) but an increase in turbidity (15-890 NTU). This was due to the metabolic growth activities of the increasing colony forming units of yeast.

Table 4.1. Quality indices of Niagara juices treated with individual antimicrobials at day 0

Juice	Treatment	pH	Brix	Turbidity (NTU)
Fresh Niagara		3.19	15.05	0.88
Niagara with low yeast inoculum	Control	3.19	15.04	0.85
	0.05% sorbate/benzoate	3.34	15.17	0.93
	250 ppm Velcorin™	3.20	15.06	0.80
	0.1% MicroGard™ 730	3.25	15.14	71.10
	0.2% MicroGard™ 730	3.31	15.21	173.00
	10 ppm Natamax™	3.17	15.07	0.99
	20 ppm Natamax™	3.16	15.07	0.94
Niagara with high yeast inoculum	Control	3.19	15.07	1.30
	0.05% sorbate/benzoate	3.35	15.17	1.10
	250 ppm Velcorin™	3.18	15.06	1.00
	0.1% MicroGard™ 730	3.26	15.12	71.10
	0.2% MicroGard™ 730	3.32	15.20	174.00
	10 ppm Natamax™	3.19	15.05	1.37
	20 ppm Natamax™	3.20	15.07	1.41

Table 4.2. Quality indices of all Niagara juices from antimicrobial combination study and carbonation study at day 0

Juice	Treatment	pH	Brix	Turbidity (NTU)
Niagara with low yeast inoculum	Control	3.11	15.13	1.31
	0.05% sorbate/benzoate	3.28	15.24	1.52
	250 ppm Velcorin™ + 10 ppm Natamax™	3.12	15.13	1.15
	250 ppm Velcorin™ + 5 ppm Natamax™	3.13	15.31	1.32
Niagara with high yeast inoculum	Control	3.09	15.15	0.91
	0.05% sorbate/benzoate	3.24	15.23	1.01
	250 ppm Velcorin™ + 10 ppm Natamax™	3.11	15.12	1.04
	250 ppm Velcorin™ + 5 ppm Natamax™	3.11	15.15	1.04
Carbonated Niagara with high yeast inoculum	Control	3.13	15.21	0.95
	0.05% sorbate/benzoate	3.29	15.37	1.03
	250 ppm Velcorin™	3.15	15.35	1.05

Shelf Life

Upon addition to the juice matrix, MicroGard™ 730 significantly increased the initial juice turbidity compared to the control and other test matrices (Table 4.1), indicating that this treatment might not be suitable for clear beverages. However, perhaps as a result of its poor solubility, as outlined in Figure 4.1, MicroGard™ 730 supplement exhibited the poorest capacity for juice preservation as the addition of up to 0.2% (w/v) MicroGard™ 730 could not effectively extend shelf-life beyond that of the negative control in Niagara grape juices.

When examining the juice matrix, the use of Velcorin™ alone or in combination with either concentration of Natamax™ was as effective as the sorbate/benzoate treatments. Each of these treatment regimens prevented spoilage in Niagara grape for up to six months at 21°C, both at low and high yeast inoculation levels (Figure 4.1 and Figure 4.2). The discrepancy in microbial counts toward the end of storage from Niagara juice with high yeast inoculums treated with either Velcorin™ or sorbate and benzoate treatments (Figure 4.1B) could be due to the sample to sample variation since separate bottles were used for each sampling time. The increased shelf life observed for Niagara juice treated with Velcorin™ alone or in combination with Natamax™ could be due to a synergistic effect with the residual free SO₂ originally presented in Niagara grape juice. Previous studies (Ough and others 1988; Threlfall and Morris 2002; Costa and others 2008) also reported this synergic effect of DMDC and SO₂ in grape juices and wines.

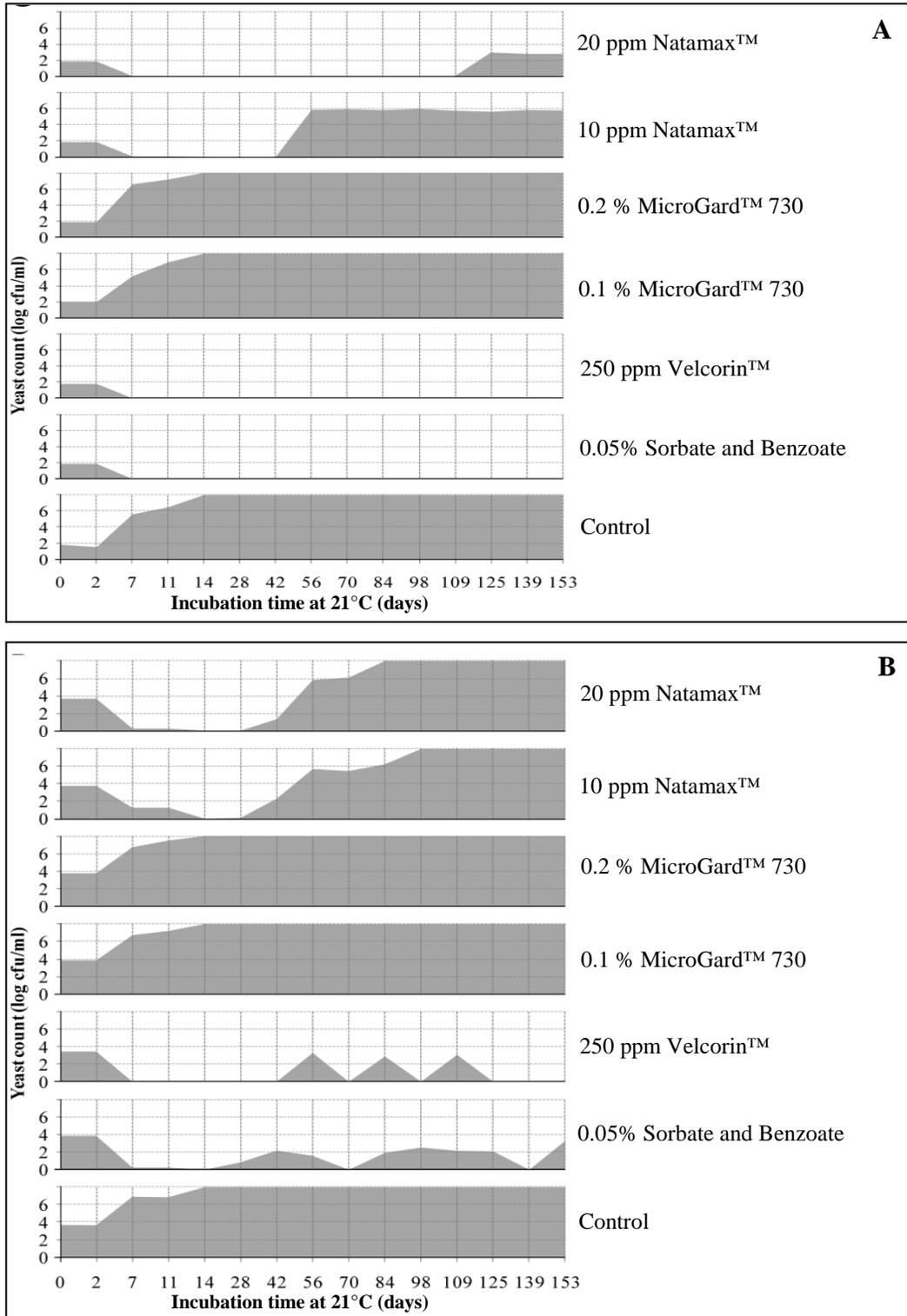


Figure 4.1. Microbial stability of cold-filled Niagara juices with low yeast inocula of 10^2 cfu/ml (A) and high yeast inocula of 10^4 cfu/ml (B) treated with different antimicrobials

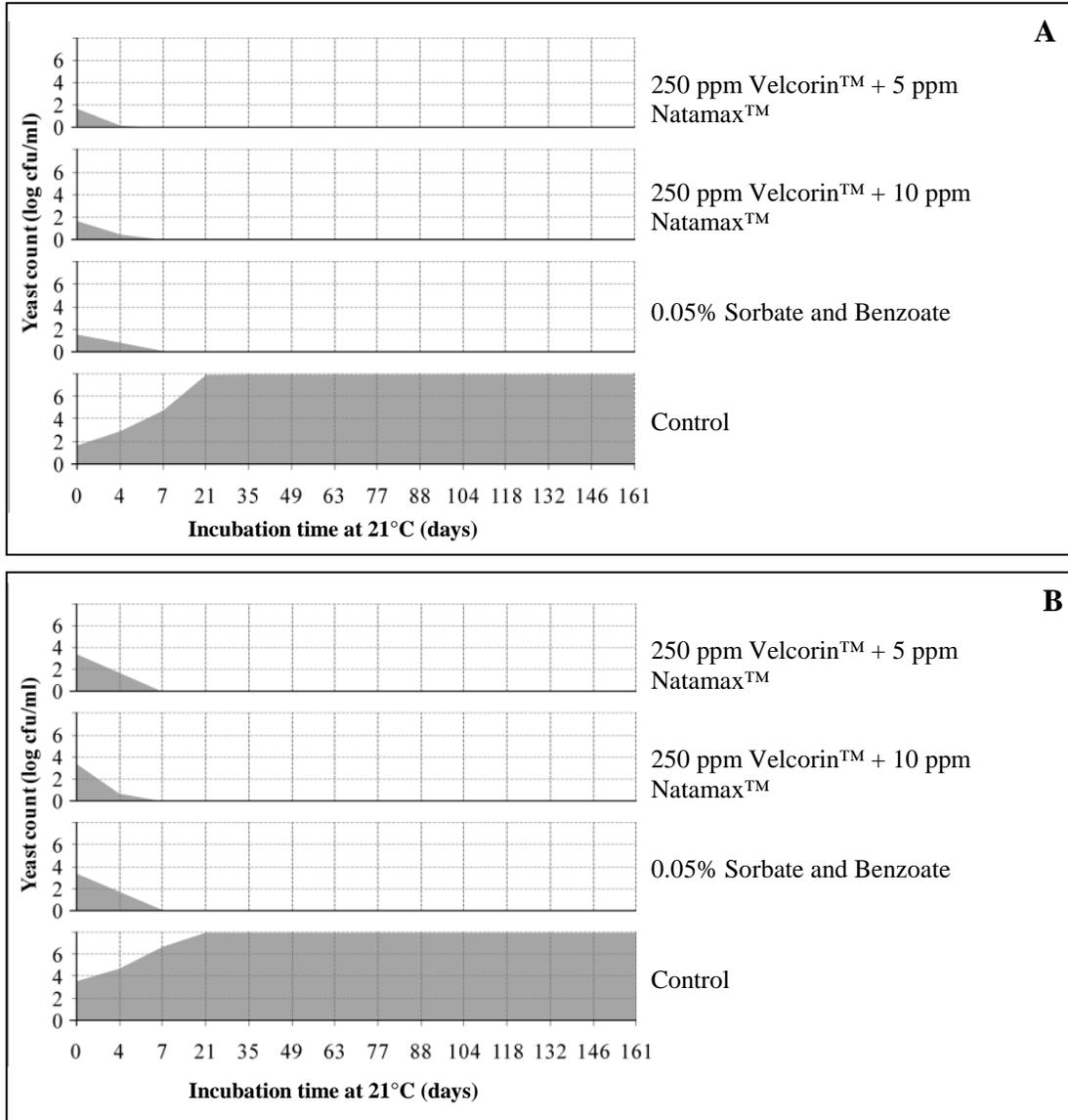


Figure 4.2. Microbial stability of cold-filled Niagara juices with low yeast inocula of 10^2 cfu/ml (A) and high yeast inocula of 10^4 cfu/ml (B) treated with different antimicrobial combinations

However, this hypothesis would be contradictory to the observations of Terrell and others (1993) in which the combination treatment of DMDC and SO₂ for Venus grape juice at a 21°C incubation was found to be less effective for delaying fermentation as compared to DMDC alone.

Low level of Natamax™ alone (10 ppm) could extend the shelf-life at 21°C for up to 56 days at low yeast counts while high level of Natamax™ (20 ppm) was as effective as sorbate/benzoate, suggesting that 10 ppm Natamax™ was not sufficient to prevent yeast growth with low yeast inoculum. The yeast counts of Natamax™ treated Niagara juice with either low or high yeast inoculum was not detectable at early storage before it started to increase. This suggested that the inoculated yeast were initially inhibited by Natamax™ but recovered and resumed growth during storage when its antimicrobial effect diminished or the yeast became resistant to the Natamax™. When challenged Natamax™ treated Niagara juice with the high yeast inoculation, their shelf-life extension was reduced approximately 2-3 fold, suggesting that Natamax™ at the concentration tested might not be sufficient to prevent yeast growth at high yeast levels. The results observed for Niagara grape juice treated with Natamax™ are comparable to previous studies with pineapple juice preservation (Thomas and Delves-Broughton 2001) that demonstrated an addition of 10 and 20 ppm of natamycin delayed the growth of multiple strains of *Saccharomyces cerevisiae* (35 to over 72 days) and *Zygosaccharomyces bailii* CRA229 (over 72 days).

