ABSTRACT

LEAVENS, JE'VELLE BONIQUE. Effects of High Hydrostatic Pressure and Thermal Processing on the Antioxidant and Sensory Characteristics of Blueberry Juice. (Under the direction of Dr. Leon Boyd.)

Blueberries are recognized for their potential health benefits and high antioxidant levels. Increased dietary consumption of blueberry products may reduce risk factors associated with cancer, heart disease, and other degenerative diseases. Blueberries are often processed into juices or wines. However during blueberry processing, the levels of antioxidants may be altered resulting in a change in antioxidant and sensory qualities. Due to the high antioxidant levels found in blueberries, blueberry processors are seeking effective processing techniques such as High Hydrostatic Pressure (HHP) to further optimize the amount of antioxidants retained in the final product. Therefore, the objectives of this study were to determine the effects of different processing techniques on the antioxidant properties of blueberry juice and consumer acceptability of blueberry juices developed from selective processing techniques (i.e. cold processing versus hot processing).

Individually quick frozen Croatan blueberries were treated with enzyme (Rapidase ^{TM}) and pressed at 22^{o}C, 43^{o}C and 75^{o}C, followed by further pasteurization of the initial cold press and hot pressed (43^{o}C) juices at 75^{o}C. In addition, trial studies were conducted using HHP processing at 400 MPa for 10 min, 20 min, and 30 min holding times. Antioxidant levels were measured by changes in total anthocyanins, total phenols, and Oxygen Radical Absorbance Capacity (ORAC) values. Results indicated the hot-pressed juice (75^{o}C) was significantly higher in antioxidant activity compared to the hot-pressed juice (43^{o}C) followed by the cold-pressed juice (22^{o}C). Addition of heat to the cold and hot-pressed juices caused an increase in the overall antioxidant activity. However, juice samples

pressed at 75^{o}C resulted in the greatest levels of total anthocyanins, phenols, and antioxidant capacity. There was a substantial amount of anthocyanins and phenolics recovered in the blueberry juice processed using HHP. The HHP_{30min} resulted in a higher antioxidant activity than the HHP_{20min} with the HHP_{10min} exhibiting the lowest antioxidant activity. In comparison to thermal processing, there were no significant differences between the hot pressed juice (43^{o}C) and the pressurized treatments at 20 min and 30 min holding times. Overall, equivalent antioxidant activity was achieved between the HHP_{20min} and the hot pressed juiced (43^{o}C), with a similar ORAC value for the HHP_{30min}. These results suggest that selective processing can be used to improve the antioxidant yield in blueberry juice. Though the addition of heat to the juices resulted in the highest levels of antioxidant activity, the application of greater levels of HHP may be an effective non-thermal processing technique to maximize antioxidant activity while minimizing sensory loss during blueberry juice processing.

Evaluation of heat treated blueberry juices by consumers (n=79) was based on the following attributes: overall acceptability, blueberry flavor intensity, blueberry flavor liking, sweetness intensity, sweetness liking, overall flavor liking, overall acceptability and overall appearance liking. Results from the consumer acceptability test indicated the juice extracted at 75^{o}C and 43^{o}C was more readily accepted by consumers than the cold- pressed juice with mean hedonic ratings of 6.33, 6.05, and 4.78, respectively. Overall, the thermally processed blueberry juices yielded a product with relatively high levels of antioxidants with a deep rich blue-purplish color that was appealing to consumers.

EFFECTS OF HIGH HYDROSTATIC PRESSURE AND THERMAL PROCESSING ON THE ANTIOXIDANT AND SENSORY CHARACTERISTICS OF BLUEBERRY JUICE

by
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DEDICATION

I would like to dedicate this thesis to my parents, grandparents, aunts and uncles for their continued love, support, and prayers throughout this professional endeavor. In some point in my life, you all have encouraged me to pursue and accomplish personal and professional goals that I have set for myself. I am very blessed to have a loving family who is very supportive of me. With much gratitude, this is for you.

BIOGRAPHY

Je'Velle Leavens was born in Washington DC, but spent the majority of her life growing up in Beulaville, NC a small town located in eastern North Carolina. Je'Velle received her Bachelor of Science degree in Chemical Engineering in 2001 from North Carolina Agricultural and Technical State University. With her interest in the science discipline, she later pursued a degree in Chemistry from the University of North Carolina at Greensboro. In 2003, Je'Velle continued her education by entering graduate school at North Carolina State University in pursuit of a Master of Science degree in Food Science.

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Chapter I

Literature Review

LITERATURE REVIEW

Historical Significance

Blueberries are one of the few crops native to North America. They belong to the *Ericaceae* family, genus *Vaccinum*, which include edible fruits such as cranberries, bilberries, and ligonberries. For centuries, Native Americans used blueberries for fresh consumption, ingredients in food preparation for meats, stews, and soups as well as utilization of the blueberry leaves and roots for medicinal purposes. Though there are several blueberry species and hybrids native to North America, the three primary species of commercial importance are the highbush (*Vaccinum corymbosum*), lowbush (*Vaccinum angustifolium*), and rabbiteye (*Vaccinum ashei*) blueberries (Bowling, 2000). Highbush blueberry varieties are commonly grown in the Mid-Atlantic, Midwest and Pacific Northwest regions of the United States and Canada, whereas the lowbush blueberries are native to the cold climates of northeastern United States and Canada. However, the rabbiteye varieties are more heat tolerant than the highbush and lowbush varieties, thus more prevalent throughout the southeastern regions of the United States. The highbush variety can grow approximately six to eight feet tall whereas the lowbush blueberry variety typically grows approximately two feet tall or less, producing a smaller fruit with a black to bright blue color (Otto, 1995). The rabbiteye variety, the most vigorous species, can grow approximately five to twenty feet tall producing berries with larger seeds and tougher skin than the highbush variety (Bowling, 2000 and Otto, 1995). Physicochemical characteristics, skin toughness and firmness determined by puncture tests and shearing, showed that rabbiteye varieties (Climax, Tifblue, and Premier cultivars) were greater in skin toughness and firmness than highbush varieties (Jersey and Bluecrop cultivars) (Silva et al., 2005). Through plant breeding, blueberries can

be enhanced to optimize the desirable flavor, texture, and color to achieve superior quality for consumers and food processing industries.

United States Blueberry Industry

North America accounts for the majority of the blueberry production, primarily localized in the United States and Canada. Among the states, Maine is the largest producer of lowbush "wild" blueberries whereas Michigan leads the production of cultivated highbush blueberries. According to the United States Department of Agriculture's (USDA) Economic Research Service, the states of New Jersey, Oregon, Georgia, North Carolina and Washington combined produce over 40% of the United States total production of cultivated blueberries (USDA Economic Research Service: Fruit and Tree Outlook, 2003). Furthermore, blueberry industries are developing globally in various countries including New Zealand, Australia, Germany, and Chile (Bowling, 2000).

The blueberry industry is divided into two market categories: fresh market and processed market blueberries. The USDA Economic Research Service reported over 90% of fresh blueberries and 50% of frozen blueberries harvested in the United States are exported to the Canadian market (USDA Economic Research Service: Fruit and Tree Outlook, 2003). Blueberries are either hand or mechanically harvested. Mechanically harvested blueberries are picked with a blueberry harvester that is designed to plow through fields using mechanical rods or vibrators to shake the plants, thus dropping the blueberries into the conveyor. Harvested blueberries are rushed to nearby processing facilities where the blueberries are treated and inspected for the removal of debris. Blueberries are then transported to the fresh and processed market channels. Generally, blueberries are hand picked for fresh market produce to ensure the best quality of fruit is being collected. Most of

the machine harvest blueberries are harvested to be individually quick frozen (IQF), canned, or dried to be used as ingredients in the development of other processed food products such as cereals, snacks, dairy products and baked goods as well as jams, jellies, juices, concentrates, wines and purees.

North Carolina Blueberry Industry

Blueberry production in North Carolina has increased over the past decade. According to the North Carolina Department of Agriculture (NCDA) Statistics, in 2001 the blueberry farm acreage consisted of 78% highbush and 22% rabbiteye blueberry varieties. Among the highbush varieties grown, Croatan and Reveille were the most prevalent varieties in North Carolina representing 40% and 16.2% of the total blueberry acreage respectively and 51% and 21% of the total highbush varieties grown. In addition, rabbiteye varieties, Premiere and Tifblue cultivars represented 10% and 8% of the total blueberry acreage respectively, representing a combined total of approximately 81% of the total rabbiteye varieties. In 2005, North Carolina ranked third in the nation for production value and fourth in total acres. North Carolina produced 26 million pounds of blueberries valued at $36 million, consisting of 16.1 million and 9.9 million pounds of fresh and frozen blueberries respectively (Cline and Ballington, 2006). The blueberry industry will continue to increase in production as consumers become more aware of the potential health benefits and high antioxidant properties of blueberries.

Phenolic Compounds and Anthocyanins

Phenolic compounds are secondary metabolites found in plants. They are responsible for necessary functions in the reproduction and growth of plants as well as acting as a defense mechanism against pathogens, parasites and predators (Liu, 2004). Phenolics are a

large family of natural compounds present in a variety of flowers, fruits, berries and vegetables. They have an essential role in food quality as it relates to appearance, taste, and flavor of plant-derived food products. Furthermore, the role of phenolic compounds in the human diet have been recognized as promoting health benefits by reducing the risk factors associated with certain types of cancer, heart disease, and other degenerative diseases. Among the phenolic compounds, flavonoids represents two thirds of the phenolics in the human diet and the remaining one third is from phenolic acids (Liu, 2004). Flavonoids have a common generic structure consisting of two aromatic rings (A and B ring) linked by an oxygen heterocyclic ring (C ring) (Figure 1). Substituents attached to the numbered positions classify the different flavonoid compounds as flavones, flavonols, flavanones, flavanols, anthocyanidins, and isoflavones (Figure 2) (Hollman and Arts, 2000).

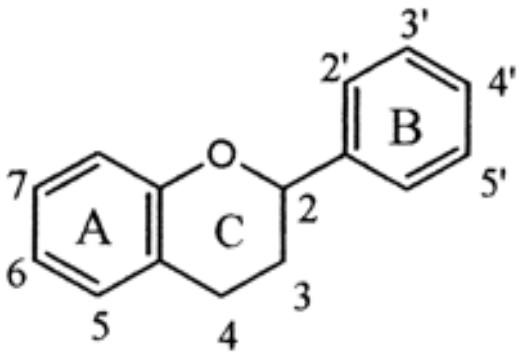


Figure 1. Generic structure of flavonoids. (Liu et al., 2004)