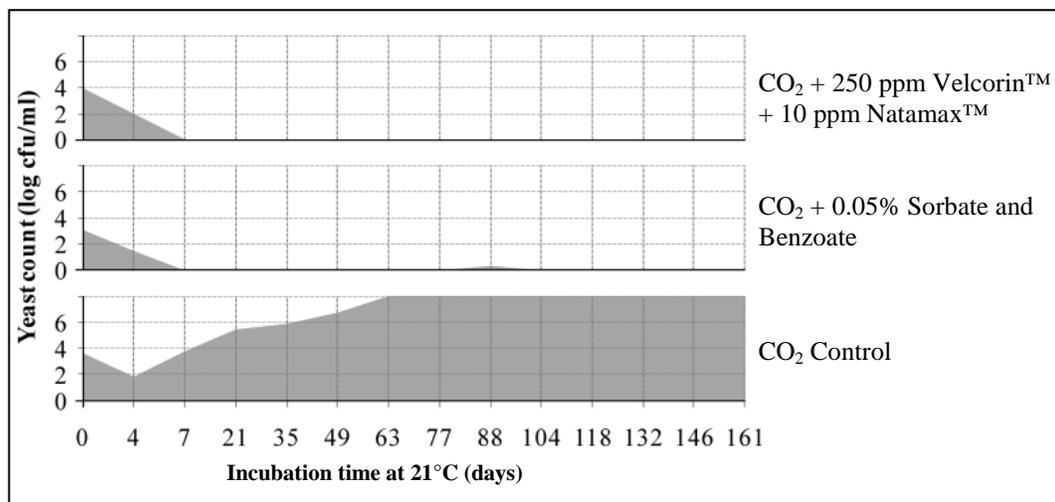


Figure 4.3. Microbial stability of carbonated Niagara juices with high yeast inocula of 10^4 cfu/ml treated with different antimicrobials

Dissolved CO₂ reduced the pH of carbonated juices (Table 4.2) and exerted an additional protective effect thereby extending the shelf life even further than what was observed in the still juices. Shelf life extension for the Velcorin™ + Natamax™ treatment in carbonated Niagara juices was identical to positive control and greater than in similar treatments in still juice matrices (Figure 4.3).

Conclusions

The most promising result of this study is the identification of a universally efficacious protocol designed to increase the shelf life of both still and carbonated cold-filled Niagara grape juice matrices to times comparable to that of the traditional sorbate and benzoate additives (>161 days). To that end, we report that the addition of 250 ppm Velcorin™ alone or in combination with 5 ppm or 10 ppm Natamax™ effectively results in a stable Niagara grape juice product using less deleterious additives or natural antimicrobials. The use of this preservative system warrants further investigation, but has the potential to enhance the shelf life of these juices while appealing to consumers interested in all-natural beverages. Further studies need to address the confounding factor of residual sulfites in white grape juices and investigate the effects of the natural preservatives alone. Additionally, it will be of tremendous importance to also perform a tandem sensory evaluation of these juices to assure organoleptic acceptability.

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CHAPTER 5

EVALUATION OF CROSSFLOW MICROFILTRATION OF NEW YORK STATE WINES AND TANK BOTTOMS AS ALTERNATIVE TO DEPTH FILTRATION

ABSTRACT

Standard diatomaceous earth (DE) depth filtration, traditionally used in wine processing, has several drawbacks and operational challenges. Cross-flow microfilters (CFMF) represent a more sustainable, easier to operate system shown to match or improve wine quality in selected studies. We evaluated ceramic and polymeric CFMF against traditional DE filtration to assess improvements in the overall quality of 4 NY red and white wines—Cabernet Franc, Riesling, Concord, and Aurora. Aurora and Cabernet Franc wines from tank bottoms (9-16% spin solids, compared to 4-6% initially) were also tested. Polymeric (0.45- μm pore size) and ceramic membranes (0.45-, 0.2- μm) were evaluated. Wines were filtered at 25°C and optimized pressure. Samples were analyzed for color (white-420 nm, red-520 nm), turbidity, phenolics, monomeric anthocyanins, polymeric color, and antioxidant capacity (ORAC assay). Microbiological analysis and sensory evaluations were also conducted.

Turbidity of CFF wines was 0.3-1.2 NTU, significantly lower than DE filtered wines (0.7-9.8). Phenolic and anthocyanin content and ORAC values of wines were not affected by filtration type and all filtrations effectively eliminated spoilage microorganisms. Ceramic membrane-filtered wines were not perceived significantly different than DE filtered wines. Polymeric membrane-filtered red wines were perceived differently and received the lowest preference ratings. Sensory evaluation showed no difference between DE and ceramic membrane-filtered tank bottoms.

Filtering tank bottoms required 2-7 times more DE than regular wine, creating significant loss of wine and increased processing cost and waste disposal; while ceramic CFMF conditions remained constant.

Using ceramic CFMF instead of DE filtration produced wine with equal or better quality and represented more sustainable operations.

Introduction

Color, clarity, and health benefits from polyphenols in wine are important for consumer acceptability. Wine components can react over time producing haze that may settle out and precipitate; thus, filtration is necessary to produce commercially clear products. Standard diatomaceous earth (DE) filtration, traditionally used in wine and juice processing for final filtration (polish filtration), is highly efficient and very effective (McLellan and Padilla-Zakour 2005). However, there are several drawbacks due to safety restrictions in handling the material, operation challenges, and cost of storage, delivery, and waste disposal (Starbard 2008). Product quality relies on the grade and amount of DE, causing its quality to be very variable. In addition, the system can plug up easily and need to be disassembled for cleaning, resulting in significant losses of valuable wines and increased operational costs (Starbard 2008). Starbard (2008) reported that roughly 567 g of wine is absorbed into every 454 g of DE used for filtration.

Cross-flow filtration (CFF), a pressure-driven membrane technology, specifically microfiltration (MF) and ultrafiltration (UF) are commonly used in the beverage industry, primarily for biological stabilization and sterilization to increase

product yield and/or quality (Zydney 1996). The advantages of CFF over traditional clarification methods include reduced processing time and enzyme usage, increased yield, elimination of filter aid and filter presses with a reusable single unit operation that is easy to clean and operate (Zydney 1996; Cheryan 1998, 1998). They also act as microbiological filters to reduce or eliminate spoilage microorganisms, depending on the pore size of the membrane, and can be used as cold-sterilizing microfilters; therefore, eliminating the need for heat pasteurization (Zeman 1996; Zydney 1996). It also minimizes volatilization or destruction of flavor-producing compounds during heat treatment and thus, improving the quality of the final product (Cheryan 1998). Zydney (1996) compared the economics of UF and conventional filtration for juice processing and reported that overall cost savings with UF process are estimated to be comparable to the capital investment required for installation of new UF system. In addition, the increased yield for UF process (approx. 5%) can contribute to additional annual revenue. DE and labor costs are major contributors to the overall cost of conventional processes.

With regard to MF, the wine industry has been one of the fastest growing beverage markets in recent years (Starbard 2008). The 0.45 μm membrane is typically used in the US and Australia, while European wineries do not filter below either 0.65 or 1.0 μm , particularly if treating red wines (Starbard 2008). Wine stabilization has been achieved using MF systems to remove spoilage wild yeast and bacteria causing acid fermentation of alcohol (Zydney 1996). MF has almost completely replaced the use of heat pasteurization and adsorptive depth filtration in white wines produced with extended shelf-life (Zydney 1996). The MF membranes can also be used as sterile

filters, as a final step before bottling (Cheryan 1998; Starbard 2008). UF membranes have been used in wine production to remove off-pigments or precipitated potassium tartrate; thus reducing tartrate crystallization during storage (Kosikowski 1986). After fermentation and before storage, UF system can be used to improve the stability of finished wine by removing large polyphenolic and haze-causing proteins; thus, reducing browning, oxidation, and haze formation while minimizing flavor loss (Kosikowski 1986; Cheryan 1998). Overall yields in UF process are typically 5-8% higher than in conventional processes (Cheryan 1998).

Membranes can be categorized by their materials: polymeric and inorganic. Polymeric UF membranes, with pore sizes ranging from 500 to 750 kDa MWCO, are available in the market, while inorganic or ceramic membranes are more common in MF system with pore sizes of 0.1-0.6 μm . Ceramic membranes are extremely versatile and offer advantages such as resistance to abrasion and chemical tolerance. Ceramic membranes can be sterilized and operated at high temperatures and pressures, are autoclavable, and have backflushing capability, and operate over a wide pH range with longer life than polymeric membranes (Padilla-Zakour and McLellan 1993; Cheryan 1998; Starbard 2008). They are better for maintaining flavor and color, as they are chemically inert. Ceramic membranes are reusable and have virtually an unlimited life, representing a more sustainable system. The main disadvantage of the CFF is the high initial investment in the unit, leading to the higher cost per membrane area compared to polymeric membrane (Cheryan 1998; Starbard 2008). Ceramic MF units are being used in the wine industry and even though its capital costs (\$0.088/hL of wine) are double that of DE filtration system (\$0.042/hL), the savings in filtering

materials alone (\$0.076 vs. \$0.263/hL) justify the ceramic systems (Short 1995). Due to improved technologies, the price of ceramic membranes has become more competitive compared to those of polymeric membranes. The price of 0.2 and 0.45 μm ceramic membranes with 0.13 to 2.5 m^2 filtering area adequate for small-scale filtration systems of 12 to 700 liters per hour, is approximately 1.7 to 2.6 times the price of hollow fiber polymeric membranes with the same pore size and filtering area (HilCo Division, Hilliard Corp., Elmira, NY; GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Even though the cost of ceramic membranes is higher their longer life compensates for the larger initial investment.

Ceramic membranes with pore sizes ranging from 0.1-1.0 μm can be used to recover wine from tank bottoms or fermentation lees (Starbard 2008). Cross-flow filtration of tank bottoms, which represented 2-3% of the wine volume from a winery, with a Flavy Leestar from Bucher Vaslin (Zurich, Switzerland) was reported to yield 75% with a return on investment from 2-3 years and reduce operating costs compared to the traditional depth filtration (Busher Vaslin Group 2011). According to Vinvicta Products Pty Ltd (2011), currently filtration of tank bottoms is being conducted using a vacuum rotating filter or a press filter in which heavy wine losses of up to 40% are generated and final turbidity of filtered tank bottoms ranged from 25-45 NTU; therefore, a second fining and a subsequent filtration were needed.

This project aimed to assess the feasibility of replacing wine and tank bottoms DE filtration with CFF. Four wines representing New York State industry; Cabernet Franc, Riesling, Concord, and Aurora, were selected. We evaluated the efficiency of

ceramic and polymeric CFF in producing wines with improved quality and decreasing wine filtration losses and waste.

Materials and Methods

Materials

Four wines made from red and white wine grapes (*Vitis vinifera*)—Cabernet Franc and Riesling, red native American grapes (*Vitis labrusca*)—Concord, and white hybrid grapes (*Vitis labrusca* x *Vitis vinifera*)—Aurora, were selected. Unfiltered cold stabilized wines were purchased from local wineries (Finger Lakes region, NY) in 2010 and stored at 2°C prior to filtration.

Wine Filtration

All wines at 38 L/batch were filtered with DE filter and 3 types of CFF—0.45 µm hollow fiber polysulfone polymeric membrane with 0.12 m² surface area and 1.0 mm fiber inner diameter (Model CFP-4-E-5A, Membrane Separations Group, A/G Technology Corporation, Needham, MA), and 0.45 µm and 0.2 µm tubular ceramic membranes with 0.13 m² surface area and 2.0 mm x 2.0 mm square opening (Ceramic membrane cross-flow liquid filtration system, HilCo Division, Hilliard Corp., Elmira, NY). For DE filtration, Shriver Plate and Frame Filter size 7 (T. Shriver & Co., Inc., Harrison, NJ) together with 0.3 % Diatomite Grades FP 4, having median particle diameter of 15.0 µm (Eagle-Picher Minerals, Inc., Reno, NV), were used. This selected DE was typically used in the wine industry. Aurora and Cabernet Franc wines from tank bottoms with high solids were also studied using DE filter and 0.2 µm ceramic membranes. All wines were filtered at 25°C and optimized pressure,

circulated through membrane for 10 min before being collected, bottled in glass bottles with crown cap, and kept at 2°C until ready for analysis.

Membranes were thoroughly cleaned between each batch of wine and the water flow rate after cleaning was in the range of 85-100% of the original flow rate. Following the cleaning procedure, the membranes were flushed twice with distilled water each for 5 min, 0.5 N NaOH (pH 11 for polymeric and pH 12 for ceramic membrane) at 50°C for an hour, sodium hypochlorite (NaOCl) solution (200 ppm free chlorine) for an hour, and distilled water for half an hour. The additional step of flushing with nitric acid solution (pH 2) after flushing with chlorine solution was used for ceramic membranes.

Microbial Analysis

Microbiological analyses including total bacterial counts, spoilage bacteria (lactic acid producing bacteria and *Acetobacter* spp.), yeast and mold, and wild yeast (non *Saccharomyces*) were also conducted in filtered wines to determine the effectiveness of each filter for removing microorganisms. Samples were properly diluted and plated into Petri plate before selective tempered agar media was poured into plates. The mixture was thoroughly mixed and incubated at 30°C for 4 days prior to enumeration.

Different media were used for different microbial analysis- standard methods agar (Criterion™, Hardy Diagnostic, Santa Maris, CA) for total bacterial count, MRS agar (Criterion™, Hardy Diagnostic, Santa Maris, CA) for lactic acid producing bacteria, calcium carbonate with ethanol for *Acetobacter* spp., acidified potato dextrose agar (Criterion™, Hardy Diagnostic, Santa Maris, CA) to pH 3.5 with tartaric

acid for yeast and mold count, and lysine agar (Sigma-Aldrich, Inc., St. Louis, MO) for non-*Saccharomyces*. The media for *Acetobacter* spp. count was prepared by autoclaving the mixture of 0.1% yeast extract (Difco™, Becton, Dickinson, and Company, Sparks, MD), 2% calcium carbonate (Fisher Scientific, Agawam, MA), and 2% agar (Difco™, Becton, Dickinson, and Company, Sparks, MD) before adding 3% ethanol to the final mixture.

Physical and Chemical Evaluation

Unfiltered wines were analysed for reducing sugar using Clinitest® (Bayer Corp. Elkhart, IN), and ethanol content using gas chromatography with flame ionization detector (GC-FID) method (Zoecklein and others 1995). Percent settled solids in unfiltered wines were determined using the spin solids method (Padilla-Zakour and McLellan 1993). All wines were analyzed for pH, titratable acidity (TA, as %w/w tartaric acid), Brix, free and total SO₂, turbidity, color, total phenolics, and antioxidant capacity.

pH was measured with a pH meter model Orion 3 Star Series pH Benchtop (Thermo Electron Corp., Beverly, MA) and a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) was used to measure Brix levels. Free and total SO₂ were measured following the method from FIAstar™ 5000 Application Note 5270 (FOSS 2008) using FIAstar™ 5000 (FOSS Analytical A/S, Eden Prairie, MN) and express in mg/L. Turbidity was measured with a Hach 2100P turbidimeter (Hach Co, Loveland, CO) and reported in nephelometric turbidity units (NTU). The Hunter L, a, and b color components were measured in 2 cm glass cuvettes with a HunterLab Ultra Scan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) with transmittance

mode (TTRAN mode). Total phenolic content was determined using the protocol for Folin-Ciocalteu (FC) reagent in colorimetric analysis described by Singleton and Rossi (1965) and expressed as mg gallic acid equivalent (GAE)/100 g. Antioxidant capacity was determined using an oxygen radical absorbance capacity (ORAC) assay (Huang and others 2002; Held 2005) and the ORAC value was expressed as μM Trolox Equivalent Antioxidant Capacity (TEAC)/g.

Brown color of white wines, Aurora and Riesling, were measured as the absorbance at 420 nm with 1.0 cm cuvettes (Fisher Scientific CO, Agawam, MA), and color of red wines, Cabernet Franc and Concord, was measured at 520 nm with a 1.0 mm pathlength cuvette (SpecVette™, ALine, Inc. Rancho Dominguez, CA) using a Barnstead Turner SP830 Spectrophotometer (Barnstead International, Dubuque, IA). For red wines, anthocyanins content, polymeric color, color intensity, and hue were also measured. Total monomeric anthocyanin content was measured using a pH-differential method (Niketic-Aleksic and Hrazdina 1972; Giusti and Wrolstad 2001) and expressed as mg malvidin-3-glucoside equivalent (MGE)/100 g. Percent polymeric color was measured using the bisulfite bleaching method as described by Giusti and Wrolstad (2001). Color intensity, expressed in AU, was a sum of absorbance at 420 and 520 nm and hue was calculated using the ratio of absorbance at 420 and 520 nm (Zoecklein and others 1995).

Sensory Evaluation

Sensory evaluations of filtered wines were conducted using preference ranking test (Lawless and Heymann 1999) and R-Index analysis for multiple comparison (O'Mahony 1992; Lee and Hout 2009). The Friedman test was used to analyze

preference ranking data and mean comparison was made using the Least Significant Ranked Difference (LSRD) test at 95% confidence interval (Lawless and Heymann 1999). R-index was used to discriminate between the DE filtration and other filtration treatments. All wine samples were compared to the DE samples as a reference. Panelists were asked to taste the reference first and rank all samples in term of difference to the reference. The reference and samples could be retasted as often as desired. The frequencies for each rank are counted and the R-index was calculated by the R-index response matrix method (O'Mahony 1992, Lee and Hout 2009). R-index was compared to the critical value at 5% level of significance ($p \leq 0.05$) obtained from a significance table developed by Bi and O'Mahony (2007). All wines were randomly served at room temperature to 24 panelists.

Triangle test (Lawless and Heymann 1999) was used to assess differences in filtered wines from tank bottoms study. The number of correct judgment from triangle test was compared to the minimum numbers of correct judgments with $p \leq 0.05$ and power of the test of 95% (Lawless and Heymann 1999). Paired preference test (Lawless and Heymann 1999) was also used for tank bottoms study and the number of agreeing judgment was compared to the minimum numbers necessary to establish significance at $p \leq 0.05$.

Statistical Analysis

Two batches of wine were prepared for each processing treatment and two analytical replicates were performed for each measurement. Results were reported as mean \pm standard deviation of each processing treatment. Data were subjected to analysis of variance (ANOVA) and means were compared with Tukey-Kramer HSD at

95% confidence interval using the JMP® 7.0 statistical software package (SAS institute Inc., Cary, NC).

Results and Discussion

Wine Quality

Characteristics of unfiltered wines used in this study are shown in Table 5.1 and their values were typical for red and white wines except Aurora wines that had lower pH (Striegler and Morris 1984; Macaulay and Morris 1993; Darias-Martin and others 2003; Chang and others 2009). The pH and TA level of filtered wines were not significantly affected by filtration type and were in the same range with their corresponding unfiltered wines (data not shown). The legal limit for maximum SO₂ in the US is 350 mg/L and the levels of SO₂ in all filtered wines were within this limit, with free SO₂ ranging from 7.7-26.8 mg/L and total SO₂ ranging from 53.4-271.8 mg/L. Spin solids of tank bottoms was 1.5-3.4 times higher than those of regular wines (Table 5.1).

Microbiological analysis showed that all filtered wines were commercially stable (Table 5.2). Total bacterial counts, spoilage bacteria (lactic acid producing bacteria and *Acetobacter* spp.), yeast and mold, and wild yeast (non *Saccharomyces*) in all filtered wines were ≤ 19.5 cfu/ml, ≤ 2.0 cfu/ml, ≤ 0.5 cfu/ml, ≤ 1.0 cfu/ml, and none, respectively.

Table 5.1. Characteristics of unfiltered wines

Variety	pH	Titrateable acidity (%w/w tartaric acid)	Reducing sugar (%w/v)	Ethanol content (%w/v)	Spin solids (%v/v)
Aurora	2.68 ± 0.01	1.43 ± 0.01	0.50 ± 0.00	14.29 ± 0.97	4.6 ± 0.2
Aurora, Bottom	2.70 ± 0.01	1.46 ± 0.01	0.25 ± 0.00	13.21 ± 0.07	15.5 ± 0.6
Cabernet Franc	3.31 ± 0.01	0.82 ± 0.00	0.75 ± 0.00	12.17 ± 0.19	6.3 ± 0.1
Cabernet Franc, Bottom	3.42 ± 0.01	0.81 ± 0.01	0.75 ± 0.00	12.28 ± 0.02	9.3 ± 0.1
Concord	2.96 ± 0.01	1.18 ± 0.03	0.75 ± 0.00	12.88 ± 0.03	3.0 ± 0.1
Riesling	3.23 ± 0.01	0.97 ± 0.01	4.00 ± 0.00	11.58 ± 0.01	0.1 ± 0.0

Table 5.2. Microbial analysis of wines

Variety	Treatment	Total bacteria (cfu/ml)	Lactic acid bacteria (cfu/ml)	<i>Acetobacter</i> spp. (cfu/ml)	Yeast and mold (cfu/ml)	Non- <i>Saccharomyces</i> (cfu/ml)
Aurora	Unfiltered	6.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0
	DE	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.45 µm polymeric	0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0
	0.45 µm ceramic	6.0 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	4.0 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cabernet Franc	Unfiltered	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	DE	1.5 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.45 µm polymeric	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.45 µm ceramic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Concord	Unfiltered	5.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0
	DE	19.5 ± 24.7	1.0 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.45 µm polymeric	1.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0
	0.45 µm ceramic	16.5 ± 16.3	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	5.5 ± 0.7	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Riesling	Unfiltered	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	DE	11.5 ± 13.4	1.5 ± 2.1	0.5 ± 0.7	1.0 ± 1.4	0.0 ± 0.0
	0.45 µm polymeric	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0
	0.45 µm ceramic	3.5 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	4.0 ± 5.7	2.0 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Aurora, Tank bottom	Unfiltered	25.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	DE	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	1.0 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cabernet Franc, Tank bottom	Unfiltered	3.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	DE	1.0 ± 1.4	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.2 µm ceramic	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Turbidity and clarity is one of the key characteristics in wine quality. Turbidity may develop from unstable proteins reacting with polyphenols, forming 0.3-1.0 μm diameter particles, and particles greater than 0.5 μm may settle out and form precipitates (Van Buren 1989; Girard and Fukumoto 2000). Filtration significantly reduced turbidity in all wines (Table 5.3) and turbidity values of CFF wines (0.3-1.2 NTU) were significantly lower than DE filtered wines (0.7-9.8 NTU). Among CFF, ceramic membrane produced Aurora wines with significantly lower turbidity than ones from polymeric membranes. However, different CFF type did not significantly affect turbidity in other wines.

Another important wine quality is its color. The absorbance at 420 nm was used as a browning index in white wines since an increase in the brown coloration would be detected at 400-440 nm wavelengths (Zoecklein and others 1995). The brown color in all white wines was in agreement with values previously reported (Flores and others 1990; Panagiotakopoulou and Morris 1991; Sindou and others 2008; Kallithraka and others 2009).

Filtration reduced brown color in Aurora wines but not in Riesling wines (Table 5.4). This could be explained by their initial brown color. Aurora wines had higher initial brown color and thus more brown color substances can be removed with the filtration. DE filtered Aurora wines had significantly higher brown color compared to CFF wines. However, there was no significant difference in color among filtered Riesling wines.

Table 5.3. Turbidity of wines

Treatment	Turbidity (NTU)			
	Aurora	Cabernet Franc	Concord	Riesling
Unfiltered	35.50 ± 2.55	53.40 ± 0.07	62.20 ± 0.21	1.70 ± 0.84
DE	3.30 ± 0.32 a	1.80 ± 0.49 a	9.80 ± 1.16 a	0.70 ± 0.03 a
0.45 µm polymeric	1.20 ± 0.05 b	1.00 ± 0.83 ab	0.40 ± 0.09 b	0.45 ± 0.01 b
0.45 µm ceramic	0.60 ± 0.04 c	0.41 ± 0.07 b	0.50 ± 0.05 b	0.43 ± 0.02 b
0.2 µm ceramic	0.63 ± 0.09 c	0.34 ± 0.18 b	0.34 ± 0.01 b	0.47 ± 0.06 b

For each column, values followed by different letters are significantly different at $p \leq 0.05$

Table 5.4. Color of Aurora and Riesling wines

Treatment	Color (Abs ₄₂₀)	
	Aurora	Riesling
Unfiltered	0.117 ± 0.000	0.111 ± 0.000
DE	0.089 ± 0.002 a	0.116 ± 0.004 a
0.45 µm polymeric	0.081 ± 0.004 b	0.114 ± 0.001 a
0.45 µm ceramic	0.081 ± 0.001 b	0.114 ± 0.000 a
0.2 µm ceramic	0.082 ± 0.001 b	0.113 ± 0.001 a

For each column, values followed by different letters are significantly different at $p \leq 0.05$

For red wine, color data correlated well with color intensity. Concord wines filtered with 0.2 μm ceramic membrane had the lowest color values and color intensity (Table 5.5) which could be due to smaller pore size. Furthermore, ceramic membranes produced lower color intensity Concord wines as compared to DE and polymeric membrane and color intensity of DE and polymeric membrane filtered wines was not significantly different. Salazar and others (2007) also reported a significant reduction in the color intensity of Pinot Noir wine when filtered with 0.2 μm ceramic membrane. On the other hand, 0.2 μm ceramic and 0.45 μm polymeric membrane produced Cabernet Franc wines with lowest color and color intensity but highest hue values, while DE filtered wines had highest color and color intensity but lowest hue values. A previous study also observed a significant decrease in color intensity in Cabernet Sauvignon wine filtered with 0.65 μm polyvinylidene difluoride (PVDF) membrane (Arriagada-Carrazana and others 2005). When comparing membranes with the same pore size (0.45 μm), polymeric membrane took out more color from Cabernet Franc wines resulting in lower color and color intensity than ceramic membrane.

The monomeric anthocyanin content and polymeric color of red wines were not significantly affected by filtration type (Table 5.5). Anthocyanin content of Cabernet Franc wines was 2.5 times higher than those in Concord wines and this correlated well with high color and color intensity. This could be due to the difference in juice extraction and fermentation processing. Cabernet Franc grapes were fermented with the skins; thus, alcohol helped extract more phenolics and anthocyanins into the wines. Concord wines, on the other hand, were made from fermenting previously pressed Concord grape juices.