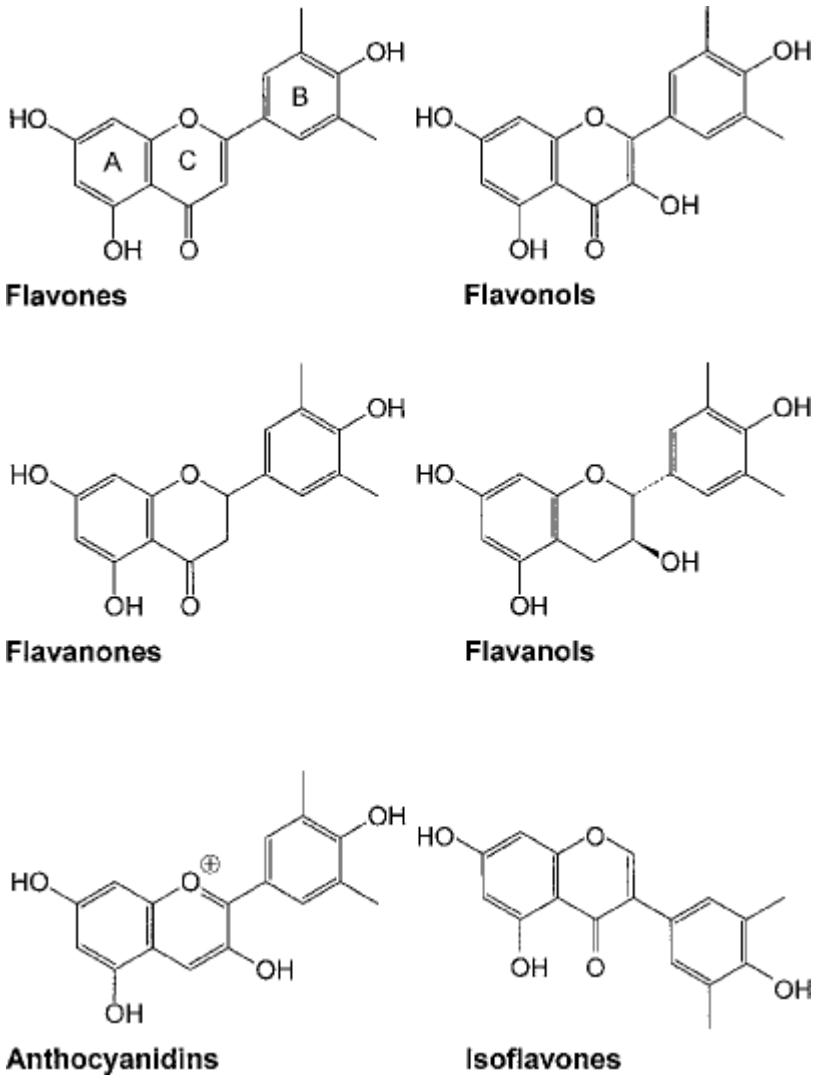
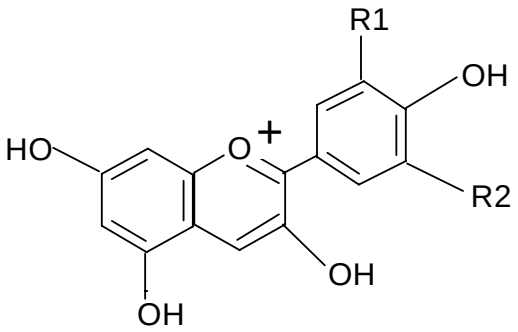


Figure 2. Subclasses of flavonoids. Classification is based on variations in the heterocyclic C-ring. (Hollman and Arts, 2000)

Flavonoids in particular anthocyanidins are of interest due to the increasing levels of anthocyanins and high phenolic content found among fruits, berries and vegetables. Anthocyanins (in Greek *anthos* meaning flower and *kyanos* meaning blue) are glycosylated anthocyandins. The anthocyandins are water soluble pigments responsible for the diverse array of colors such as red, blue, and purple that gives color to many food crops. There are several naturally occurring anthocyanidins, however only six are commonly distributed

throughout the plant kingdom: cyanidin, delphindin, malvidin, peonidin, pelargonidin, and petunidin (Figure 3) (Beattie et al., 2005).



Anthocyandin	R1	R2	Color
Pelargonidin	H	H	orange red
Cyanidin	OH	H	red
Delphinidin	OH	OH	pink
Peonidin	OCH_3	H	bluish purple
Petunidin	OCH_3	OH	purple
Malvidin	OCH_3	OCH_3	redish purple

Figure 3. Structures of major anthocyanins. (Beattie et al., 2005)

Individual anthocyanins differ due to the number of hydroxyl and methoxyl groups present; the number, identity and position of sugar attachments; and the number and identity of aliphatic or aromatic acids attached to the sugar in the molecule (Kong et al., 2003). The relative color depends upon changes in pH. With a pH between 1 and 3, the anthocyanins exist primarily as a red base color, whereas at pH 5 a colorless base is generated, and at pH 7 and 8 a blue to purple base color is formed (Bagchi et al., 2004). Anthocyanins are typically

found in plant species such as blueberries, raspberries, and red cabbage, as well as fruit juices, wines, green tea and other beverage products.

Antioxidants and Free Radicals

Antioxidants are compounds that protect cells against oxidative damaging effects known as free radicals. Examples of free radical species causing damaging effects within the human body include singlet oxygen ($^1O_2$), superoxide (O_2^-), peroxyl radicals (ROO^-), hydroxyl radical (HO^-) and peroxynitrite($ONOO^-$) (Huang et al., 2005; Kaur and Kaper, 2001). Though some free radicals occur naturally during metabolism, environmental factors such as pollution, radiation, cigarette smoke, and herbicides can also generate free radicals within the human body (Kaur and Kaper, 2001). To reduce the effect of free radicals, antioxidants act as scavengers by donating an electron to neutralize the reactive oxidative species. Though antioxidant enzymes, superoxide dismutase, catalase, and gluthathione peroxidase are produced in the body as a defense mechanism to reduce the generation of free radicals, cellular and tissue damage may still occur if the free radical production is excessive, and if there is a decrease in antioxidant enzymes.

Many fruits and vegetables contain natural sources of antioxidants such as Vitamin C and E, carotenoids, anthocyanins, as well as many other phytochemicals that can act as free radical scavengers. Furthermore, blueberries have been recognized for their potential health benefits and high antioxidant capacity. Wu et al. (2000) measured the lipophilic and hydrophilic antioxidant capacities of over 100 foods commonly consumed in the United States. Blueberries are often used for fresh consumption or processed for use as ingredients in other products. Compared to other fruits, vegetables, and nuts provided in the database, blueberries are one of the richest sources of antioxidants (Table 1). In addition, blueberries

are low in calories and fat content as well as providing a source of essential vitamins and minerals (Table 2).

Wang et al. (2000) studied the scavenging capacity of berry crops against reactive oxygen species. The antioxidant scavenging ability of fruit juices from two different blueberry cultivars of highbush blueberries, Elliot and Bluecrop were shown to have a higher inhibition against superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (HO^-) and singlet oxygen ($^1O_2$), radicals when compared to wild blueberries (Wang and Jiao, 2000). In addition, among different cultivars of small fruits, fruit juices from Hull Thornless blackberry, Earliglow strawberry, Early Black cranberry, Jewel raspberry, and Elliot blueberry had a relatively high scavenging capacity against superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen radicals (Wang and Jiao, 2000).

Table 1. Food Sources Rich in Antioxidants (Wu et al., 2004)

Food Item	**Serving Size (g)**	**Total Antioxidant Capacity/serving (μmol of Trolox equivalents)**
Small red bean (dry)	92 (1/2c)	13727
Lowbush blueberry	145 (1c)	13427
Red Kidney bean (dry)	92 (1/2c)	13259
Pinto bean (dry)	96 (1/2c)	11864
Cultivated blueberry	145 (1c)	9019
Cranberry	95 (1c)	8983
Blackberry	144 (1c)	7701
Artichoke(cooked)	84 (1c, hearts)	7904
Prune(dried)	85 (1/2c)	7291
Raspberry	123 (1c)	6058
Strawberry	166 (1c)	5983
Red Delicious Apple (peel)	138 (1 fruit)	5900
Granny Smith Apple	138 (1 fruit)	5381
Pecan	28.4 (1oz)	5095
Sweet Cherry	145 (1c)	4873
Black Plum	66 (1 fruit)	4844
Russet Potato (cooked)	299 (1 potato)	4649
Black Bean (dried)	52 (1/2)	4181
Gala Apple	138 (1 fruit)	3903

Table 2. Blueberry Composition: (USDA National Nutrient Database for Standard Reference Release 18 (2005)

Blueberry Composition (Raw)	**Amount/100g**
Food Energy	57.00 kcal
Water	84.21 g
Protein	0.74 g
Total Lipids (Fat)	0.33 g
Ash	0.24 g
Total Carbohydrates	14.49 g
Sugars	9.96 g
Total Dietary Fiber	2.4 g
Vitamin C	9.7 mg
Sodium	1.0 mg
Potassium	77.0 mg
Calcium	6.00 mg
Iron	0.28 mg
Folate	6.00 mg

Phenolic Compounds, Antioxidants, and Health Benefits

Researchers and medical professionals have indicated the significance of phenolic compounds as having health benefits. A review of flavonoids by Nijveldt et al. (2001) summarized the biological properties of flavonoids and their beneficial health effects. Increased dietary consumption of fruits and vegetables rich in antioxidants may reduce risk factors associated with cancer, heart disease, and other degenerative diseases. Fruits and vegetables contain many different antioxidant components contributing to their antioxidant activity other than vitamin C, vitamin E, and β carotene (Wang et al., 1996). During the last decade, much interest has been focused on blueberries as well as other small fruit crops due to their high levels of anthocyanins and antioxidant capacity. Prior et al. (1998) reported a significant correlation between the antioxidant capacity and the total content of anthocyanins and phenolics among blueberries. Numerous researchers have reported the antioxidant

activity including anthocyanins and total phenolic content of berries, fruit juices and wines. However, studies have shown that the phenolic compounds, including anthocyanins, in blueberries vary widely. A study conducted by Sellappan et al. (2002) evaluated the phenolic content of different cultivars of blueberries from rabbiteye and southern highbush blueberry species and found significant differences in the phenolic content among the different cultivars, however the overall content of anthocyanins, phenolics, and antioxidant capacity were higher in the rabbiteye blueberries than in the southern highbush blueberries.

Anthocyanins have been associated with the antioxidant properties of many common small fruit crops and have been characterized as having significant beneficial effects on various diseases. For example, an in vitro study investigating anthocyanidins found cyandin and delphindin to be potent inhibitors against the growth of human tumor cells (Meiers et al., 2001). Furthermore, Yi et al. (2005) investigated the bioactivity of phenolic compounds of different rabbiteye blueberry cultivars, Brightblue, Tifblue, and Powderblue. From the study, anthocyanins had the greatest antiproliferation effect with an inhibition of greater than 50% as opposed to the phenolic acids, flavonols, and tannins (Yi et al., 2005). In a similar study, anthocyanins were found to have the greatest inhibitory effect on HepG2 cancer population growth at 50% inhibition with 150ug/ml of anthocyanins from Tifblue and 70ug/ml of anthocyanins from Brightblue and Powderblue blueberries (Yi et al., 2006). Anthocyanins have been reported to possess significant biological effects including anti-inflammatory, anti-carcinogenic, anti-mutagenic, and other vasodilator properties. Phenolic compounds from berry extracts have been reported to exhibit a broad range of protective health benefits that may reduce the risk factors associated with certain types of cancer, cardiovascular disease as well as other degenerative diseases (Bagchi et al., 2004). Both blueberry and cranberry

juices are known to be useful in preventing harmful bacteria such as *Escherichia Coli*, from anchoring itself to the wall of the bladder and urinary tract, thus these juices have been recommended for reducing the risk associated with urinary tract infections (Kalt, 1997). In addition, berry phenolics were shown to be protective against the oxidation of low density lipoproteins (LDL) and liposome oxidation (Heinonen et al., 1998). Several studies have investigated the anticancer potential of lowbush blueberries during the initiation, promotion and progression stages of carcinogenesis and indicated that blueberry flavonoids and other phenolic compounds can be characterized as having anticancer protection against multiple stages of carcinogenesis (Kraft et al., 2005 and Smith et al., 2000).

Absorption and Metabolism In Humans after Blueberry Consumption

Increased consumption of fruits and vegetables have been suggested due to the positive association and biological effects of flavonoids with reducing risk factors associated with cancer, heart disease, and other degenerative diseases. However research is ongoing to determine the bioactivity of dietary flavonoids, their bioavailability, absorption and metabolism in humans. Cook and Samman (1996) summarized the chemistry, metabolism, cardioprotective effects, and dietary sources of flavonoids. Pedersen et al. (2000) investigated the effect of blueberry and cranberry juice consumption on plasma phenolic content and antioxidant capacity in nine healthy female volunteers between 23 and 41 years of age. After consumption of 500ml of either blueberry or cranberry juice, blood samples were collected at different intervals up to four hours as well as urine samples collected after four hours of consumption. Though the antioxidant activity of the blueberry juice was rich in phenolics with a high antioxidant capacity, there was not a significant increase in the antioxidant capacity in the plasma. After consuming cranberry juice, the plasma antioxidant

capacity increased significantly with a 30% increase in Vitamin C from 0.5 hours to 4 hours when compared to those individual who consumed blueberry juice. It was concluded that the fortification of Vitamin C in the cranberry juice was a factor in the increased antioxidant capacity in the plasma as opposed to the organic blueberry juice which did not contain Vitamin C fortification (Pedersen et al., 2000).