Table 5.5. Monomeric anthocyanin content and color of Cabernet Franc and Concord wines

Variety	Treatment	Monomeric anthocyanin content (mg MGE/100 g)	Polymeric color (%)	Color (Abs ₅₂₀)	Color intensity (Abs ₄₂₀ +Abs ₅₂₀)	Hue (Abs ₄₂₀ /Abs ₅₂₀)
Cabernet Franc	Unfiltered	252.1 ± 17.5	36.87 ± 5.61	5.18 ± 0.00	8.06 ± 0.01	0.56 ± 0.00
	DE	249.2 ± 9.0 a	32.96 ± 4.86 a	5.71 ± 0.06 a	8.68 ± 0.06 a	0.52 ± 0.01 c
	0.45 µm polymeric	250.1 ± 4.3 a	32.80 ± 2.48 a	4.23 ± 0.04 c	7.14 ± 0.06 c	0.69 ± 0.00 a
	0.45 µm ceramic	247.3 ± 18.4 a	29.98 ± 2.05 a	4.96 ± 0.04 b	7.80 ± 0.07 b	0.57 ± 0.00 b
	0.2 µm ceramic	230.7 ± 10.0 a	35.25 ± 7.06 a	4.29 ± 0.01 c	7.25 ± 0.04 c	0.69 ± 0.01 a
Concord	Unfiltered	95.1 ± 4.6	52.20 ± 8.00	1.40 ± 0.01	2.33 ± 0.04	0.66 ± 0.01
	DE	97.9 ± 1.3 a	40.59 ± 0.72 a	1.41 ± 0.03 a	2.30 ± 0.00 a	0.63 ± 0.03 a
	0.45 µm polymeric	94.4 ± 2.7 a	36.56 ± 0.43 a	1.37 ± 0.04 a	2.25 ± 0.02 a	0.65 ± 0.03 a
	0.45 µm ceramic	99.5 ± 3.6 a	40.03 ± 0.86 a	1.33 ± 0.04 a	2.15 ± 0.05 b	0.62 ± 0.01 a
	0.2 µm ceramic	95.7 ± 6.0 a	37.61 ± 2.55 a	1.24 ± 0.02 b	2.06 ± 0.04 c	0.66 ± 0.00 a
Cabernet Franc, Tank bottom	Unfiltered	247.4 ± 1.8	33.47 ± 1.49	5.48 ± 0.60	8.69 ± 0.86	0.59 ± 0.02
	DE	247.0 ± 18.7 a	32.32 ± 1.83 a	5.80 ± 0.49 a	9.15 ± 0.75 a	0.58 ± 0.01 a
	0.2 µm ceramic	230.7 ± 19.7 a	32.91 ± 3.37 a	5.74 ± 0.23 a	9.12 ± 0.20 a	0.59 ± 0.03 a

For each wine in each column, values followed by different letters are significantly different at $p \leq 0.05$

The phenol content and antioxidant capacity as ORAC values of all wines, except Cabernet Franc, were not significantly affected by filtration type (Table 5.6). Phenolic content and ORAC values in Cabernet Franc wines were 1.5-3 times higher than Concord wines, and 4-8 times higher than white wines. ORAC values of Cabernet Franc wines from 0.45 μm polymeric membrane were significantly higher than wines from other filters. Filtering Cabernet Franc wines with either 0.45 μm polymeric membrane or 0.2 μm ceramic membrane produced wines with the highest phenolic content while DE filtered wines had the lowest. Weinand and Krueck (1989) also reported that crossflow microfiltration produced wines with higher polyphenols and color intensity than wines from conventional processing. This study also observed a greater retention of high molecular weight compounds in wines filtered with ceramic membrane than from polysulfone membranes.

The R-index for multiple comparison expressed the probability of distinguishing between 2 samples, the control or DE samples and the other filtered samples. R-index = 50 indicated chance discrimination or signified no detection. The deviation of R-index score from 50% was compared to the critical value developed by Bi and O'Mahony (1995, 2007) to test the significance at $p \leq 0.05$. Ceramic membrane filtration gave a very promising result. According to Table 5.7, ceramic membrane-filtered wines, except 0.45 μm ceramic membrane filtered Concord wine, were not perceived differently to DE filtered wines. All polymeric membrane filtered wines, except Riesling, were perceived differently than DE filtered wines. Similar result was found with preference ranking test (Table 5.8). Polymeric membrane filtered wines received the lowest preference ratings, while DE and ceramic membrane filtered wines

were not preferred differently. It was worth noting that the filtration type did not significantly affect neither the R-index test nor the preference rating in Riesling wines.

Tank Bottoms Study

Aurora and Cabernet Franc wines from tank bottoms, having 9.3-15.5% spin solids as compared to 4.6-6.3 % from top of the tank (Table 5.1), were used. Turbidity of Aurora wines from ceramic membrane was significantly lower than DE filtered wines (Table 5.9). Other Aurora and Cabernet Franc wines quality attributes were not significantly affected by filtration type. This result was confirmed by triangle and pair preference tests in which both wines from DE and ceramic membrane filter were preferred equally and not perceived differently.

Filtration Efficiency

Filtration efficiency of CFF was determined using filtration flux ($L/m^2/h$) which was calculated as permeate flow rate per membrane area. Permeate flow rate was measured every 10 min during filtration. According to Figure 5.1, flux of all membranes dropped quickly after the first 20 min and remained stable after that. Flux from different membranes of the same wine was not significantly different. Riesling wine had the highest filtration flux, followed by Cabernet Franc, Concord, and Aurora, respectively. Flux of Riesling wine was not significantly different than that of Cabernet Franc, but flux of both wines were significantly higher than that of Aurora.

Table 5.6. Phenolic content and antioxidant capacity of wines

Variety	Treatment	Phenolic content (mg GAE/100 g)	ORAC (μM TEAC/g)
Aurora	Unfiltered	36.30 \pm 0.14	8.79 \pm 0.27
	DE	34.45 \pm 0.78 a	8.38 \pm 0.09 a
	0.45 μ m polymeric	35.25 \pm 0.21 a	8.45 \pm 0.16 a
	0.45 μ m ceramic	34.00 \pm 1.98 a	8.90 \pm 0.64 a
	0.2 μ m ceramic	34.15 \pm 0.78 a	8.52 \pm 0.26 a
Cabernet Franc	Unfiltered	185.0 \pm 0.2	32.91 \pm 4.55
	DE	181.5 \pm 3.3 c	33.58 \pm 1.70 b
	0.45 μ m polymeric	247.3 \pm 0.4 a	38.41 \pm 0.52 a
	0.45 μ m ceramic	205.6 \pm 5.5 b	33.30 \pm 1.24 b
	0.2 μ m ceramic	256.4 \pm 4.0 a	35.03 \pm 0.95 b
Concord	Unfiltered	87.15 \pm 1.77	20.29 \pm 1.62
	DE	86.55 \pm 0.64 a	19.09 \pm 1.24 a
	0.45 μ m polymeric	88.20 \pm 2.55 a	18.95 \pm 0.36 a
	0.45 μ m ceramic	87.40 \pm 2.83 a	19.28 \pm 0.01 a
	0.2 μ m ceramic	88.05 \pm 1.20 a	19.24 \pm 1.66 a
Riesling	Unfiltered	35.20 \pm 0.42	8.31 \pm 0.81
	DE	34.80 \pm 1.41 a	7.80 \pm 0.08 a
	0.45 μ m polymeric	34.75 \pm 0.50 a	8.02 \pm 0.15 a
	0.45 μ m ceramic	34.10 \pm 0.85 a	8.09 \pm 0.06 a
	0.2 μ m ceramic	33.80 \pm 0.14 a	8.02 \pm 0.42 a

For each wine in each column, values followed by different letters are significantly different at $p \leq 0.05$

Table 5.7. Comparison between control, DE filtered, and other filtered wines using the R-index analysis

Treatment	R-index value			
	Aurora	Cabernet Franc	Concord	Riesling
DE	50.00	50.00	50.00	50.00
0.45 µm polymeric	69.44*	74.74*	70.05*	53.82
0.45 µm ceramic	47.92	51.82	33.07*	57.55
0.2 µm ceramic	56.25	50.00	42.19	56.42

* Indicate the significant value compared to critical value = 15.49 (Bi and O'Mahony 2007) at $p < 0.05$, two-tailed, $N = 24$

Table 5.8. Preference ranking test results of filtered wines

Treatment	Preference ranking score			
	Aurora	Cabernet Franc	Concord	Riesling
DE	2.17 ± 0.96 b	2.25 ± 1.11 b	2.54 ± 0.88 ab	2.42 ± 1.25 a
0.45 µm polymeric	3.00 ± 1.10 a	3.21 ± 1.06 a	3.25 ± 1.07 a	2.75 ± 1.19 a
0.45 µm ceramic	2.50 ± 1.14 ab	2.21 ± 0.98 b	2.13 ± 1.15 b	2.50 ± 1.02a
0.2 µm ceramic	2.33 ± 1.17 ab	2.33 ± 1.09 b	2.08 ± 1.02 b	2.33 ± 1.05 a

For each column, values followed by different letters are significantly different at $p \leq 0.05$

Table 5.9. Quality attributes of tank bottoms wines

Variety	Treatment	Phenolic content (mg GAE/100g)	ORAC (μM TEAC/g)	Turbidity (NTU)
Aurora	Unfiltered	38.75 \pm 0.92	9.59 \pm 0.37	32262 \pm 484
	DE	44.35 \pm 0.07 a	10.69 \pm 0.32 a	1.70 \pm 0.03 a
	0.2 μ m ceramic	47.00 \pm 3.11 a	11.03 \pm 0.11 a	0.70 \pm 0.05 b
Cabernet Franc	Unfiltered	212.0 \pm 0.7	33.78 \pm 0.64	810 \pm 4
	DE	215.3 \pm 2.7 a	33.88 \pm 0.77 a	2.70 \pm 1.80 a
	0.2 μ m ceramic	222.7 \pm 13.6 a	33.47 \pm 0.67 a	0.96 \pm 0.42 a

For each wine in each column, values followed by different letters are significantly different at $p \leq 0.05$

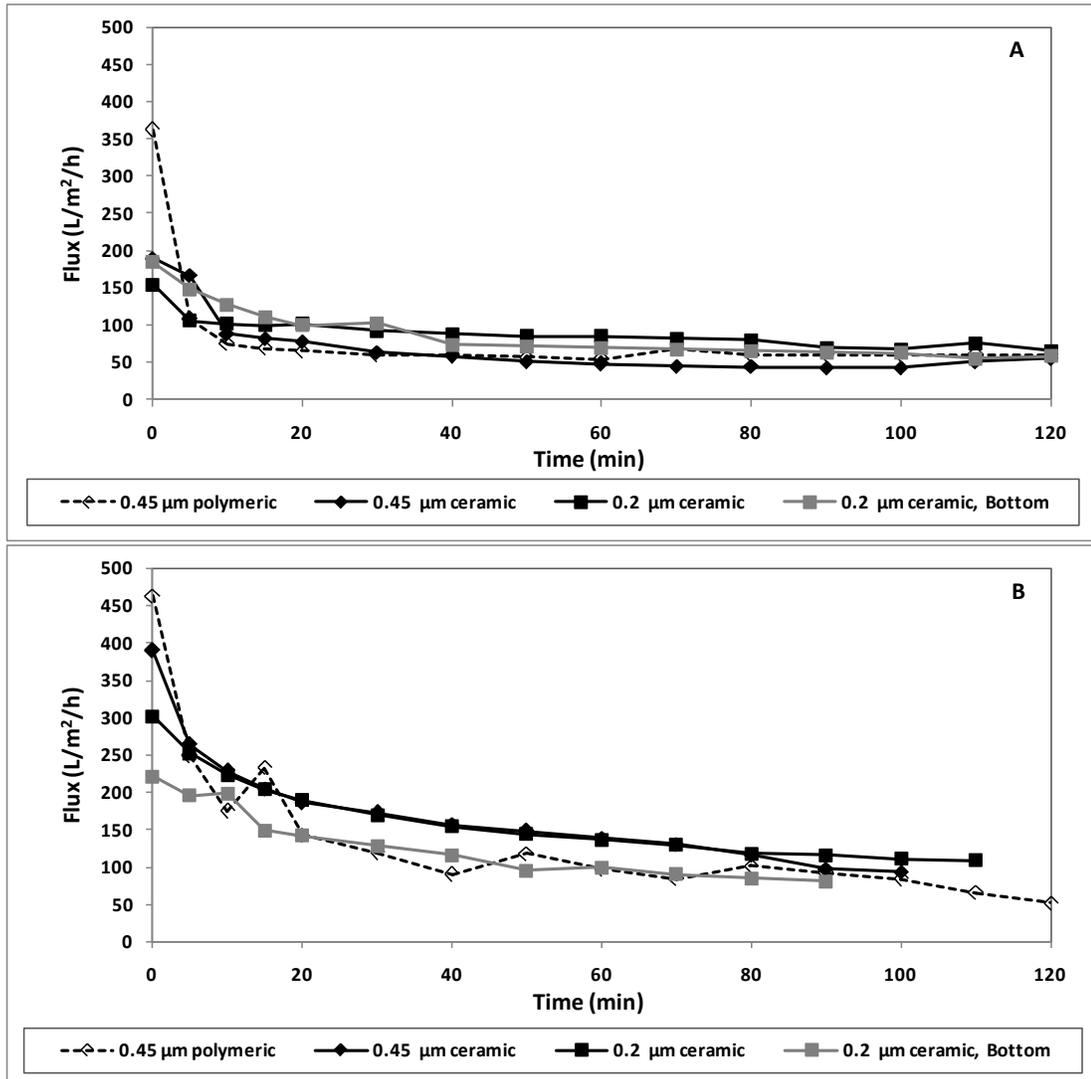


Figure 5.1. Cross-flow filtration flux for Aurora (A) and Cabernet Franc (B) wines

Even though tank bottoms contained 1.5-3.7 times higher spin solids than regular wines, flux of CFF tank bottoms was not significantly different than those of regular wine while other operating conditions remained the same. In contrast, filtering tank bottoms with DE filtration required 2-7 times more DE material, adding more operational cost as well as creating more waste disposal. Furthermore, significant losses of tank bottoms with DE (up to 22%) was observed while the loss of tank bottoms from ceramic membrane filtration was the same as those from regular wines.

Conclusions

Ceramic CFMF is a viable alternative to traditional DE filtration to produce wine with equal or better quality and represented more sustainable operations especially for filtration of difficult material such as tank bottoms. CFMF improved wine quality in terms of turbidity and white wine color than DE filter. Furthermore, phenolic and anthocyanin content and antioxidant capacity were not significantly affected by filtration type. Based on the sensory evaluation results, ceramic membrane filtered wines were not perceived differently to DE filtered wines while polymeric membrane filtered wines were perceived differently and received the lowest preference ratings. For high solids wines, such as tank bottoms, cross-flow filtration with ceramic membranes represents a viable and effective option.

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CHAPTER 6

ANTHOCYANIN LOSS DURING COLD STABILIZATION IN MODEL JUICES

ABSTRACT

Anthocyanins losses with potassium bitartrate (KHT) crystal during cold stabilization were studied in juice models containing 250 ppm anthocyanins. Model juice solutions with different combinations of tartaric acid concentration (0.02 M and 0.04 M) and potassium ion concentration (0.02 M and 0.04 M) at 5 different pH values (2.35, 2.70, 2.95, 3.20, and 3.40) were tested. The amount of KHT crystal precipitated during cold stabilization increased as the concentration of tartaric acid and potassium increased. Anthocyanins impeded bitartrate crystal growth, resulting in the lower amount of bitartrate crystal. Anthocyanins in bitartrate crystal were identified and quantified using HPLC analysis. The amount of anthocyanins per crystal weight reduced as the concentration of potassium ion in a model solution increased, indicating that both compounds were competing for coprecipitation. The critical factor for anthocyanin coprecipitation was mainly due to the pH which governed the charge of bitartrate crystal. The loss of anthocyanin with bitartrate crystal was minimized at lower pH ($\text{pH} \leq 2.95$) which was likely due to neutralization of the negatively charged KHT crystal surface. Delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside were identified and the ratio of individual anthocyanins in model solutions and bitartrate crystals were significantly different. The enrichment of delphinidin-3-glucoside and cyanidin-3-glucoside and the depletion delphinidin-3-rutinoside and cyaniding-3-rutinoside in bitartrate crystals were

observed, indicating that the preferential loss depended more on sugar molecule attached to aglycone rather than the aglycone itself.

Introduction

The color of red and purple grapes is due primarily to anthocyanin pigments, and is critical to consumer acceptance of grape-derived products like juices and wines (Morris and Striegler 2005). Additionally, the anthocyanins along with other polyphenols and their metabolites have been implicated as important phytonutrients capable of reducing the incidence of chronic disease (Zafra-Stone and others 2007; Iriti and Faoro 2009; Pandey and Rizvi 2009). Due to their overall importance to the acceptability of fruit juices and related products, several publications have considered the impact of production practices on anthocyanin stability (Rommel and Wrolstad 1993; Skrede others 2000; Sacchi and others 2005).

Grapes are uniquely high in tartaric acid compared to other fruits, 2-14 g/kg, or 0.01M – 0.07 M (Amerine and Ough 1980), and also contain high concentrations of potassium, 0.01 – 0.06 M (Mattick and others 1972; Zoecklein and others 1999). These concentrations are at or above the solubility of potassium bitartrate (KHT) in pure water at 0°C (0.01M). Although the polyphenolic constituents of grapes will inhibit crystallization (Balakian and Berg 1968; Boulange-Petermann and others 1999), grape juices and wines will generally precipitate KHT crystals during cold storage. As a result, a cold-stabilization step is usually performed on grape juices and wines prior to bottling to avoid precipitation of KHT post-bottling (Konja and Lovric 1993). Factors affecting the kinetics and thermodynamics of KHT precipitation are

well studied (Dunsford and Boulton 1981a, 1981b; Gerbaud and others 1996). Several grape derived compounds can inhibit both nucleation and crystal growth, particularly anthocyanin pigments, proteins, and polysaccharides.

The cold stabilization step is well known to result in a loss of anthocyanin pigments as well as other polyphenolic species, e.g. hydroxycinnamic acids and flavonols, due to coprecipitation with KHT (Ingalsbe and others 1963; Correagorospé and others 1991; Vernhet and others 1999; Alongi and others 2010). For example, a 20-40% loss of total anthocyanins was reported during cold stabilization of Concord grape juice (Ingalsbe and others 1963), with similar losses reported elsewhere (Vernhet and others 1999; Alongi and others 2010). This process results in enrichment of the anthocyanins in the KHT precipitate compared to the remaining solution by about an order of magnitude (Vernhet and others 1999; Alongi and others 2010).

The mechanism for the loss of anthocyanins during cold stabilization is not well understood. Occlusion of anthocyanins within the crystal is unlikely to occur, as the proportions of coprecipitating compounds are different than their proportions in solution (Balakian and Berg 1968). Incorporation of anthocyanins into the crystal lattice does not appear to occur, either. Rather, the interaction of anthocyanins and KHT appears to be adsorptive in nature (Correagorospé and others 1991), a process which also inhibits crystal growth and increases the apparent solubility of KHT in grape products vs. pure water (Balakian and Berg 1968). The interactions between the KHT crystal face and phenolics are variously proposed to be ionic, hydrogen-bonding, or charge-transfer in nature (Rodriguezclemente and Correagorospé 1988; Celotti and others 1999). X-ray crystallography data indicates that the {010} face is populated by

the bitartrate species, and it was hypothesized that this would result in a positive surface charge on the {010} face created by excess potassium ions, and consequentially the adsorption of Lewis bases, e.g. the neutral forms of anthocyanins (Rodriguezclemente and Correagorospé 1988). In contradiction, Alongi and others (2010) observed that anthocyanin species which favored the flavylium cation form (lower pK_h value) were more likely to be lost via coprecipitation, indicating that the anthocyanins may interact directly with bitartrate at the surface.

Regardless of the mechanism, the loss of anthocyanins and other polyphenolics during bitartrate precipitation is undesirable to the wine and grape industries, but strategies to reduce these losses are largely unknown. However, a recent study by our group showed that anthocyanin coprecipitation is significantly less in juice concentrate as compared to single strength juice (Alongi and others 2010). Cold-stabilization of single-strength Concord juice prior to concentration resulted in modest losses (~20%) of anthocyanins, similar to previous reports, while concentration prior to cold-stabilization (so-called “direct to concentrate”) resulted in no significant loss of anthocyanins. Compositional analysis of KHT crystals yielded similar results – although comparable losses of KHT occurred in both systems, the precipitate from the direct to concentrate had a lower anthocyanin content (0.13% vs. 0.80% w/w). The improved anthocyanin stability achieved in concentrate did not appear to result from increased co-pigmentation. Because anthocyanin species that existed more in charged forms (higher pK_h values) were more likely to coprecipitate, it was hypothesized that the reduction in coprecipitation in concentrate could be credited to the lower pH of concentrate. The pH of concentrate (2.5) is lower than single-strength juice (pH = 3.1),

which should result in a neutralization of the surface charge of the KHT surface (Celotti and others 1999). However, because concentrate differs from juice in many other respects (greater ionic strength, lower water activity, etc), this was not conclusive.

The current study used model juices to study the effects of juice parameters (pH, K⁺ concentration, tartaric acid concentration) on coprecipitation of anthocyanins with KHT. The primary goal was to determine if pH could explain the minimal coprecipitation observed during cold stabilization of concentrate. A secondary goal was to determine if the selectivity of co-precipitation was tunable, that is, whether the relative proportion of anthocyanins in the precipitate could be altered by altering juice parameters.

Materials and Methods

Chemicals: Black currant powder containing 20% anthocyanin as cyanidin-3-glucoside equivalents was used as an anthocyanin source (Artemis International Inc Fort Wayne, IN). Malvidin-3-glucose was purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Citric acid monohydrate and anhydrous sodium hydrogen phosphate were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). D-glucose, D-fructose, L-(+)-tartaric acid, potassium chloride, sodium chloride, and 0.01 M hydrochloric acid were purchased from Thermo Fisher Scientific Inc. (Fair Lawn, NJ). Water from a Nanopure water purifier (Barnstead Thermolyne, Boston, MA) was used throughout the study.

Preparation and Cold-Stabilization of Model Juice Systems: A full factorial design was used to produce model juice systems with varying pH values, K^+ concentrations, and tartaric acid concentrations. All model juices contained 80 g/kg glucose, 80 g/kg fructose, and 250 mg/L anthocyanin as cyanidin-3-glucoside equivalents (similar to red grape juice). Five pH values were used: 2.35, 2.70, 2.95, 3.20, and 3.40, and were prepared by appropriate combination of 0.1M citric acid and 0.2M sodium hydrogen phosphate buffer solutions. Two K^+ concentrations were used: 0.02M and 0.04M, added in the form of KCl. Two tartaric acid concentrations were used: 0.02M and 0.04M. The total number of model juice systems investigated was 5 pH x 2 K^+ x 2 tartaric = 20 systems. Each juice system was prepared in duplicate. Cold stabilization was performed by storing all model juices at $-3^{\circ}C$ for 7 weeks without any bitartrate crystal seeding. The pH of model juices was measured before and after cold stabilization using a pH meter model Orion 3 Star Series pH Benchtop (Thermo Electron Corp., Beverly, MA).

Characterization of anthocyanins in KHT crystals: KHT crystals were collected by filtration on a glass fiber filter (Type A/E, PALL Corp, Ann Arbor, MI), followed by a washing step with cold 95% ethanol to remove any loosely adhering material on the crystal surface. The crystals were dried to constant weight in an oven at $60^{\circ}C$ and weighed prior to anthocyanin analysis.

Fifty mg of KHT crystals were dissolved at room temperature in 3 mL of 1M NaCl acidified with HCl (0.01 M). When less than 50 mg of precipitate was formed, a proportionally reduced amount of the acidified NaCl solution was used for dissolution.

After dissolution (approximately 15 minutes) each sample was immediately filtered through a 0.2 µm regenerated cellulose membrane (Sigma-Aldrich, St. Louis, MO) in preparation for HPLC analysis.

Anthocyanins in each redissolved KHT sample were analyzed by an Agilent 1100 series HPLC system with inline degasser, autosampler and diode array detector (Agilent Technologies, Santa Clara, CA). A 250 mm x 4.6 mm Varian LiChrospher RP-18 endcapped column (particle size 5 µm, pore size 100 Å; Varian, Inc, Palo Alto, CA) was maintained at 30°C by an Eppendorf CH-30 external column heater. A 50 µl aliquot of each sample was injected on to the HPLC system. Mobile phase A consisted of a water: phosphoric acid buffer (99.5:0.5) and mobile phase B consisted of an acetonitrile: water: phosphoric acid buffer (50:49.5:0.5). Analytes of interest were resolved over a 38 min gradient elution profile starting at 0% B for 2 min, increasing to 20% B over 5 min, increasing to 36% B over 15 min, increasing to 100% B over 6 min, holding at 100% B for 2 min, followed by an 8 min return to starting conditions. The flow rate was 1 mL/min. The eluent was monitored at 520 nm. Analytes were identified based on comparison of relative retention times to those previously reported for anthocyanins in blackcurrant juice (Slimestad and Solheim 2002). Quantification of each anthocyanin was based on a malvidin-3-glucose standard curve and thus reported in units of malvidin-3-glucoside equivalents. The total anthocyanin content was calculated as the sum of all major anthocyanins identified by HPLC analysis and expressed in units of malvidin-3-glucoside equivalents. Two analytical replicates were performed on each sample.

The stability of the anthocyanins in the acidified NaCl solution preparation was evaluated by repeatedly analyzing one sample from each pH solution at 0, 24, 48, 72 hours. No significant differences were observed.

Statistical analysis: Results were reported in mean \pm standard deviation. Data were subjected to analysis of variance (ANOVA) and means were compared with Tukey-Kramer HSD at 95% confidence interval using the JMP® 8.0 statistical software package (SAS institute Inc., Cary, NC).

Results and Discussion

Formation of Potassium Bitartrate Crystals: The concentrations used in this study for potassium (0.02M, 0.04M) and tartaric acid (0.02M, 0.04M) are within the range ordinarily encountered in grape juice: 0.01 – 0.06 M for potassium (Mattick and others 1972; Zoecklein and others 1999) and 0.01 – 0.07 M for tartaric acid (Amerine and Ough 1980). The pH range, 2.35-3.4, was selected to bracket the range typically observed in single strength grape juice (pH = 3.0-3.5) as well as in 59 Brix juice concentrate (pH = 2.5).