Wu et al. (2002) studied the absorption and metabolism of anthocyanins in healthy elderly women between 60-70 years of age. Four subjects were given 12 g of elderberry extract that contained 720 mg of anthocyanins blended with 500 ml of water and six subjects were given 189 g of frozen lowbush blueberries, containing 690 mg of anthocyanins. Anthocyanin recovery and metabolites in urine excretions were detected from the consumption of elderberry and blueberry, however, the anthocyanin concentrations were relatively low (0.004%) in the consumption of blueberries compared to those individuals who consume elderberry extracts (0.077%) (Wu et al., 2002). Though more research studies are needed to fully understand the bioactivity and metabolism of phenolic compounds found in blueberries, they have been identified as a significant source of phytochemicals, particularly phenolic compounds and anthocyanins that can act as antioxidants; thus contributing to the potential health benefits.

Effects of Thermal Processing on Antioxidant Properties

Blueberries are generally processed into juice, juice concentrates, wines, and other blueberry products. Due to their possible health benefits, there has been an increased interest in the phenolic content and antioxidant capacity of blueberries and blueberry products after processing in order to better assess the dietary information of the processed products (Kader et al., 2002; Lee et al., 2002). There have been several investigations on the effect of

processing on phenolic compounds and anthocyanins in many fruits and berries. Various parameters such as heat, pH, oxygen, light and storage conditions are recognized as having an effect on anthocyanins. A study investigated by Kalt et al. (2000) determined that the extraction of blueberry fruit at 60^{o}C resulted in a higher anthocyanin recovery and antioxidant capacity as well as an increase in the total phenolic content compared to the extraction at 25^{o}C. The anthocyanin content of the berries increased by 15-fold in the extraction at 60^{o}C after 60 minutes compared to the extraction at 25^{o}C. The phenolic content and antioxidant capacity increased approximately 2-fold after 65^{o}C extraction (Kalt et al., 2000). The increase in phenolic compounds may have been due to the increase in permeability of membranes in the maceration of the berries (Spanos et al., 1990; Kalt et al., 2000). However, substantial losses in anthocyanins and in the total antioxidant capacity occurred under storage conditions of 20^{o}C from extracts obtained at 60^{o}C. Rossi et al. (2003) showed that fruit blanching increased the phenolic content and radical scavenging activity of blueberry juice obtained from blanched blueberries opposed to the juice extracted from non blanched blueberries. Thermal processing treatments, hot pressing, cold-enzyme pressing, and hot-enzyme pressing on blueberry juice yield and composition was studied by Fuleki and Hope (1964). Results showed that treatment of fresh or frozen lowbush blueberries with pectinolytic enzyme at 63^{o}C prior to pressing the berries improved juice yields, soluble solids and color pigmentation of the blueberry juice (Fuleki and Hope, 1964).

Anthocyanins and other phenolic compounds are easily oxidized and therefore susceptible to degradation in various processing steps. Previous research studies have shown that polyphenol oxidase (PPO) when not inactivated will cause phenolic compounds to undergo degradation during fruit processing (Skrede et al. 2000; Lee et al., 2002; Kader et

al., 2002). Lee et al. (2002) investigated the anthocyanin and polypehnolic content of blueberry juice by comparing two pretreatments, heat and sulfur dioxide (SO_2). It was reported that a significant amount of anthocyanins and polyphenols were loss in the pretreatments as well as the control during the processing steps: thawing, crushing, depectinization and pressing of the berries, however there was a higher anthocyanin yield from the heat and SO_2 treatments, but the polyphenolic content was relatively similar to the control sample (Lee et al., 2002). Skrede et al. (2000) reported substantial losses in anthocyanins and polyphenolics of highbush blueberries during juice processing, and concluded that different classes of phenolic compounds had varying susceptibility to degradation which was thought to have been caused by activating PPO during the milling and depectinization processing steps. In addition to PPO, another natural enzyme peroxidase (POD), an iron containing enzyme, was shown to have involvement in anthocyanin degradation as well as a possible factor in the development of browning during storage (Kader et al., 2002).

Food processing procedures are recognized as a contributing factor to changes in natural phytochemicals of fruits and vegetables after processing, thus having a possible effect on the antioxidant capacity and sensory attributes of the processed food product. Though domestic processing and storage conditions can have a negative effect on the quality of fruits and berry products, better extraction of phenolic compounds and anthocyanins have been achieved with the addition of heat and higher extraction temperatures. Due to the high antioxidant levels found in blueberries, researchers and blueberry processors are seeking effective processing techniques to optimize the amount of antioxidants obtained in the final

product, while preserving the natural phytochemicals and sensory qualities of the blueberry product.

Effects of High Hydrostatic Pressure on Antioxidant Properties

Much attention has been given towards high hydrostatic pressure (HHP), also known as high pressure processing, a non-thermal processing technique in which the food is subjected to high pressures with or without the addition of heat to achieve microbial and enzymatic inactivation. Temperatures in the range of 0^{o}C to 100^{o}C with pressure treatments between 100-1000 MPa can be applied from a few seconds to over 20 minutes (Butz and Tauscher 2002; Senorans et al. 2003). The pressure and temperature ranges commercially used are between 400-600 MPa at 18-22^{o}C (Zabetakis et al., 2000; Suthanthangjai et al., 2005). High hydrostatic pressure has been used to produce value added products with excellent sensory and nutritional benefits as well as preserving the natural quality of the food product. Commercially pressurized products have been introduced into the markets of Japan, France, Spain and the United States (Butz and Tausher 2002). Commercially available food products treated by high pressure processing include guacamole and oysters in the United Sates, jams, jellies, fish meat products, sliced ham, salad dressings, rice cakes, juices and yogurt in Japan and Europe (Sizer et al., 2002).

Though thermal processing has been the processing method of choice for foods, HHP has been introduced as an alternative non-thermal processing technology. Compared to thermal processing, HHP has been found to produce a quality product with minimal impact on food quality attributes such as flavor, texture, pigmentation, and nutritional value by limiting the detrimental effects caused by extreme temperatures during thermal processing. Several studies have investigated the effect of HHP on processed fruit products. The quality

of pressurized strawberry jam was efficient in preserving the fresh like characteristics and anthocyanin content, however the thermally processed jam maintained the highest quality for several months while stored at room temperature (Kimura et al., 1994). Another study investigated the effect of post processing on the total antioxidant capacity of orange juice treated with HHP and thermal pasteurization and reported overall, the antioxidant retention was higher in the pressure treated orange juice than the thermally processed juice under various storage conditions (Polydera et al, 2004).

Furthermore, when applying HHP to food products it is essential to know the effect it has on enzyme activities. Natural enzymes peroxidase (POD) and polyphenoloxidase (PPO) are known to cause deterioration of flavor and color in fruits and berries. Palazon et al. (2000) determined that the most cost effective inactivation of POD was achieved with 600 and 800 MPa at 15 minutes in both strawberries and red raspberries. In contrast, a significant inactivation of PPO was achieved at 800MPa for 15 minutes in red raspberries where as 600 MPa for 15 minutes and 800 MPa for 10 minutes were the most cost effective conditions for inactivating PPO in strawberries (Palazon et al., 2004). Similar results reported by Zabetakis et al. (2000) concluded the highest stability of anthocyanins was observed in fruit juices prepared from strawberries pressurized at 800 MPa for 15 min and stored at 4^{o}C. Furthermore, the highest stability of anthocyanins reported in puree made from red raspberries was observed at 4^{o}C as opposed to 20^{o}C and 30^{o}C (Suthanthangjai et al., 2005). The higher storage temperatures caused an increase in enzymatic activity, thus leading to higher anthocyanin degradation.

Although many of the research studies investigating the effects of HHP have been related to other fruits and berries, using high pressure could be an effective processing

technology method in the recovery of anthocyanins and other phenolic compounds as well as preserving the nutritional and sensory qualities of blueberries and blueberry products.

Characterization and Measurement of Antioxidant Activity

High Performance Liquid Chromatography (HPLC) is commonly used as an analytical technique to identify and determine phenolic compounds present in berries and other phenolic containing products. Food phenolics are generally detected using UV-vis and photodiode array detectors. The identification and quantification of individual anthocyanin and other phenolic fractions are compared with the retention times and spectral data of known standards of phenolic compounds. Mazza and Miniati (1993) reviewed the anthocyanin composition of various blueberry species, thus indicating derivatives of delphindin, malvidin, and petunidin as major anthocyanin pigments present in blueberries. Kader et al. (1994) studied the phenolic profile of Coville blueberries and identified 15 anthocyanins presents. Derivatives of delphindin and malvidin accounted for 37% and 31% respectively of the total anthocyanin composition, thus the derivatives of 3-monoglucosides, galactosides, and arabinosides accounted for 31.5%, 40.9% and 27.6% of the anthocyanin composition of Coville blueberries (Kader et al., 1994).

Sapers et al. (1984) investigated the color and composition of highbush blueberry cultivars and identified only 10 anthocyanins present in Coville blueberries. The variation in the anthocyanin profile may be due to the environmental conditions such as berry ripeness as well as other conditional factors such as post harvest handling, storage conditions, different extraction procedures, and mobile phases. The anthocyanin fraction of blueberries is comprised of more than 16 individual anthocyanin components (Sapers et al., 1984; Prior et al., 2001). Taruisco et al. 2004 identified 3-glucosides and 3-galactosides of delphindin and

malvidin as the major anthocyanin pigments, quercetin as the primary flavonol and chlorogencic acid as the primary phenolic acid present in half-highbush and highbush blueberry varieties.

Total Anthocyanins

Anthocyanin pigments contribute to the color quality of blueberries as well as many other fresh and processed fruits and vegetables. The characterization and measurement of the total anthocyanin content and pigment degradation indices is essential in determining the color quality of food and beverage products rich in anthocyanins. The total anthocyanin assay is based on a pH-differential method which measures the absorbance at two different pH values, pH 1.0 and pH 4.5 buffer solutions (Giusti and Wrolstad, 2001). Although the assay does not determine the quantity of individual anthocyanins, the method is rapid and easy for quantifying the total anthocyanin content and degradation indices of fresh and processed food and beverage products. The total anthocyanin content of a sample is expressed in terms of the specific anthocyanin, however if the identity of the anthocyanin pigments is unknown, the anthocyanin content is based on cyanidin-3-gluocoside, thus expressed as cyanidin-3-gluocoside equivalents.