During cold storage of our model juices, as with real juices, we observed precipitation of KHT. Previous work has shown that KHT precipitation occurred in 2 stages, the initial induction stage, in which the concentration of bitartrate nuclei increased due to chilling, and the crystallization stage where crystal growth and development occurred (Rodriguezclemente and Correagorospé 1988; Zoecklein and others 1999).

The amount of KHT precipitate recovered by filtration from the cold-stabilized model juices are reported in Table 6.1. Unsurprisingly, increasing K^+ and tartaric acid concentrations increased the amount of precipitate recovered. At each pH, the 0.04M K^+ and 0.04M tartaric acid juices had significantly more precipitate than those juices with lower K^+ or tartaric acid. For example, the amount of KHT precipitated from the 0.02M/0.02M model juices ranged from 0.22-0.37 mmoles, over an order of magnitude less than the precipitate recovered from the 0.04M/0.04M juices (range = 4.2-9.33 mmoles). pH also affected the amount of KHT precipitated. This was especially apparent for the 0.04M/0.04M solutions, where the pH 2.95-3.4 model juices precipitated 8.63-9.33 mmoles of KHT, significantly greater than the amount precipitated at pH 2.35 (4.2 mmoles) and pH 2.7 (6.98 mmoles). This likely resulted from the low concentration of the bitartrate at the low pH values, as pK_{a1} (tartaric acid) = 2.98.

Interestingly, although the concentration of bitartrate is predicted to be at a maximum at pH 3.65, the pH 3.4 model juice did not produce the largest amount of precipitate. Instead, the model juices followed the order pH 2.95 > pH 3.2 > pH 3.4 > pH 2.7 > pH 2.35. This is likely due to increasing adsorption of anthocyanins to the growing crystal faces at higher pH. This coprecipitation effect is well known to limit crystal growth and the extent of precipitation (Balakian and Berg 1968; Rodriguezclemente and Correagorospé 1988; Correagorospé and others 1991). Evidence for this phenomenon is presented in the next section.

Table 6.1. Weight of bitartrate crystal (mean \pm standard deviation) after cold stabilization and total anthocyanin content in bitartrate crystal calculated as malvidin-3-glucoside. Items in parentheses had too little crystal to permit further characterization

pH	Tartaric acid (M)	K ⁺ (M)	Crystal weight		Anthocyanin of crystal (% w/w)
			(g)	(mmoles as KHT)	
2.35	0.02	0.02	(0.041 \pm 0.003)	0.22 \pm 0.01 ij	-
		0.04	(0.033 \pm 0.008)	0.17 \pm 0.04 j	-
	0.04	0.02	(0.038 \pm 0.000)	0.20 \pm 0.00 ij	-
		0.04	0.790 \pm 0.000	4.20 \pm 0.00 d	0.01 \pm 0.00
2.70	0.02	0.02	(0.040 \pm 0.003)	0.22 \pm 0.01 ij	-
		0.04	(0.041 \pm 0.001)	0.22 \pm 0.00 ij	-
	0.04	0.02	(0.049 \pm 0.017)	0.26 \pm 0.09 hij	-
		0.04	1.314 \pm 0.013	6.98 \pm 0.07 c	0.02 \pm 0.00
2.95	0.02	0.02	(0.041 \pm 0.003)	0.22 \pm 0.02 ij	-
		0.04	0.075 \pm 0.007	0.40 \pm 0.04 ghi	0.04 \pm 0.00
	0.04	0.02	0.651 \pm 0.017	3.46 \pm 0.09 e	0.05 \pm 0.00
		0.04	1.756 \pm 0.001	9.33 \pm 0.01 a	0.02 \pm 0.00
3.20	0.02	0.02	(0.041 \pm 0.006)	0.22 \pm 0.03 ij	-
		0.04	0.085 \pm 0.011	0.45 \pm 0.06 gh	0.21 \pm 0.00
	0.04	0.02	0.366 \pm 0.009	1.95 \pm 0.05 f	0.21 \pm 0.00
		0.04	1.748 \pm 0.018	9.29 \pm 0.09 a	0.11 \pm 0.00
3.40	0.02	0.02	(0.069 \pm 0.020)	0.37 \pm 0.11 ghij	-
		0.04	(0.040 \pm 0.006)	0.21 \pm 0.03 ij	-
	0.04	0.02	0.109 \pm 0.001	0.58 \pm 0.01 g	0.39 \pm 0.00
		0.04	1.623 \pm 0.016	8.63 \pm 0.09 b	0.19 \pm 0.00

Values followed by different letters in the same column are significantly different at $p \leq 0.05$

Balakian and Berg (1968) previously observed that tartaric additions tend to result in a larger increase in the amount of precipitate formed than K^+ additions. In our own work we observed the similar effect at $pH \geq 2.95$: the 0.02M K^+ / 0.04M tartaric acid model juices had 10-fold more precipitate than the 0.04M K^+ / 0.02M tartaric acid model juices.

Effects of Juice Composition on Anthocyanin Coprecipitation: In initial work, we attempted to use commercial grape anthocyanin extracts in our model juice systems. However, we observed very little coprecipitation of anthocyanins during these preliminary experiments. A possible explanation is that the commercial anthocyanin extract had already undergone cold-stabilization, resulting in the loss of the species most prone to coprecipitation.

As an alternative, we chose to use commercially available black currant extract as our source of anthocyanins. An HPLC chromatogram of the original black currant extract is shown in Figure 6.1. Black currants contain four dominant anthocyanins (Slimestad and Solheim 2002) which account for >99% of the total anthocyanin content, which simplifies the chromatographic separation. Identifications were performed by comparison against previous reported identifications (Slimestad and Solheim 2002). Two of these anthocyanins are major anthocyanin species in grapes (cyanidin-3-glucoside and delphinidin-3-glucoside), while the other two (cyanidin-3-rutinoside and delphinidin-3-rutinoside) are not observed in grapes. However, the inclusion of the rutinosides permitted us to evaluate the effect of the sugar moiety in comparison to the effect of the aglycone, as described later in the paper.

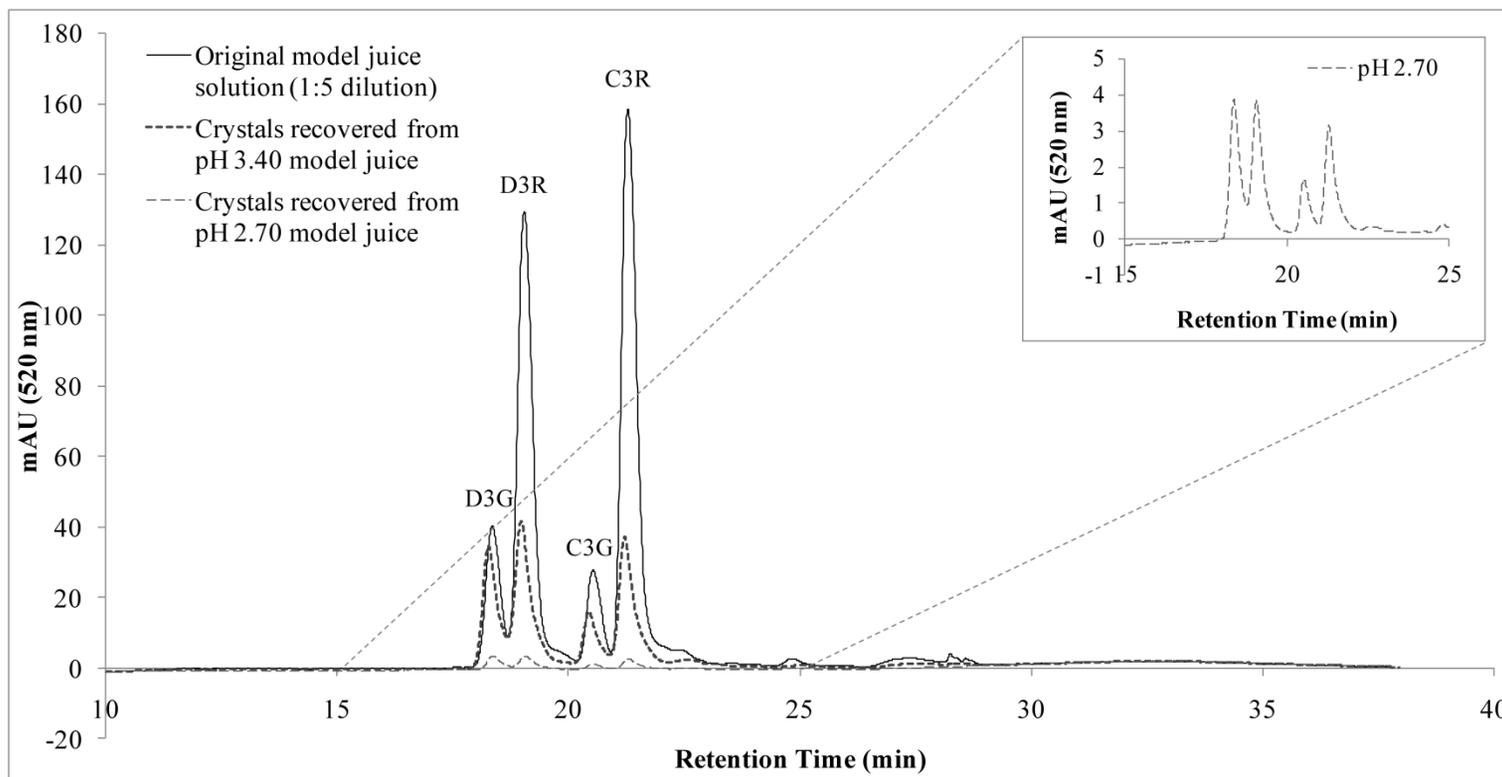


Figure 6.1. HPLC chromatogram of individual anthocyanins from original model juice and KHT precipitate recovered from pH 2.70 and pH 3.40 model solutions at 520 nm. The KHT precipitate was prepared by dissolving 50 mg of filtrate in acidified NaCl.

The KHT precipitates were collected by filtration, washed and redissolved prior to characterization of anthocyanins by HPLC. The total anthocyanin content was calculated as the sum of four major anthocyanins, and the %w/w anthocyanin content of the crystals calculated. These values are reported in Table 6.1. The highest contents (0.39%) were less than the 0.8% w/w content of KHT recovered from cold stabilization of single strength Concord grape juice (Alongi and others 2010). The difference between our model system and Concord may be due to the higher concentrations of coumarylated anthocyanins in Concord, which are more likely to coprecipitate (Ingalsbe 1963; Alongi and others 2010).

An ANOVA of the effects of the different factors on anthocyanin content was performed. Unfortunately, several model juices produced too little precipitate (< 70 mg) to facilitate analyses. Specifically, nearly all samples with low tartaric acid concentrations (0.02 M), and samples with low K^+ (0.02M) and low pH (2.35 or 2.7) could not be evaluated by HPLC. As a result, the effect of tartaric acid on the %w/w anthocyanin content of crystals could not be evaluated statistically, and the ANOVA only considered the effects of pH and K^+ for $pH \geq 2.95$. The results of the ANOVA are shown in Table 6.2. All terms (pH, K^+ , $pH \times K^+$) explained a significant portion of the variance, but pH was the most critical (Table 6.2). The %w/w anthocyanin content of the precipitate followed the trend $pH\ 3.4 > pH\ 3.2 > pH\ 2.95$. For example, at $K^+ = 0.02M$, tartaric acid = 0.04M, the anthocyanin content of the pH 3.4 model juice was 0.19% w/w, compared to 0.11% w/w at pH 3.2 and 0.02% w/w at pH 2.95. These differences appear to occur independently of the amount of precipitate that is formed,

Table 6.2. Analysis of variance (ANOVA) of total anthocyanin of bitartrate crystal (%w/w) from 0.04 M tartaric acid samples at pH 2.95 ± 0.01 , 3.20 ± 0.01 , and 3.40 ± 0.00

Factor	Sum of squares	F-ratio	p value
pH	0.130	9.71×10^{15}	<.0001*
K concentration	0.036	5.42×10^{15}	<.0001*
pH x K concentration	0.015	1.09×10^{15}	<.0001*

since all model juices produced comparable amounts of precipitate (8.63-9.33 mmoles).

The effects of pH on the amount of anthocyanin coprecipitation is readily appreciated in Figure 6.2, which shows pictures of the filtrate from pH 2.95 and pH 3.4 model juice. The pH 2.95 filtrate appears slightly colored, while the pH 3.4 filtrate is intensely pigmented as the concentration of anthocyanins in the latter is almost 10 times higher (Table 6.1). The HPLC chromatograms of the re-dissolved crystals from these experiments are shown in Figure 6.1.

The observation that a decreasing pH results in decreased coprecipitation supports the hypothesis previously advanced by Alongi and others (2010) to explain differences in KHT precipitation from concentrate and single strength juice. The authors observed that KHT precipitation from juice concentrate resulted in negligible losses as compared to precipitation from single strength juice. The higher pH of juice (3.1) could result in a negative surface charge of KHT and thus increase interactions between the flavylium form of anthocyanins and the deprotonated sites of the bitartrate crystals, while at the low pH of concentrate (2.5) the KHT surface would be neutralized and interactions would diminish. For the 0.04M K⁺ / 0.04M tartaric

samples, a sharp increase in the % w/w anthocyanin content was observed at pH >2.95 (Table 6.1 and Figure 6.3), while negligible coprecipitation (0.01-0.02% w/w) was observed at pH 2.95 and lower. This suggests that the transition from neutral to charged surface occurs around pH 3.0 for KHT. This is below the pH range usually observed for red grape juices and wines, which may explain why this phenomenon had not been previously observed. According to Celotti and others (1999), a transition in the surface charge at low temperature appears to occur between pH 2.8 and 3.0, as measured by streaming potential experiments. Surprisingly, the authors report that the surface charge became more negative with decreasing pH, an observation at odds with what typically occurs to surface charge with decreasing pH.

The K^+ concentration had a smaller but still significant effect on coprecipitation (Table 6.2). The higher K^+ concentration (0.04M) model juices had 2-3 fold lower anthocyanin content than their corresponding low K^+ concentration model juices (0.02M). This is likely a result of competition between K^+ and anthocyanins for negatively charged bitartrate at the crystal surface. Raising the K^+ concentration will increase the amount of KHT formed without increasing the amount of anthocyanin, and crystals should be able to grow larger before anthocyanin adsorbance blocks further growth.



Figure 6.2. Picture of bitartrate crystal precipitated from 0.04 M tartaric acid and 0.04 M potassium model juice at pH 2.95 (left) and pH 3.40 (right)

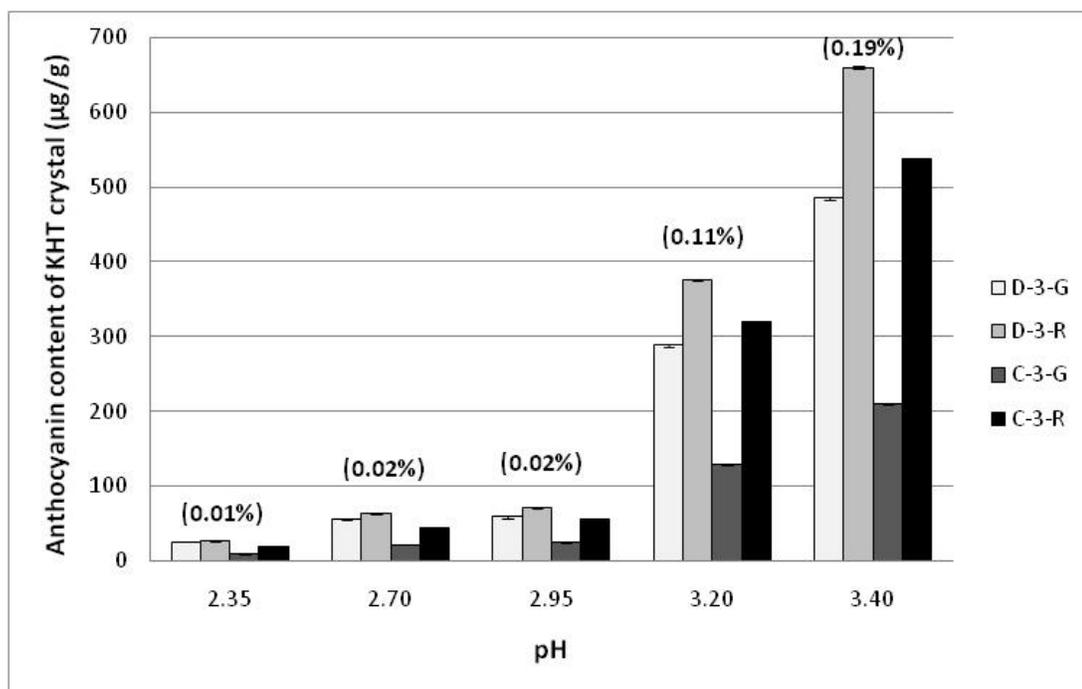


Figure 6.3. Changes of anthocyanin content in bitartrate crystal recovered from 0.04 M tartaric acid and 0.04 M KCl samples at different pH solutions. D-3-G: delphinidin-3-glucoside; D-3-R: delphinidin-3-rutinoside; C-3-G: cyanidin-3-glucoside; C-3-R: cyanidin-3-rutinoside. The number in parenthesis represented the total anthocyanin in bitartrate crystal (%w/w).

Selectivity of Anthocyanin – KHT Coprecipitation

The distribution of anthocyanins found in the KHT crystals was significantly different than the model juices (Figure 6.4) and this difference was more pronounced than when comparing anthocyanin distribution in KHT crystals from different pH solutions. The HPLC chromatograms for coprecipitated anthocyanins from two of the experiments (pH 2.7 and pH 3.4) are shown in comparison to the distribution in the original juice in Figure 6.1. As mentioned above, a sharp increase in the anthocyanin content of the KHT precipitate was observed at $\text{pH} > 2.95$.

In the original black currant extract, the major anthocyanin species was predominantly C-3-R (45%), and the anthocyanin content of the original juice followed the order C-3-R > D-3-R > D-3-G > C-3-G. In the pH 3.4 precipitate, the major species was D-3-R (35%), and the distribution of the anthocyanins followed the order D-3-R > C-3-R > D-3-G > C-3-G. The amount of anthocyanins with rutinoside group was significantly higher than those with glucoside group. This was a result of high amount of anthocyanins with rutinoside originally in model solutions.

The differences in the relative concentrations of species are better appreciated by plotting the ratio of the % concentration in the precipitate to the % concentration in the original juice, shown in Figure 6.5. An ANOVA of the effects of the different factors on the differences in the relative concentrations of anthocyanin species was also performed, and the data presented in Table 6.3. Both sugar and anthocyanidin factors explained a significant portion of the variance ($p < 0.05$), with the sugar having a greater effect. pH, on the other hand, only explained a slight portion of the variance compared to the other two factors and K^+ did not have a significant effect ($p \leq 0.05$).

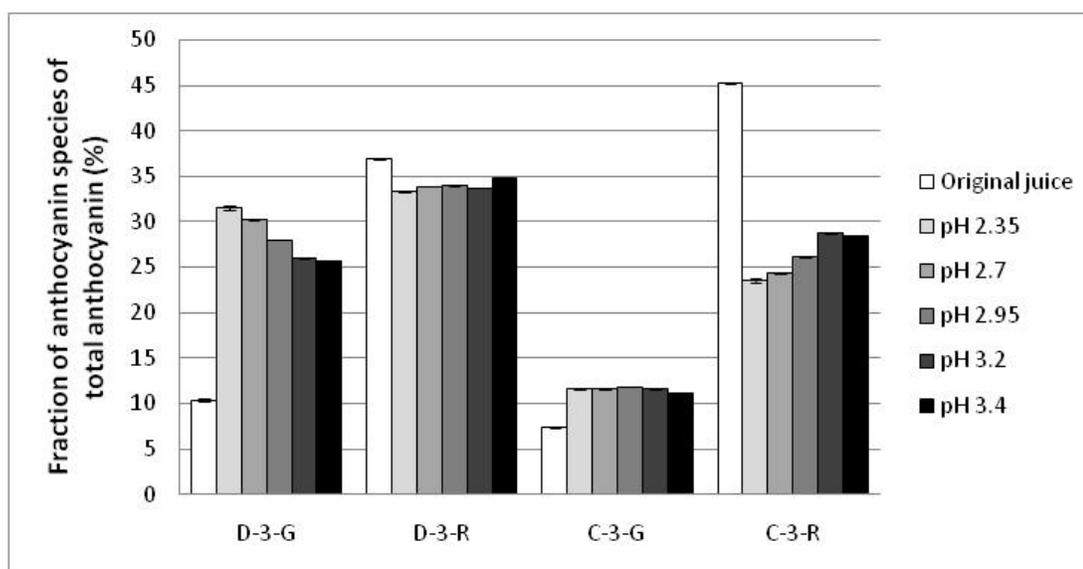


Figure 6.4. Fraction of anthocyanin species of total anthocyanin in bitartrate crystal recovered from 0.04 M tartaric acid and 0.04 M KCl samples at different pH solutions. D-3-G: delphinidin-3-glucoside; D-3-R: delphinidin-3-rutinoside; C-3-G: cyanidin-3-glucoside; C-3-R: cyanidin-3-rutinoside

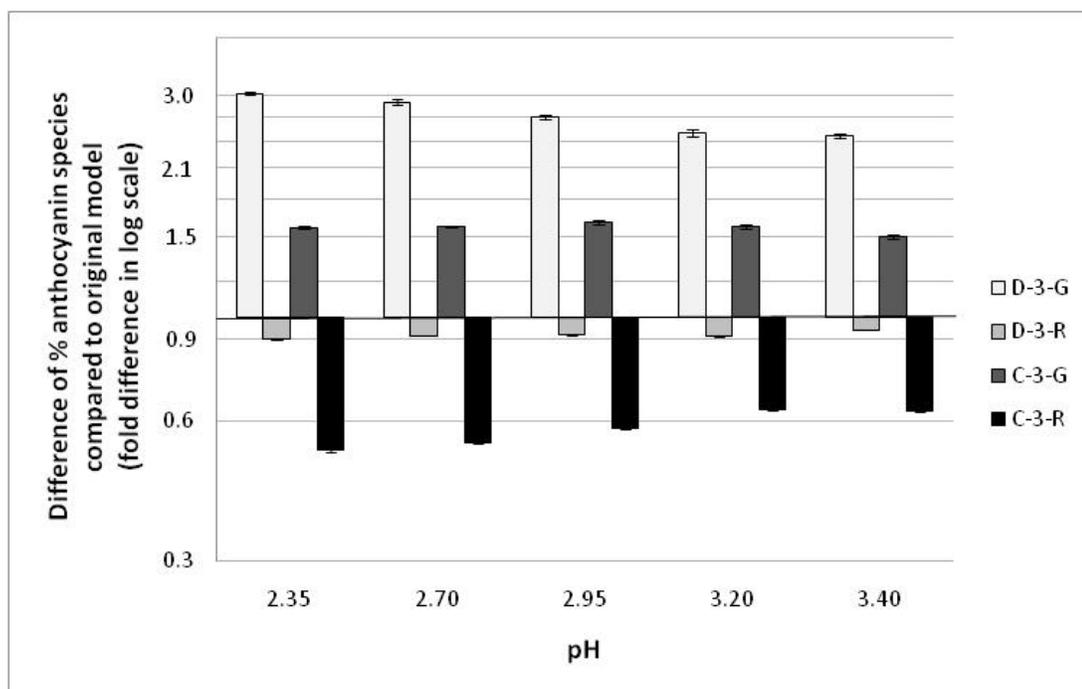


Figure 6.5. Difference in fraction of anthocyanin species (%) of total anthocyanin in bitartrate crystal recovered from 0.04 M tartaric acid and 0.04 M KCl samples compared to the original juice model. D-3-G: delphinidin-3-glucoside; D-3-R: delphinidin-3-rutinoside; C-3-G: cyanidin-3-glucoside; C-3-R: cyanidin-3-rutinoside

Table 6.3. Analysis of variance (ANOVA) of fold difference of fraction of anthocyanin species (%) in bitartrate crystal recovered from 0.04 M tartaric acid samples at pH 2.95 ± 0.01 , 3.20 ± 0.01 , and 3.40 ± 0.00 compared to the original juice model. Since potassium concentration were not significantly affect the ratio ($p \leq 0.05$), this factor was taken out from the analysis

Factor	Sum of squares	F-ratio	p value
Sugar	19.930	30449.23	<.0001*
Anthocyanidin	4.757	7268.32	<.0001*
pH	0.036	27.46	<.0001*
Sugar*Anthocyanidin	1.278	1952.66	<.0001*
Sugar*pH	0.089	68.36	<.0001*
Anthocyanidin*pH	0.009	7.01	0.0027*
Sugar*Anthocyanidin*pH	0.003	2.14	0.1321

As a result, K^+ was taken out from the statistical analysis. The glucosides D-3-G and C-3-G were enriched by a factor of 1.5 to 3, while the corresponding rutinoides were depleted by 10-50%. The preferential loss of glucosides may be due to steric hindrance of the rutinoides (monosaccharide vs disaccharide).

We also observed that the delphinidin derived anthocyanins were more likely to coprecipitate than the cyanidin based anthocyanins. This result is in contradiction to our previous work (Alongi and others 2010), where we observed a greater loss of C-3-G from Concord grape juice (15% decrease) than D-3-G (3%) from single strength juice during cold stabilization. Previously, we had explained these differences in terms of the hydration constant (pK_h) value of anthocyanins. The published pK_h value of delphinidin-3-glucoside ($pK_h = 2.36$) was lower than that of cyanidin-3-glucoside ($pK_h = 3.01$) (Figueiredo and others 1996; Stintzing and others 2002).

Therefore, at the same pH, anthocyanins with cyanidin aglycone would be in the flavylium ion form more than those with delphinidin aglycone, leading to more coprecipitation with bitartrate crystal (Alongi and others 2010). One possible explanation of this contradiction was that the interaction of anthocyanin coprecipitation could be in the neutralized form of anthocyanin rather than in the flavylium cation form. We do not observe differences in behavior among the two anthocyanins as pH is changed, i.e. the sharp decrease in cyanidin and delphinidin anthocyanins both occur at pH 2.95. This observation seems better explained by neutralization of KHT surface charge at lower pH, as described above, which should simultaneously affect all anthocyanins. This was also supported by the changes of the differences in the relative concentrations of anthocyanin species over different pH.