Total Phenols Assay

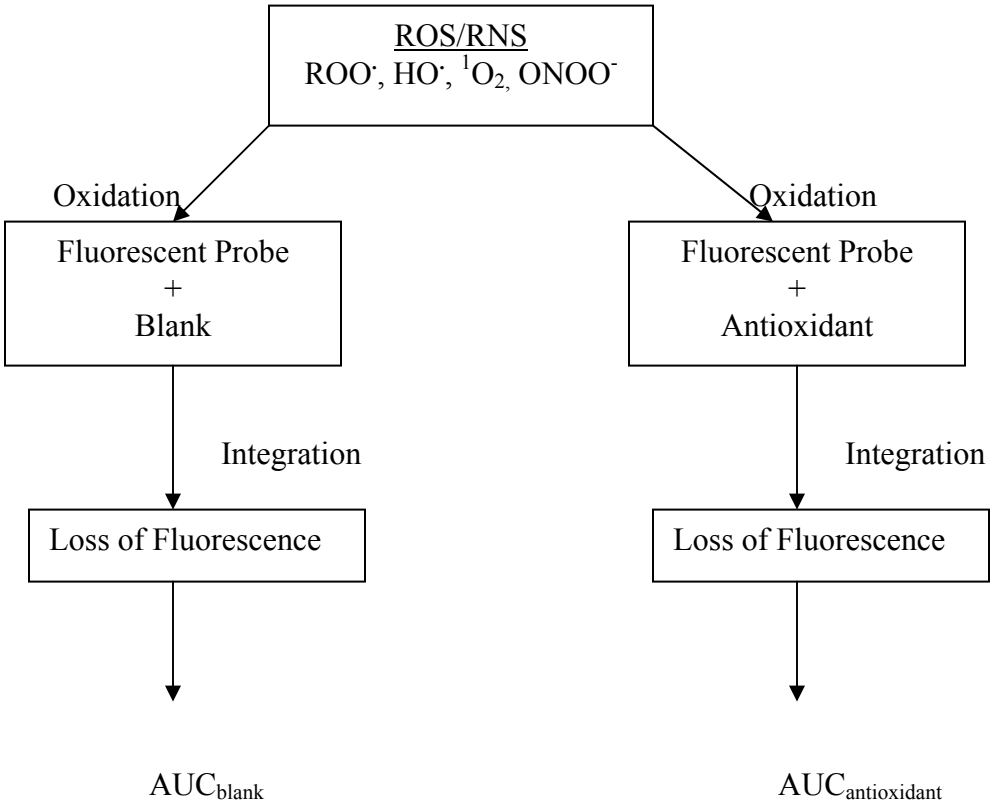
The total phenolic content of blueberries and blueberry products has been reported in several research studies. The Folin-Ciocalteu Assay method modified from Singleton and Rossi 1965 is a commonly accepted assay among research laboratories in analyzing dietary antioxidants in various food applications such as fruit extracts, juices, wines and many other antioxidant containing products (Amerine and Ough, 1980). The assay is based on colorimetric properties, in which the amount of color formation depends on the amount of

hydroxyl groups (-OH) or potentially oxidizable groups present. Gallic acid is used as a standard to quantify the total phenolic content of the sample based on the total phenolic content of the standard at different concentrations. The absorbance reading is measured at 765 nm after heating at 45^{o}C for 15 minutes (Shandi and Naczk, 2004) or at 20^{o}C (i.e. approximately room temperature) for two hours (Amerine and Ough, 1980). The total phenolic content of the sample is expressed as gallic acid equivalents.

Oxygen Radical Absorbance Capacity

There are various methods used to evaluate the total antioxidant capacity such as ferric reducing ability of plasma (FRAP), trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP), and oxygen radical absorbance capacity (ORAC). Among the methods, (ORAC) has been widely accepted as a standardized technique used among researchers in food, nutraceutical and pharmaceutical industries to measure the antioxidant capacity of various foods, beverages and natural products. The assay evaluates the antioxidant scavenging activity against an oxidation agent such peroxyl radical generator 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Trolox, a Vitamin E analogue, is used as the control standard for which antioxidant activity of the sample is compared to. The chemical assay combines a fluorescent probe such as fluorescein with a test sample and an oxidizing agent, AAPH (Ou et al., 2001). The intensity of the fluorescein decreases as it is damaged by free radicals. In the presence of an antioxidant, the florescence slowly decreases over time as the antioxidant scavenges the free radical to provide protection to the probe (Figure 4) (Haung et al., 2002). Once the reaction has gone to completion, quantitative data of the antioxidant protection can be calculated by the area under the curve, which combines the inhibition time as well as the inhibition percentage of

the free radical damage by the antioxidant into a single quantity. The ORAC value is expressed as Trolox equivalents.



$$\text{Antioxidant Capacity} = AUC_{antioxidant} - AUC_{blank}$$

Figure 4. Schematic diagram of the ORAC assay principle. (Huang et al., 2002)

Sensory Evaluation of Blueberry Juice Quality

Sensory evaluation of foods includes evaluating food attributes such as flavor, texture, color and appearance. Sensory techniques can be used to optimize a developing product as well as the quality of the final product (Lawless and Heyman, 1998). Although much of the focus has been towards the biological properties including antioxidant capacity and health benefits of blueberries and blueberry products, very few studies have evaluated

the sensory characteristics. The juice industry has increased their production of blended juices as a means of providing consumers with variety and better flavored juice products (Roberts et al., 2004). A wide range of blended juices have included berry juices blended with traditional juices such as cranberry, apple, and grape juices. The cost of blueberries is relatively expensive in producing a 100% full strength blueberry juice; therefore a blueberry juice blend would be more efficient and cost effective for large scale blueberry juice production.

Value-added products produced from IQF blueberries, for example blueberry juice, should maintain the qualities of fresh blueberries. Blueberry juice has a strong flavor and low sugar content, thus making a juice blend is an effective way to provide a more palatable juice beverage. Tipton et al. (1999) investigated grape juice as a sweetener for blueberry juice. Juice blends were evaluated based on blueberry flavor, color, and aroma as well as astringency and body. Several formulations were prepared for sensory evaluation of the juice blends using a trained panelist. It was concluded that the intensity of flavor, color, and aroma increased with an increase in concentration of blueberry juice for blends prepared at 20%, 40%, 60%, and 80% blueberry, water, and high fructose corn syrup or Thompson Seedless concentrate (Tipton et al., 1999). In addition, 100% juice blends were prepared with 60% blueberry juice and 40% grape juice. The Venus and Concord blends were rated low in aroma and flavor intensities; however the Concord blend was perceived as rich in color, but predominate in grape flavor thus lacking the flavor profile of blueberry characteristics (Tipton et al., 1999).

However in consumer testing, a juice blend containing 60% blueberry juice, 20% Concord grape juice, 15% high fructose corn syrup and 5% water had the highest rating in

flavor liking as oppose to the other blueberry juice blends (Price et al., 1993). Main et al. (2001) investigated the quality and stability of blueberry juice blends formulated at 70%, 50%, and 25% blueberry with apple, grape, and cranberry juice. In this study, blueberry juice blended with apple and cranberry juice cocktail, overwhelmed the blueberry characteristics, however the Concord grape juice blend was perceived as having blueberry like characteristics, though as the concentration increased, the Concord grape juice dominated the blueberry aroma, flavor, and color. Furthermore, the quality and stability of all the juice blends decreased in color pigmentation after three months of storage at 37^{o}C (Main et al., 2001). Furthermore, Roberts et al. (2004) evaluated the utilization of dried apple pomace as a press aid to improve the quality of several berry juices, including blueberry juice. Sensory evaluation showed differences between juices pressed with conventional press aids, rice hulls and paper, than those pressed with dried apple pomace. It was concluded from the flavor analysis that the differences could have been due to off-flavors from the conventional press aids used in making berry juices (Roberts et al., 2004). Taking in consideration the formulation blend as well as the juice type, it is hypothesized that blueberry juices can be blended with other juices to produce a quality and value-added product with blueberry characteristics. Therefore, the purpose of our research study was to determine the effects of selected processing techniques (i.e. thermal processing and high hydrostatic pressure) on the antioxidant activity and sensory characteristics of blueberry juice prepared from North Carolina "Croatan" blueberries.

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Chapter II

Effects of Processing on the Antioxidant Properties of Blueberry Juice

INTRODUCTION

Fruits and vegetables contain natural sources of antioxidants. Antioxidants work by neutralizing potentially dangerous free radicals that can damage cells. Several studies have reviewed the epidemiological evidence of fruit and vegetable consumption, dietary flavonoids as antioxidants and disease prevention (Neuhouser 2004; Block et al., 1992; Cook and Sammon 1996). Increased dietary consumption of fruits and vegetables rich in phytochemicals has been associated with promoting health benefits thus reducing the potential risk factors associated with certain types of cancers, heart disease, and other degenerative diseases.

Blueberries are recognized for their potential health benefits and high antioxidant capacity. The health promoting benefits are attributed to phenolic compounds, in particular anthocyandins. Anthocyanins are glycosylated anthocyandins responsible for the diverse array of colors found among many fruits and berry crops. Blueberries are one of the richest sources of antioxidants. The presence of significant amounts of anthocyanins and other phenolic compounds contributes to the powerful antioxidant activity of blueberries (Prior et al., 1998). Phenolic compounds found in blueberries are mainly distributed throughout the pomace or skin of the berry. Anthocyanin flavonoids and hydroxycinnamic acids such as chlorogenic acid are major contributors to the overall phenolic composition of blueberries (Kalt et al., 2000). The phenolic composition of berries is widely influenced by the cultivar selection, environmental conditions and maturity of the blueberries at harvest as well as processing and storage conditions. (Prior et al., 1998; Howard et al., 2003; Zadernowski et al., 2005).

Blueberries are generally processed into juices, juice concentrates, wines, and as ingredients for the production of other blueberry products. The phenolic compounds present in blueberry and blueberry products may be beneficial to the human diet. However during processing, the levels of antioxidants may be altered resulting in a change in the antioxidant content of the final product. Basic fruit juice processing involves crushing, partial maceration with pectinolytic enzymes, temperature adjustments and pressing to release the juice. The additions of pectin enzymes and heat have been used to improve the extractability and quality of juices as well as increase the overall juice yield (Fuleki and Hope, 1964; Kalt et al., 2000; and Buchert et al., 2005).

North Carolina is one of the leading producers of blueberries. In 2005, North Carolina ranked third in the value of blueberry production and fourth in total acreage; therefore it is important to find novel processing technologies or modified techniques that can be utilized to increase the production and optimization of "value-added" blueberry products, thus increasing the economic impact of North Carolina blueberry farms. To meet consumers' demands for products with bioactive components rich in antioxidants, it is essential for food processors to know the affect of processing procedures on the nutrient concentration and the bioactivity of compounds present in the final product. The objective of this study was to determine the effects of different processing techniques, thermal processing and high hydrostatic pressure (HHP) on the antioxidant activity of blueberry juice. Antioxidant levels were measured by changes in total anthocyanins, total phenols, and Oxygen Radical Absorbance Capacity (ORAC) values.

MATERIALS AND METHODS

Individually Quick Frozen (IQF) Croatan blueberries (7 cases of 32 lbs) were obtained from Solo Foods, Burgaw, North Carolina. The blueberries were transported to the Department of Food Science and held frozen at -23^{o}C until used.

Blueberry Juice Processing

Thermal Processing

IQF blueberries were thawed and pulverized using a hand-operated pulper. Crushed berries were treated with a pectin enzyme Rapidase (Presque Isle Wine Cellars, North East, PA) at a ratio of 0.1 ml/lb of crushed berry weight. The slurry was further macerated and chilled overnight under refrigerated conditions at 3^{o}C, prior to applying processing treatments.

Blueberry juices were obtained using the following processing techniques: cold processing and hot processing. Juices obtained using the cold processing method, involved pressing the crushed berries at room temperature (22^{o}C) whereas the hot processing method involved rapid heating to 43^{o}C and 75^{o}C for 30 sec. using a steam jacketed kettle, followed by rapidly cooling in an ice bath. The crushed blueberries were pressed using a stainless steel basket bladder press (Presque Isle Wine Cellars, Northeast, PA) with 100% cotton pressing cheesecloth followed by further pasteurization of the juice at 75^{o}C for 30 sec. After pasteurization the juice was packaged in half gallon plastic jug containers, and frozen at -23^{o}C (Appendices Figure 1). Aliquots from all treatments were taken for chemical analyses to measure changes in total anthocyanins, total phenols, and antioxidant activity using Oxygen Radical Absorbance Capacity (ORAC) values. Juice yields were calculated based on the initial weight of the crushed berries for each processing treatment.