The difference was higher at the lower pH where KHT surface charge was neutralized. As a result, the difference followed the trend pH 2.95 > pH 3.2 > pH 3.4. The differences in the previous work and our current work may stem from other variants between the model and real juice systems, and will demand further study.

Potential implications for juice processing and selective isolation of anthocyanin classes: Our study clearly demonstrates the key effect of pH on anthocyanin coprecipitation. Specifically, at $\text{pH} \leq 2.95$ in our model systems, minimal coprecipitation occurred. Potentially, the 20-40% loss in anthocyanins during cold-stabilization of grape juice and wine could be eliminated by intentionally reducing the pH to ~ 2.95 prior to production. Reducing the pH much below 2.95 is likely undesirable, at least in single strength juice, as insufficient KHT precipitation would occur. A reduction of pH can be achieved by concentration prior to cold-stabilization, as previously demonstrated (Alongi and others 2010), but this is a complex process, and would not be appropriate for wine or for juices that are intended to be bottled without concentration. Alternatively, the pH could be reduced chemically (i.e. by addition of tartaric acid) or by physical means (i.e. electro dialysis) prior to cold stabilization. Following cold stabilization, the pH could be raised by analogous chemical or physical processes. Cation-exchange could also be used to reduce pH, but the cation exchange resins are well known to adsorb anthocyanins, so an improvement to anthocyanin content probably would not be realized.

Alternatively, coprecipitation with KHT could be exploited to selectively enrich and isolate anthocyanins or other natural products. Coprecipitation via

inclusion, occlusion, or adsorption is a classic analytical strategy for enriching trace analytes (Harvey 2000), although the strategy has been used primarily for enriching cations. In the case of anthocyanins, commercial products are generally sold as crude preparations due to the cost and difficulty of purifying polyphenol compounds from complex natural sources (Kraemer-Schafhalter and others 1998; Cote and others 2010). Pure polyphenols are often unavailable commercially or else are prohibitively expensive reserved for use in research settings, e.g. for biological activity studies, or for customized applications within the food industry. Coprecipitation of anthocyanins or other natural products with KHT or other organic salts could represent a novel, benign, and cost-effective strategy for isolating and fractionating polyphenols from natural sources for use as colorants or nutraceuticals. The concentration of anthocyanins in KHT crystals can approach 1%, comparable to the loadings achievable with reversed phase resins, but with the advantage that KHT is a fraction of the cost of commercial resins. Ideally, the selectivity of coprecipitation should be tunable to allow a user to preferentially retain specific classes of anthocyanins. We observe minor changes in selectivity resulting from changes in pH, but further work will be necessary to determine what other factors can be used to tune the selectivity of the coprecipitation process.

In summary, at $\text{pH} \leq 2.95$, cold stabilization of model grape juices results in significantly less coprecipitation of anthocyanins with KHT crystals. This is likely due to neutralization of the negatively charged KHT crystal surface, and could explain our previous observation that cold-stabilization of concentrate results in negligible coprecipitation. Potentially, pH adjustments could be made to juice by chemical or

physical processes prior to cold stabilization to reduce losses of anthocyanins. Higher concentrations of K^+ also result in a lower anthocyanin content of crystals, although greater total losses of anthocyanins may be observed due to increased KHT precipitation. The glucosides were more likely to coprecipitate with KHT than rutosides, possibly due to steric effects, and the delphinidin species were more likely to coprecipitate than the cyanidins. This selectivity was more evident at lower pH, which suggests that it may be possible to tune coprecipitation to achieve selective isolation of certain anthocyanin classes. An interesting future direction for study would be to investigate whether other organic salts with sparing solubility in aqueous solvents (e.g. calcium malate) can also coprecipitate anthocyanins or other polyphenolics, and if they display different selectivity.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

New York State is the 3rd largest producing grape in the US and the grape juice and wine industry is very important to the New York State industry and economics. For that reason, we aimed to improve the quality of grape juices and wines in New York State by using alternative antimicrobials and processing techniques to reduce sulfite usage, increasing retention of important compounds such as anthocyanins, and by utilizing crossflow filtration to improve sustainability.

Niagara grape juice is the most predominant white grape juice in New York State and its light color, clarity, and unique fruity flavor constitute the quality that consumers are seeking for. Furthermore, due to an increasing interest in healthy foods and beverages, nutritional quality and healthy attributes of Niagara juice are also important to consumers. Phenolic content and antioxidant capacity have been associated to many health benefits; however, they could be reduced by various processing factors and storage parameters such as enzymatic degradation, exposure to heat, light, and oxygen. Another increasing trend in the food and beverage industry is all natural products as part of a health and wellness trend. In Niagara grape juice processing, sulfite is traditionally been used to prevent both enzymatic and non-enzymatic browning. However, a growing public awareness of sulfite sensitivity has created the need for its reduction or replacement.

Our main objective was to find alternative processing methods without sulfite addition to produce Niagara grape juice with comparable quality, focusing on

evaluating postharvest management and processing conditions. The browning reaction in Niagara grape juice can be divided into 2 categories- enzymatic and non-enzymatic. The enzymatic browning reaction, which is responsible for the majority of browning that occurs during processing, is due to polyphenoloxidase (PPO) which catalyses the oxidation of polyphenols to quinones that undergo further reactions resulting in melanins, dark colored substances. The non-enzymatic browning reaction can be from 3 different pathways- Maillard reaction, ascorbic acid decomposition, and caramelization. Sulfite is an antioxidant that helps prevent browning by acting as a reducing agent to convert quinones back to polyphenols. Sulfite also helps destabilize disulfide bridges that maintain PPO enzymes in their active form.

In order to get a better understanding of factors affecting the browning and quality of Niagara grape juices, we studied the impact of raw material, Niagara grapes, by evaluating the effect of grape maturity and harvest method, as well as processing parameters from grape crushing to bottling and storage conditions. Ascorbic acid, a natural antioxidant, was used as sulfite substitute to prevent oxidation. To inactivate the PPO enzyme early on in the process, hot-break (heating of crushed grapes to 70-80°C) was applied instead of the traditional cold-press. Another strategy that we tested was the removal of browning precursors, namely polyphenols, using cross-flow filtration and chemical addition techniques.

We found out that ascorbic acid could potentially be used as sulfite substitute if added during grape crushing. The quality of 500 ppm ascorbic acid treated juices was comparable to that of sulfited juices, and based on preference and acceptance tests, both juices were not significantly different. Different harvest methods, hot-break

temperatures, and final pasteurization temperatures (hot-pack temperature), did not significantly affect juice quality if antioxidants such as sulfite or ascorbic acid are added during grape crushing. Since ascorbic acids or Vitamin C is a natural compound, the production of a light colored juice using ascorbic acid without bisulfite addition would offer the grape juice industry a competitive advantage in today's market and a product that fits into the all-natural premium juice category; therefore, more opportunities would be available for grape growers. Further studies should be conducted on the optimum concentration of ascorbic acid alone or in combination with less than 10 ppm sulfite to prevent browning in Niagara grape juices. Based on current FDA regulations for the juice industry, juices with less than 10 ppm sulfite do not require to carry the "contain sulfite" label. Lastly, an economic feasibility study should be conducted to determine the production cost and the demand for grape products in the premium juice category.

Another promising result was found with the use of the hot-break procedure. Prevention of PPO at the beginning of the process by using the hot-break process without sulfite addition produced juices with better quality than cold-press juices; however, they darkened more rapidly over time, indicating that they were less stable over storage at 18°C. The use of hot-break process warrants further investigation but has the potential to produce high quality Niagara juices without sulfite addition and thus appealing to consumers interested in all-natural juices. Further studies need to verify and optimize the hot-break process by scaling up with a continuous heat exchanger that will allow a controlled fast heating and cooling, thus minimizing heat exposure. The combination of hot-break with a final addition of an optimized ascorbic

acid concentration prior to bottling may prove to be a viable system for high quality white grape juice production.

Another aspect that should be investigated is the application of techniques for browning precursors removal in combination with the hot-break process to reduce the brown color development over storage time. Some techniques that could be tested for browning removal are cross-flow membrane filtration or the usage of chemical additives as we have shown from our studies that these techniques could partially reduce polyphenols (browning precursors) resulting in lower brown colored juices. The producers and manufacturers can also gain the additional benefits of implementing cross-flow filtration instead of traditional diatomaceous earth (DE) filtration which is a depth filtration. Cross-flow filtration gives higher yield and is easier to operate with a more sustainable system. Niagara grape juices filtered with 500 kDa MWCO polysulfone membranes had better quality than DE filtered juices, while juices filtered with ceramic membranes (0.2, 0.01 μm) and polymeric membranes (0.45 μm) were not significantly different based on physical attributes and preference and acceptance tests. Furthermore, juices from cross-flow filtration had lower phenolic contents than that from DE filtration, indicating that cross-flow filtration is more effective in browning precursors removal. Chemical addition techniques using PVPP (fining agent) and acetaldehyde (promotes condensation of polyphenols), and aeration were not as effective in browning prevention alone as compared to traditional cold-press sulfite addition procedure, but could be combined with other techniques to increase efficiency. Another possible future study is to combine the chemical addition techniques with aeration to produce light color Niagara

juices without sulfite addition. The brown substances formed from aeration or acetaldehyde condensation could potentially be removed by the addition of fining agents such as PVPP and gelatin, thus resulting in Niagara grape juices with lighter color. In all, these suggested future studies have the potential to produce light color Niagara juices without sulfite addition in response to an increasing demand from consumers.

Antimicrobials are typically used in juices to extend the shelf-life. Growing concerns of chemical preservatives regarding their potential toxicity and allergenic reactions have led to a need to find alternative antimicrobials to satisfy consumer demands for all natural products. In order to produce a clean-label Niagara grape juice, the effectiveness of alternative antimicrobials in extending the shelf-life of all natural cold-filled Niagara grape juices was tested against traditional chemical preservatives, sorbate and benzoate. The most promising result is the identification of a universally efficacious protocol designed to increase the shelf life of both still and carbonated cold-filled Niagara grape juice matrices to times comparable to that of the traditional sorbate and benzoate additives (>161 days at room temperature). To that end, we report that the addition of 250 ppm DMDC alone or in combination with 5-10 ppm natamycin effectively results in a stable Niagara grape juice product using less deleterious additives or natural antimicrobials. The use of this preservative system warrants further investigation but has the potential to enhance the shelf life of these juices while appealing to consumers interested in all-natural beverages. Further studies need to address the confounding factor of residual sulfites in white grape juices and investigate the effects of the natural preservatives alone. Other fruit juices could also

be tested to determine the efficacy of the natural antimicrobials in different matrices. Additionally, it will be of tremendous importance to also perform a tandem sensory evaluation of these juices to assure organoleptic acceptability.

In the wine industry, DE filtration is used as the final/polish filtration prior the bottling. However, significant losses of wines with DE, safety restrictions in handling DE during operation and cost of waste disposal have created a need to find alternative filtration systems. As mentioned earlier, cross-flow membrane filtration has showed to increase product yield and lower the operation cost by eliminating the filter aid and it is also reusable and easier to operate. Furthermore, ceramic membrane cross-flow filtration is reported to better maintain flavor and color as it is chemically inert and has longer life compared to polymeric membranes. We evaluated cross-flow filtration methods against DE filtration to achieve improvements in overall quality and production efficiency of New York State red and white wines. Based on our results, ceramic membranes with 0.45 and 0.2 μm are viable alternatives to traditional DE filtration to produce wine with equal or better quality, and represented more sustainable operations especially for filtration of difficult material such as tank bottoms. Ceramic membranes improved wine quality in terms of turbidity compared to DE filtration while other wine quality attributes including phenolic and anthocyanin contents, and antioxidant capacity were not significantly different as confirmed by discrimination and preference tests. The knowledge gained could lead to a more efficient processing and more profitable operations for the wine industry. Further trials need to conduct economic feasibility studies regarding the introduction of ceramic membrane filtration to small wineries, including the break-even analysis. The

knowledge gained will help local wineries make an informed decision and promote sustainability operations.

Another part of our study was to gain a better understanding of the factors governing the loss of anthocyanins with potassium bitartrate (KHT) crystals during cold stabilization. Previous work in our group has shown a significant loss of anthocyanins during cold stabilization in Concord grape juice processing. Anthocyanins are important to Concord juice quality not only as the source of deep red-purple color that consumers are looking for, but also as a source of antioxidants which has been shown to be associated with various health benefits. We evaluated the factors affecting the anthocyanin loss in model juice system. Our work showed that pH is the critical factor governing the amount of anthocyanin loss by controlling the charge of KHT crystal. As a result, to minimize anthocyanin loss and thus improve red grape juice quality, pH of the juices should be adjusted to lower than 2.95, the point at which we believe that negatively charge at KHT surface started to neutralize. Potassium concentration reduced anthocyanin loss by competing together for coprecipitation. Therefore, raising the potassium concentration can help increase the amount of KHT formed without increasing the amount of anthocyanin in the crystal. Lastly, the enrichment of delphinidin-3-glucoside and cyanidin-3-glucoside and the depletion of delphinidin-3-rutinoside and cyanidin-3-rutinoside in KHT crystal indicated that the preferential loss of anthocyanins depended more on sugar molecule attached to aglycone rather than the aglycone itself. This preferential loss of certain class of anthocyanins with bitartrate crystal could potentially be used for individual anthocyanins selectivity upon further investigation with other organic salts.