Hydrostatic Pressure Processing

Preliminary experiments were conducted by applying pressure using a high hydrostatic pressure unit (CIP 22260, Autoclave Engineers, Erie, PA). Approximately 10 pounds of IQF blueberries were pulverized using a hand-operated pulper. Crushed berries were treated with pectin enzyme Rapidase (Presque Isle Wine Cellars, North East, PA) at a rate of 0.1 ml/lb of crushed weight, followed by maceration and chilled overnight under refrigerated conditions at 3^{o}C, prior to applying processing treatments. Crushed berries were hot sealed in plastic pouches holding approximately 300 g of enzyme-treated crushed berries. The berries were subjected to high pressure at 400 MPa at 10 min, 20 min, and 30 min holding times (Appendices Figure 2). The pressure processed slurry was pressed using a stainless steel basket bladder press (Presque Isle Wine Cellars, Northeast, PA) with 100% cotton pressing cheesecloth. Aliquots of samples were taken for chemical analyses to measure changes in total anthocyanins, total phenols, and antioxidant activity using Oxygen Radical Absorbance Capacity (ORAC) values.

Measurement of Antioxidant Activity

Total Anthocyanins and Polymetric Color

The total monomeric anthocyanin content of the juice samples were determined by the pH differential method (Guisti and Wrolstad, 2001). Samples were diluted to the appropriate concentration with 0.025M potassium chloride buffer (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5). The absorbance was measured in the Spectronic Gynesis 2 UV-Visible spectrophometer (Thermo Electron Scientific Instruments Corporation, Madison, WI) using 1 cm path length disposable cells at 520 nm and 700 nm after 15 min of incubation at

room temperature. The absorbance and anthocyanin pigment of the juice sample was calculated based on cyanidin-3-glucoside as follows:

$$A = (A_{520}-A_{700})_{pH\ 1.0} - (A_{520}-A_{700})_{pH\ 4.5} \quad (1)$$

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/ (\varepsilon \times 1) \quad (2)$$

where A=absorbance, MW=molecular weight (449.2 g/mol), DF=dilution factor and ε= molar extinction coefficient (26900 L cm^{-1} mol^{-1}). The content of total anthocyanins was expressed as milligrams of cyandin-3-glucoside equivalents (Cy-g) per liter.

The percent colormetric content of juice samples was determined by the bisulfite bleaching method as described by Guisti and Wrolstad (2001). Juice samples were diluted using the appropriate dilution factor and treated with either potassium metabisulfite solution ($K_2S_2O_5$) or distilled water. The samples were incubated at room temperature for 15 min prior to measuring absorbance readings in the Spectronic Gynesis 2 UV-Visible spectrophometer (Thermo Electron Scientific Instruments Corporation, Madison, WI) at 420 nm, 520 nm and 700 nm. The percent polymeric color was based on the color density of the control sample and the polymeric color of the bisulfite bleached sample as follows:

$$\text{Color density} = [(A_{420nm}-A_{700nm}) + (A_{520nm}-A_{700nm})] \times DF \quad (3)$$

$$\text{Polymeric color} = [(A_{420nm}-A_{700nm}) + (A_{520nm}-A_{700nm})] \times DF \quad (4)$$

$$\%\ \text{Polymeric color} = (\text{polymeric color}/\ \text{color density}) \times 100 \quad (5)$$

where A=absorbance and DF=dilution factor.

Total Phenols

The total phenolic content of the blueberry juice was determined colormetrically using the Folin-Ciocalteu method (Amerine and Ough, 1980). Juice samples were diluted to fall within the range of the calibration curve. The calibration curve was prepared using 0, 20, 100, 150, 250, 500, 750 mg/L of gallic acid equivalents (GAE). The samples were

incubated for 2 hr in the dark at room temperature prior to measuring the absorbance reading at 765 nm in the Spectronic Gynesis 2 UV-Visible spectrophotometer (Thermo Electron Scientific Instruments Corporation, Madison, WI). Quantification of the data was calculated based on the calibration curve generated using gallic acid as the standard and the results was expressed as mg/L of gallic acid equivalents (GAE).

Oxygen Radical Absorbance Capacity

The total antioxidant capacity was performed in a Tecan Safire Microplate Reader (Tecan US, Durham, NC) using the modified method of Prior et al. (2003). The antioxidant activity was measured by the ability of antioxidants to inhibit or decrease the degradation of Flurorescein (Sigma-Aldrich, Milwaukee, WI) in the presence of AAPH (2, 2'-azobis (2-amidino-propane dihydrochloride) (Wako Chemicals USA, Inc., Richmond, VA), a peroxyl radical generator which initiates the breakdown of Flurorescein. Phosphate buffer at pH 7.4 was used as a blank and Trolox, a water soluble Vitamin E analogue, (MP Biomedicals, Inc., Salon, Ohio) was used as the control standard for antioxidant activity using the following concentrations: 1.56, 3.125, 6.25, 12.5, and 25 µM. Juice samples were diluted to the appropriate concentration with phosphate buffer. Preparation of analysis was conducted using a 96-well plate (Costar) consisted of the following:

1. Blank = 130µL of buffer + 60µL of Fluorescein + 60µL of AAPH
2. Trolox = 70µL of buffer +60µL of Trolox + 60µL of Fluorescein + 60µL of AAPH
3. Sample = 70µL of buffer + 60µL of Sample+ 60µL of Fluorescein + 60µL of AAPH

The fluroresein, phosphate buffer and samples were incubated for 15 min between 37^{o}C ± 1^{o}C prior to the addition of the AAPH. The fluorescence was recorded every two minutes for approximately 80 min with an emission wavelength of 520 nm and excitation wavelength of 485 nm. Quantification of the ORAC value was determined by using a regression

equation (Y= a + bX) between the Trolox concentration and the net area under the fluorescence curve. The area under the curve was calculated using the following equation:

$$AUC= 0.5 + (R_2/R_1) + (R_3/R_1) + (R_4/R_1) +...0.5(R_n/R_1) \quad (6)$$

where R_1 is the fluorescence reading at the initiation stage of the reaction and R_n is the final measurement. The net AUC was obtained by subtracting the AUC of the blank from the AUC of the sample. The ORAC value was expressed as μmoles Trolox equivalents/Liter (μmol TE/L).

STATISTICAL ANALYSIS

The study was replicated and duplicate samples were analyzed for total anthocyanins, color indices, and total phenolic content. Triplicate samples were analyzed for total antioxidant capacity. Analyses of data were analyzed using ANOVA to determine difference between treatment means. Means were separated by Fisher's Least Significant Difference ($p<0.005$) using SAS 8.2 (Cary, NC).

RESULTS AND DISCUSSION

Effects of Thermal Processing on the Antioxidant Activity of Blueberry Juice

Juice yields were calculated based on the initial weight of the crushed berries for each processing treatment. Average juice yields for extraction temperatures 22°C, 43°C, and 75°C were 66.1%, 84.6%, and 87.8% respectively. A better juice extraction was obtained from the hot processing methods thus resulting in a better extraction yield of phenolic compounds in the juices.

The total anthocyanin content of blueberry juice was influenced by temperature changes. Figure 1 shows the total anthocyanin content for "Croatan" berry juice pressed at 22°C, 43°C, and 75°C. Berries subjected to cold processing showed the least amount of total anthocyanin content and was statistically significant from the other processing treatments.

Samples processed at 75°C contained the highest content of anthocyanins and were significantly higher in content compared to the pasteurized hot pressed juice at 43°C. Although the hot pressed juice at 43°C had slightly higher anthocyanin content than the pasteurized juice from cold processing, there were no significant differences among the two processing treatments.

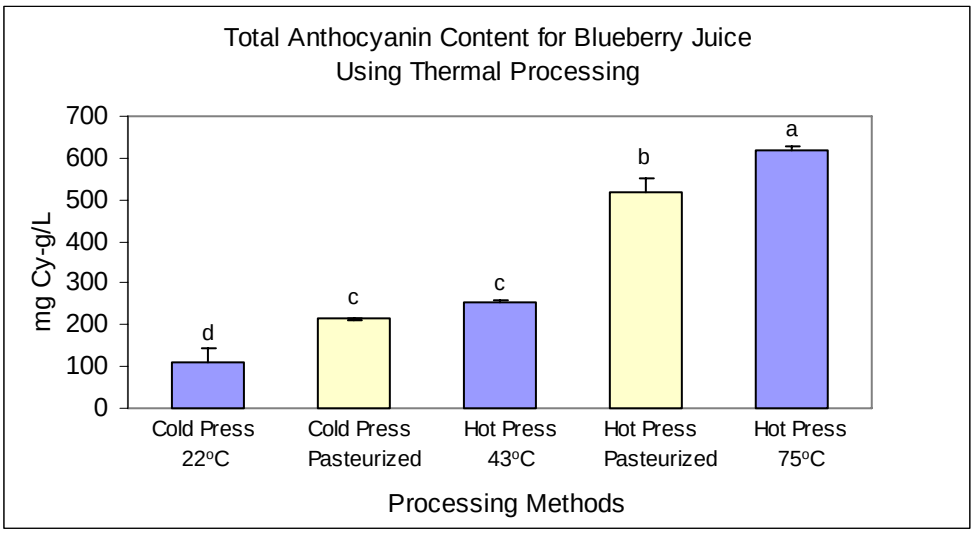

Figure 1. Total anthocyanin content of blueberry juice using thermal processing. Mean values followed by different letters are significantly different ($p<0.05$).

Mazza and Miniati reported highbush blueberries have a total anthocyanin content ranging from 25-495 mg/100 g (1993). Prior et al. (1998) reported the total anthocyanin values for several North Carolina blueberry cultivars such as Reveille, Blue Ridge, Cape Fear, Pender and Bladen. The pigment content ranged from 62.6 mg/100 g to 157.4 mg/100 g (Prior et. al, 1998). The wide range in variation is believed to be due to such factors as the genotype, growing season, and maturity of the berry at harvest (Howard et. al, 2003; Prior et. al, 1998).

Anthocyanins are glycosylated anthocyandins responsible for the diverse array of colors found among many fruits and berry crops. Differences in processing techniques can have an affect on the color quality of the juices. The effect of processing on juice color was quantified by measuring color density, polymeric color, and percent polymeric color. The percentage of polymeric color is a result of the degree of anthocyanin polymerization (Rommel et al., 1992). Thermal processing at different temperatures influenced the anthocyanin polymerization of blueberry juices. The color density increased due to the increase in polymeric color (Table 1). There was a significant increase in the color density and polymeric color at 75°C. The percentage of polymeric color from the hot processing at 43°C was not significantly different from the hot processing at 75°C or the pasteurized juices.

Table 1. Color Indices of Blueberry Juice Using Thermal Processing

Juice Samples	Color Density Index	Polymeric Color Index	% Polymeric Color
cold press 22°C	10.9d	1.9d	17.5c
cold press pasteurized	16.6cd	3.4cd	20.7cb
hot press 43°C	22.0c	5.1c	23.2ab
hot press pasteurized	31.3b	7.9b	25.3ab
hot press 75°C	39.9a	10.8a	27.2a

Statistical analysis ANOVA and Fisher's LSD was performed using SAS 8.2. Mean values in a column followed by different letters are significantly different ($p<0.05$).

Ju et al. (2003) investigated the effects of solvent and temperature on anthocyanins and phenolic compounds from dried red grape skin. Increase in color density and polymeric color resulted in the greatest contribution of polymers to color formation, thus indicating a higher anthocyanin degradation at extreme temperatures greater than 100°C (Ju et al., 2003). Based on the current study and previous work (Carlson, 2003) if processing conditions include temperatures above 90°C, it is believed that there will be a decrease in the total

anthocyanin content thus indicating the occurrence of anthocyanin degradation in the blueberry juice.

The total phenolic content of the blueberry juice increased with increasing temperature (Figure 2). Hot processing at 75°C had the greatest total phenolic content with an average value of approximately 2490 mg of Gallic acid equivalents per liter (GAE/L); however the amount of total phenolics did not differ significantly from the pasteurized juice obtained from the hot processed berries at 43°C. The hot pressed juice (43°C) had a higher phenolic content than the pasteurized juice from cold processing but a lower phenolic content compared to the pasteurized juice from hot pressed berries at 43°C.

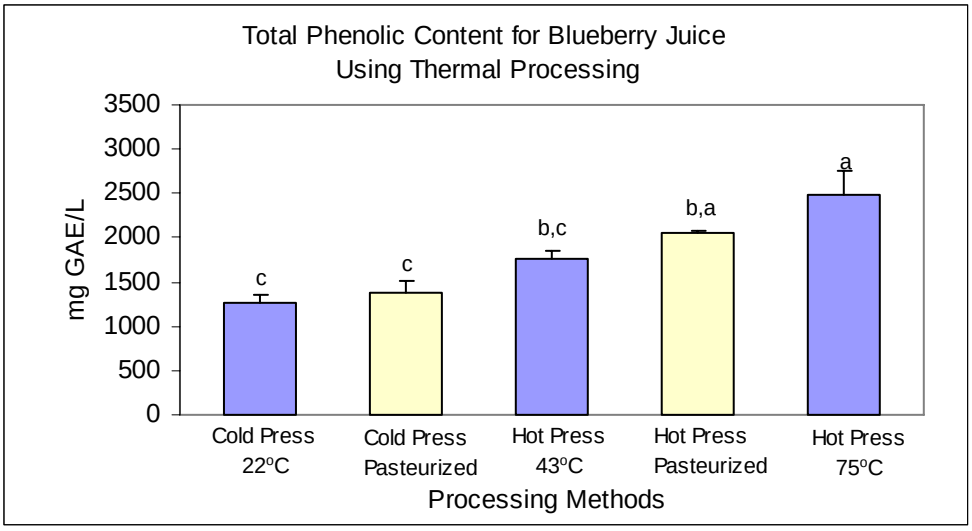

Figure 2. Total phenolic content of blueberry juice using thermal processing. Mean values followed by different letters are significantly different ($p<0.05$).

It has been shown that the application of heat can increase the phenolic content in blueberry juice (Kalt et al., 2000). In our study, the phenolic content increased approximately 50% in the juice extraction at 75°C compared to the extraction at 22°C. The increase in phenolic content may have been due to the increased permeability of the cell

membranes within the skin during the maceration of the blueberries using heat at elevated temperatures (Spanos et al., 1990; Kalt et al., 2000; Rossi et al., 2003).

Thermal processing treatment at 75°C increased the total antioxidant activity of the blueberry juice (Figure 3). Results indicated the same trend for the extraction of juices with increasing temperatures as in the total phenols. Hot processing at 75°C had the highest antioxidant capacity as well as total phenolic content. The total antioxidant activity increased approximately 3.5-fold at 75°C compared to the juice extraction from the cold processing at 22°C. Although there was a slightly higher antioxidant capacity at 75°C, there was not a significant difference in ORAC values compared to the pasteurized juice obtained from hot processing at 43°C. Cold processing procedures exhibited the lowest antioxidant activity and differed significantly from the other treatments.

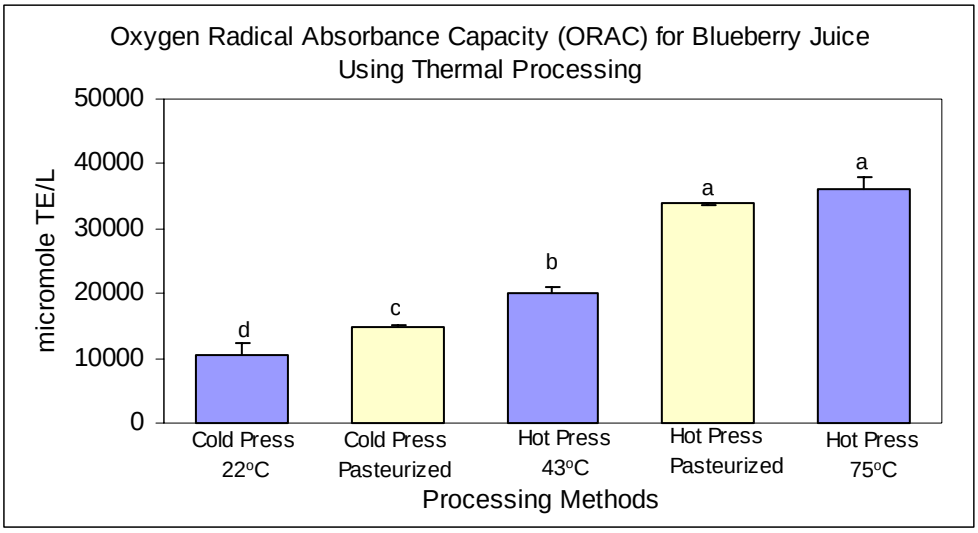

Figure 3. Total antioxidant capacity of blueberry juice using thermal processing. Mean values followed by different letters are significantly different ($p<0.05$).

Overall, the total antioxidant capacity of the blueberry juice was affected by the different extraction temperatures. Furthermore, pasteurization of the cold and hot pressed

juice, 22°C and 43°C, respectively caused an increase in antioxidant activity. In our study, the cold pressed juice (22°C) showed the lowest antioxidant activity; therefore cold pressing was not as efficient in releasing phenolic compounds thus having an effect on the overall total antioxidant capacity. A similar trend was observed for the total antioxidant capacity of blueberry juice pressed at elevated temperatures ranging from 22°C to 90°C (Carlson, 2003). These findings indicate that through selective processing methods, blueberry juice can maintain substantial amounts of phenolic compounds, thus contributing to their increasing levels in antioxidant activity after processing as determined by ORAC values.

Effects of High Hydrostatic Pressure on the Antioxidant Activity of Blueberry Juice

Thermal processing of food or beverage products using high temperatures can involve the loss of valuable nutrients and sensory qualities. However high hydrostatic pressure (HHP) can be used to minimize the detrimental effects caused by thermal processing. The effect of HHP on the antioxidant properties of blueberry juice was investigated. Berries were subjected to 400 MPa for 10, 20 and 30 minute holding times and pressed for juice at room temperature. The antioxidant activity was measured by changes in total anthocyanins, total phenols, and ORAC values.

The total anthocyanin content of the blueberry juice prepared from crushed berries pressurized at 400 MPa for 30 minutes was significantly different from the 20 min and 10 min holding times. Results showed the highest amount of anthocyanins was recovered from the 30 min holding time, with an average anthocyanin content of 197 mg/L of cyandin-3-glucoside equivalents (Figure 4). The HHP_{10min} and HHP_{20min} extractions were not as effective in maximizing antioxidant yield in the blueberry juice. However, using a pressure

greater than 400 MPa for 10 to15 minutes may increase the anthocyanin yield in the blueberry juice.

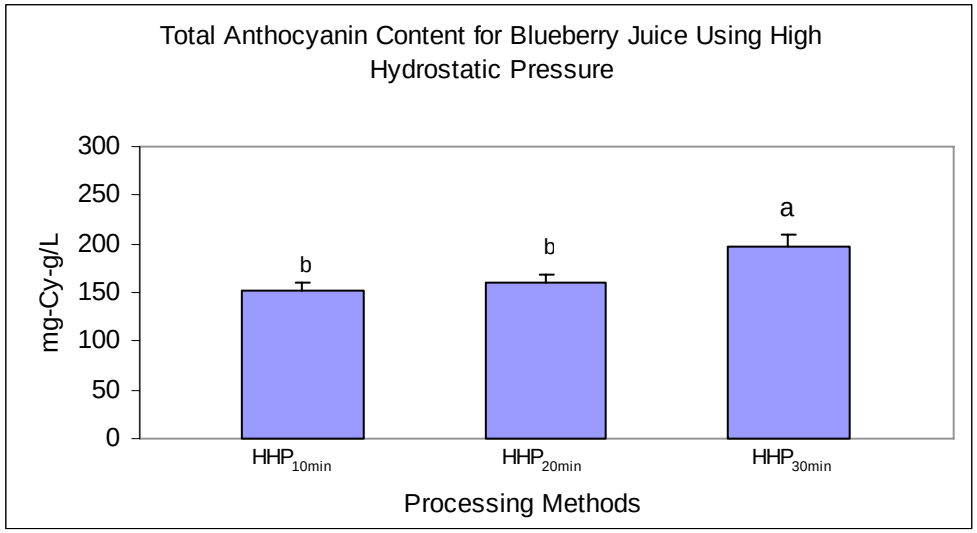

Figure 4. Total anthocyanin content of HHP blueberry juice at 400MPa for 10, 20, and 30min. Mean values followed by different letters are significantly different ($p<0.05$).

Suthanthangjai et al. (2005) investigated the effects of high hydrostatic pressure on the stability of anthocyanins in raspberries. Fruit puree was subjected to 200, 400, 600, and 800 MPa for 15 min at ambient temperatures between 18-22^{o}C. The stability of anthocyanins was investigated over a period of time at various storage conditions, 4^{o}C, 20^{o}C, and 30^{o}C. Samples were analyzed for anthocyanin content after 1, 2, 4, 7, and 9 days of storage using HPLC analysis. The highest stability of anthocyanins was achieved at 200 and 800 MPa stored at 4^{o}C. The percentage of cyandin-3-glucoside losses were 18.6% and 25.0% respectively (Suthanthangjai et al., 2005). Storage at 30^{o}C resulted in a major loss of anthocyanins after nine days of storage. The percentage of anthocyanin losses at 200 MPa and 800 MPa in day one was approximately 11.9% and 13.9%, and after nine days of storage, the losses were 62.8% and 70.9% respectively (Suthanthangjai et al., 2005). In our study, the

effect of HHP on the total anthocyanin content was evaluated on the basis of processing time to determine the effects of various holding times on the anthocyanin pigment content. Previous studies conducted by our research group compared the effects of storage under refrigerated conditions on the phenolic content and antioxidant activity of blueberry juice produced using traditional thermal processing methods and microwave processing at various temperatures (Carlson, 2003). From the storage study, there was an increase in the total anthocyanin content of blueberry juices after 18 days of storage under refrigerated conditions.

In addition to the anthocyanin content, the percent colorimetric contents of the juice samples was determined by measuring the indices of color density and polymeric color to determine the effects of HHP on polymerization of anthocyanins. The color density showed a similar trend over time (Table 2) compared to thermal processing. In the HHP processing there was a relative increase in the percentage of polymeric color. However the extraction of the juice from the pressurized berries at 20 min was not statistically different from the 10 or 30 min processing treatments. The percentage of polymeric color for the HHP processing at 10, 20 and 30 min holding times were similar in value to the pasteurized cold pressed juice and the hot pressed juice (43^{o}C).

Table 2. Color Indices of Blueberry Juice Using High Hydrostatic Pressure

Juice Samples	Color Density Index	Polymeric Color Index	% Polymeric Color
HHP_{10min}	17.8a	3.6a	20.4b
HHP_{20min}	18.11a	3.9a	21.9ab
HHP_{30min}	20.5a	4.5a	22.5a

HHP: High Hydrostatic Pressure 400MPa at 10 min, 20 min, and 30 min. Mean values in a column followed by different letters are significantly different ($p<0.05$).

The HHP_{20min} did not differ significantly in total phenolics from HHP_{10min} or the HHP_{30min} treatments. However, the HHP_{30min} had a slightly higher phenolic content than the HHP_{20min} followed by the HHP_{10min} (Figure 5). The HHP_{30min} yielded an average phenolic content of 1820 mg of Gallic acid equivalents per liter (GAE/L). This value was within the range of values reported for the hot pressed juice at 43°C and the pasteurized juice with an average phenolic content of 1735.5mg GAE/L and 2049.1 mg GAE/L respectively. Statistically, equivalent total phenolic content was achieved between the HHP_{30min} and the hot processed 43°C blueberry juice. The cold processing method and the HHP_{10min} exhibited the lowest phenolic content and did not differ significantly from the pasteurized cold pressed juice or the HHP_{20min}. In contrast, the hot pressed juice at 75°C was superior in recovering more phenolic compounds than the HHP treatments (Figure 6).

The total antioxidant capacity did not show any significant differences between the 20 min and 30 min treatments (Figure 7). HHP_{30min} had the greatest antioxidant capacity, followed by HHP_{20min} with an increase of approximately 2-fold in antioxidant capacity compared to the processing treatment HHP_{10min}. In comparison to the thermal processing, the antioxidant capacity of HHP_{20min} did not differ significantly from the hot pressed juice (43°C), and equivalent antioxidant capacity was achieved between the two treatments. Furthermore, ORAC value for the HHP_{30min} was similar to the hot pressed juice (43°C), but was not superior to the total antioxidant capacity in the hot pressed juiced at 75°C based on the ORAC value (Figure 8).

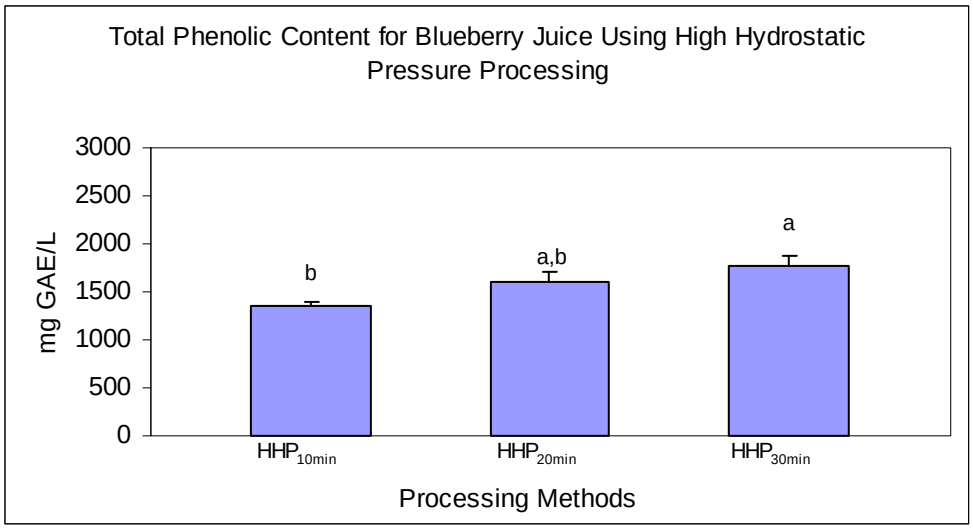

Figure 5. Total phenolic content of HHP blueberry juice at 400 MPa for 10, 20 and 30min. Mean values followed by different letters are significantly different ($p<0.05$).

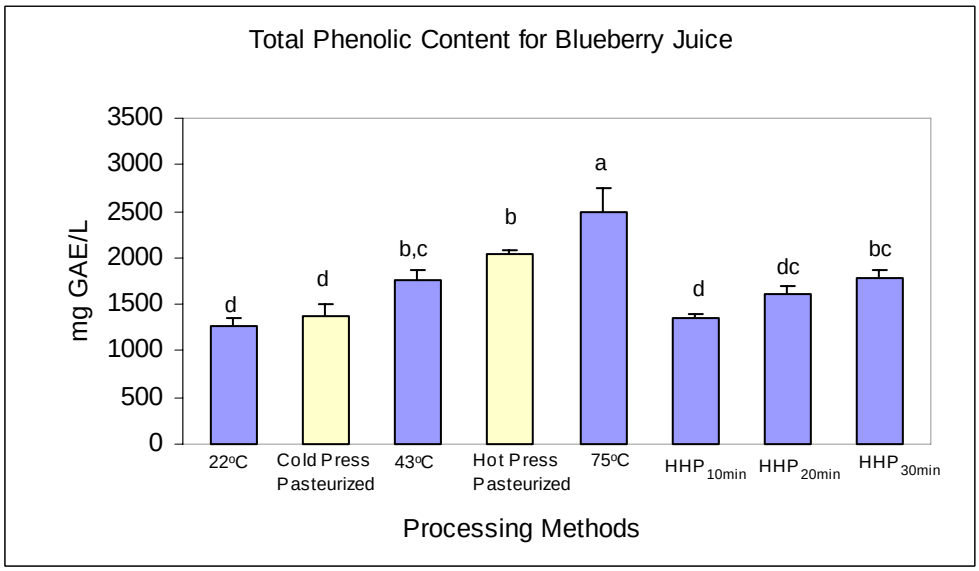

Figure 6. Comparison of the total phenolic content of thermal processed and HHP blueberry juice at 400 MPa for 10, 20, and 30min. Mean values followed by different letters are significantly different ($p<0.05$).

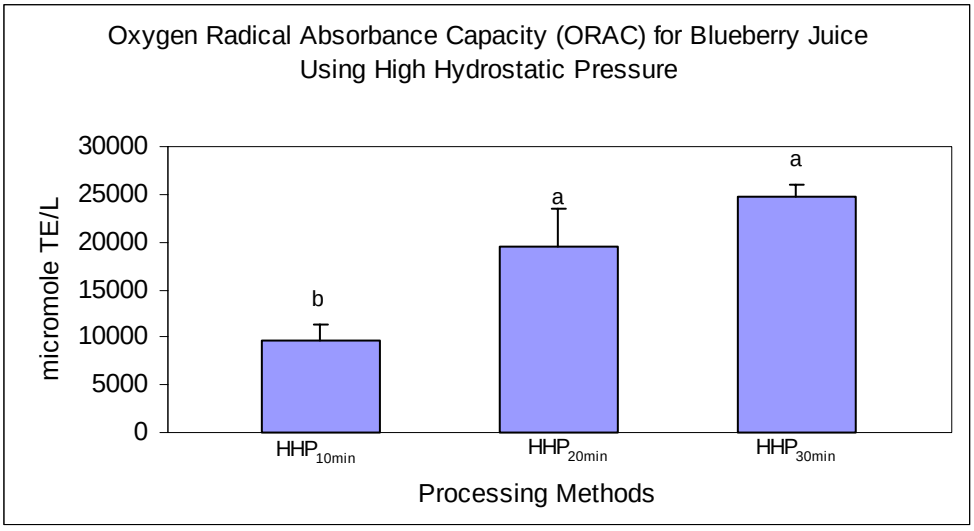

Figure 7. Total antioxidant capacity of HHP blueberry juice at 400 MPa for 10, 20, and 30min. Mean values followed by different letters are significantly different ($p<0.05$).

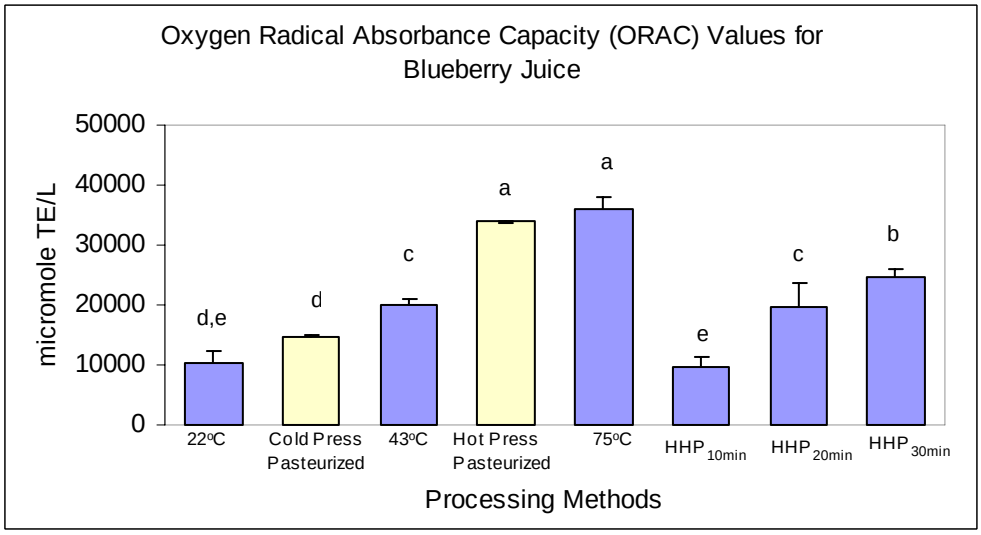

Figure 8. Comparison of the total antioxidant capacity of thermal processed and HHP blueberry juice at 400 MPA for 10, 20, and 30min. Mean values followed by different letters are significantly different ($p<0.05$).

The pressure and temperature ranges commercially used for HHP are between 400-600 MPa at 18-22^{o}C (Zabetakis et al., 2000 ; Suthanthangjai et al., 2005). Though using high pressure 400 MPa for 20 min and 30 min holding times resulted in high antioxidant capacity in the blueberry juice, long duration of holding times would not be feasible for a large scale production of blueberry juice. Combining pressures of 700 MPa and temperatures of 70^{o}C-90^{o}C has been utilized to sterilize low acid foods (Ramaswamy et al., 2005). Cano et al. (1997) investigated the effects of high pressure treatment (50-400 MPa) combined with heat treatment of 20-60^{o}C on enzyme inactivation in strawberry and orange juice. The optimal inactivation of polyphenoloxidase (POD) in strawberry puree was obtained using 230 MPa and 43^{o}C for 15 min (Cano et al., 1997). A study conducted by Kimura et al. (1994) reported the quality of pressurized strawberry jam was efficient in preserving the fresh like characteristics and anthocyanin content, however the thermally processed jam maintained the highest quality for several months after processing under room storage conditions.

The use of HHP has several advantages over thermal processing. HHP can reduce processing time instantaneously while retaining sensory attributes such as flavor, texture, appearance, and color. High hydrostatic pressure has been applied to various applications within the food industry to achieve food preservation, enzyme inactivation, and starch gelatinization (Ludikhuyze et al., 2003). Overall, HHP can be used to improve the retention of various nutrients and phytochemicals that are naturally present in blueberries. However there are some challenges associated with applying high pressure to food or beverage products such as maintaining the quality of the product under various storage conditions and the affect pressure has on enzyme activities. Though thermal processing is generally

considered the most effective processing method to inactivate polyphenoloxidase (PPO) and inhibit enzymatic browning, the presence of PPO is still a concern to food processors due to the deterioration and enzymatic browning of food and beverage products after processing and storage conditions (Weemaes et al., 1998; Ludikhuyze et al., 2003). However, the combined use of limited temperature applications and HHP may provide optimal conditions in producing a blueberry juice containing superior sensory qualities while minimizing loss of color.

CONCLUSION

Antioxidant activity of blueberry juice was measured by changes in total anthocyanins, total phenols and Oxygen Radical Absorbance Capacity (ORAC) values. There was a greater extraction of anthocyanins and phenolic compounds with increasing temperatures, thus contributing to the high antioxidant capacity as determined by ORAC. The HPP_{10min} juice differed significantly from the HHP_{20min} and the HHP_{30min} juices in its ability to scavenge free radicals based on ORAC values. However, the antioxidant capacity of HHP_{20min} did not differ significantly from the hot pressed juice (43^{o}C), and equivalent antioxidant capacity was achieved between the two treatments. Furthermore, the ORAC value for the HHP_{30min} juice was similar to the hot pressed juice (43^{o}C), but was not as high in total antioxidant capacity as the hot pressed juiced at 75^{o}C. The application of greater levels of HHP may be an effective non-thermal processing technique to maximize antioxidant activity while minimizing sensory losses during blueberry juice processing. Additional research is needed to further develop a "value-added" quality blueberry juice product that could impact the economic growth of North Carolina blueberry farms and industry. Future studies should include a storage study to determine the optimum processing

and storage conditions for blueberry juice that would maintain stability of anthocyanins and other phenolic compounds while minimizing the detrimental effects on sensory characteristics.

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Appendices

Blueberry Juice Processing Flow Diagram

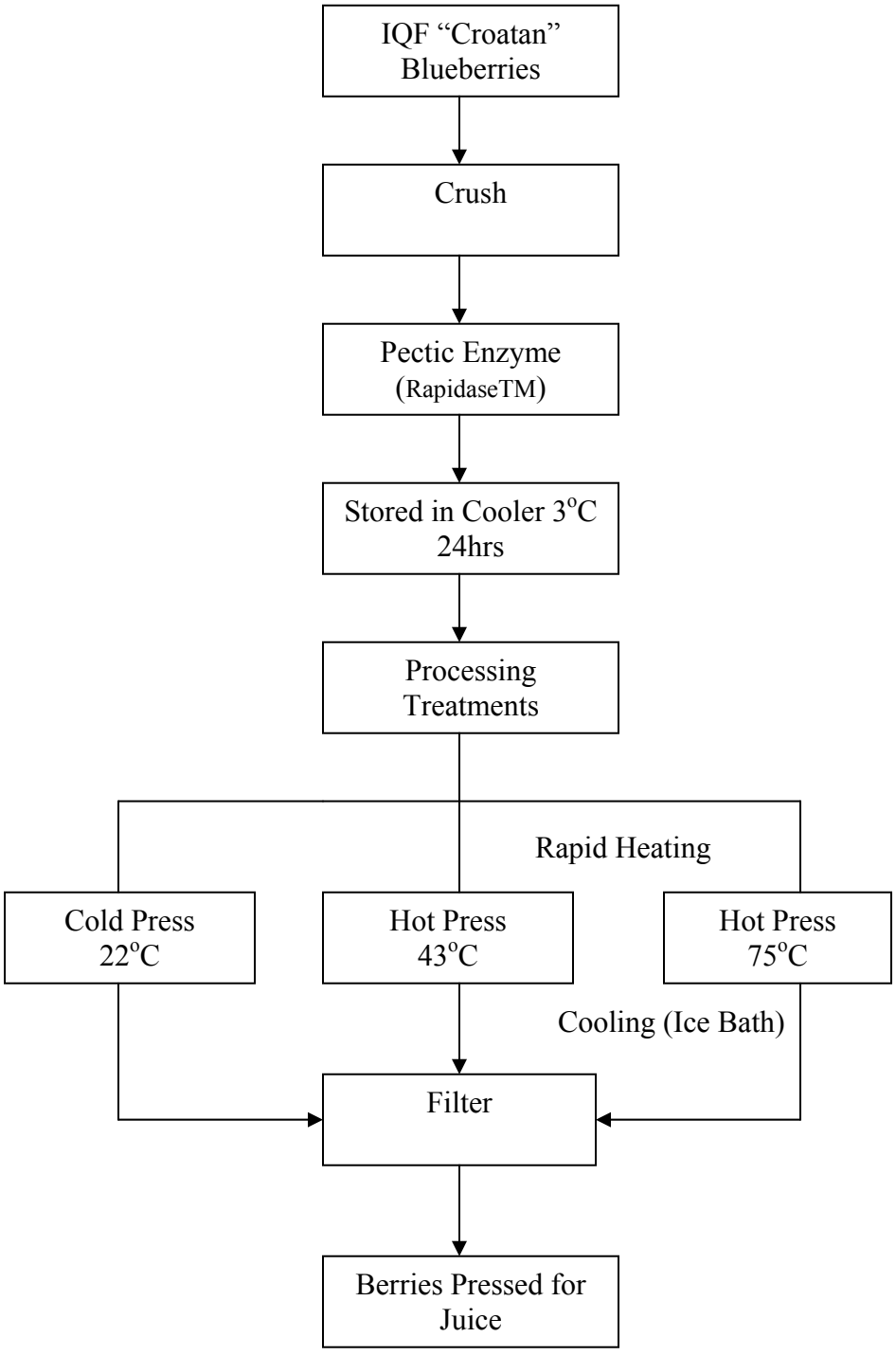
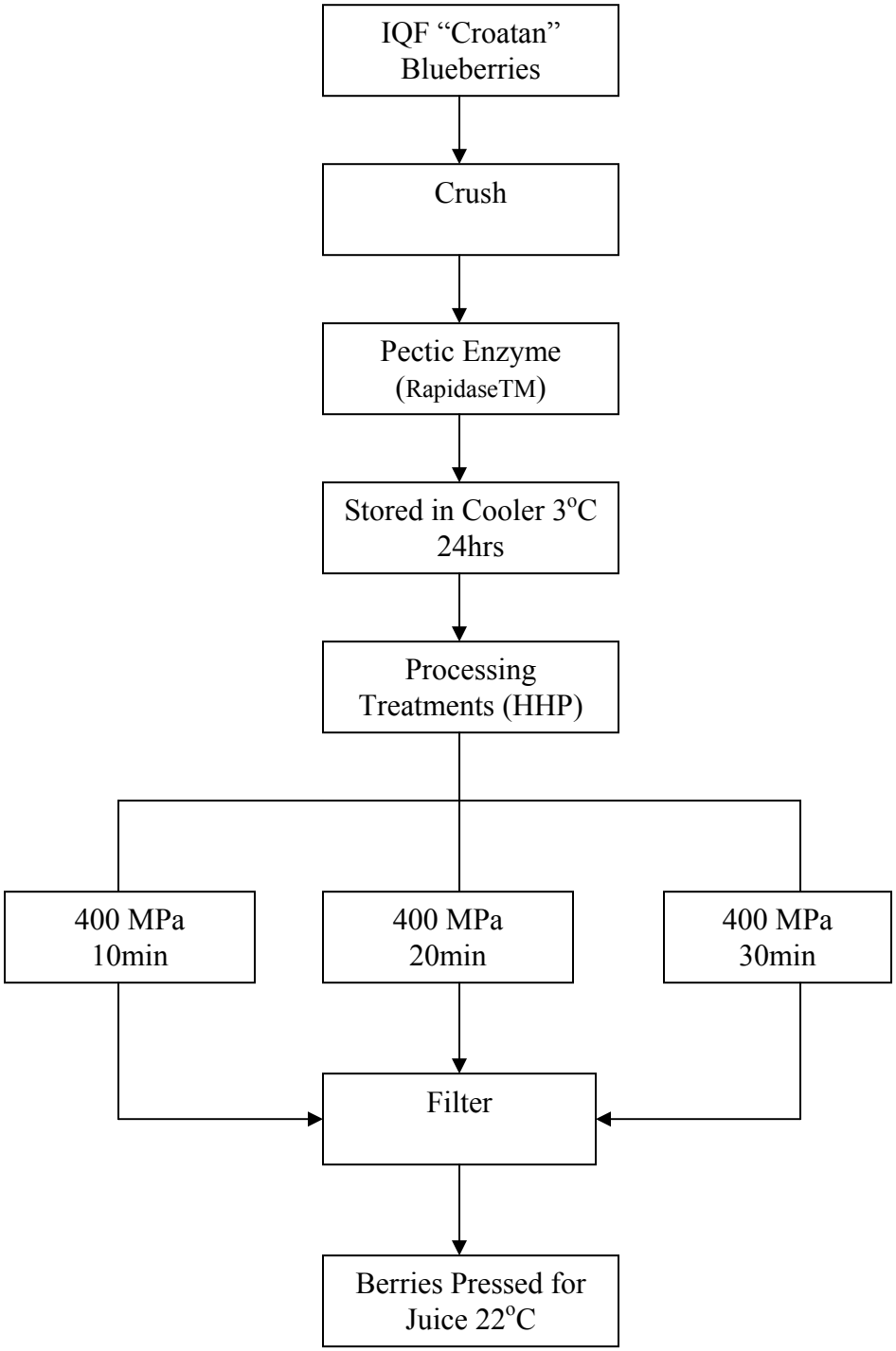


Figure 1. Blueberry juice processing flow diagram using thermal processing.

Blueberry Juice Processing Flow Diagram



Aliquots of Samples Taken, Stored in Freezer -23°C

Figure 2. Blueberry juice processing flow diagram using high hydrostatic pressure.

Chapter III

Consumer Acceptability of Processed Blueberry Juice

INTRODUCTION

Fruits and berries such as blueberries rich in phytochemicals are increasingly popular in the human diet. Through research studies and epidemiological evidence, consumers are becoming more knowledgeable of the relationship between the nutritional composition of the food or beverage product, rich in phytochemicals and other nutrients, and the health promoting attributes that may reduce risk factors associated with certain types of cancer, heart disease, and other degenerative diseases. The current market for blueberries includes processed products such as juices, juice concentrates, and wines. A variety of fruit and berry juices are processed and marketed as refrigerated, shelf-stable, and frozen products. In 2000, the United States retail sales of fruit juice beverages were approximately $15 billion with and estimated projection of $18 billion in 2005 (McLellan and Padilla- Zakour, 2005).

The utilization of selective processing techniques using elevated temperatures has improved extraction of anthocyanins and other phenolic compounds from blueberries. However, differences in processing methods and storage conditions may influence the sensory characteristics and nutritional content of the juice product. During processing, structural changes of the fruit occur as well as changes in the composition of flavor compounds. Phenolic compounds play an active role in food flavor as it relates to taste and aroma. The four main groups contributing to flavor characteristics of fruits are organic acids, sugars, bitter or astringent attributes, and volatile flavor constituents (Taylor, 2001; Tomás-Barberán and Espín, 2001). The organic acids (i.e. citric, malic, or lactic acids) provide tartness to the fruit whereas sugars contribute to the sweetness and body. Furthermore, certain phenolic compounds (i.e. tannins) are associated with the bitterness and astringency and can also contribute to the composition of aroma volatile profiles (Taylor, 2001).

The attractive color of fruit and berry juices is one of the key factors influencing consumer perception when evaluating products (Rein et al., 2004). Processing fruit juices can have detrimental effects on the product such as anthocyanin degradation and browning. The methods of processing and fruit variety are essential factors when developing a quality juice product. Therefore the objective of this study was to determine consumer acceptability of blueberry juices developed from selective processing techniques.

MATERIALS AND METHODS

Physiochemical Analyses

Total soluble solids (TTS) were determined as degree Brix using a CHASE hand-held refractometer (Thomas Scientific Incorporated). The pH was determined using a pH meter (ORION Research model 601A/digital analyzer). Titratable acidity was determined by diluting 10 ml of juice to 100 ml with deionized water and titrating to an endpoint of 8.2 with 0.1N sodium hydroxide (NaOH). Results were expressed as percent citric acid.

Juice Sample Preparation

Samples subjected to sensory evaluation included juices prepared from individually quick frozen (IQF) Croatan blueberries using thermal processing methods, cold processing at 22^{o}C, and hot processing at 43^{o}C and 75^{o}C prior to pressing. Following pressing, the juices were pasteurized at 75^{o}C. A blueberry juice blend was prepared with 100% apple juice from frozen concentrate (pasteurized) to optimize the strongly flavored pure blueberry juice. The 100% apple juice concentrate was purchased from a local grocery store within two weeks of the consumer evaluation and stored in the freezer until used. In addition, a commercial 100% blueberry juice product was purchased from a local grocery store to use in the sensory evaluation.

A blueberry juice blend was prepared containing 80% blueberry juice and 20% apple juice made from concentrate. The 100% apple juice was reconstituted according to manufacture's instructions. The juice blend was prepared within 24hrs of the sensory evaluation. Samples containing 30 ml of juice were placed in 4oz clear plastic cups, labeled with three-digit random codes, with lids and stored under refrigerated conditions until the day of the consumer testing. A total of four blueberry juice blend samples were evaluated for consumer acceptability.

Sensory Evaluation Procedure

The study was approved by the Institutional Review Board at North Carolina State University. Seventy-nine panelists consisting of faculty, staff, and students were recruited from the Department of Food Science at North Carolina State University via electronic mail and fliers. Prior to evaluating the samples, subjects were presented with an informed consent form and a consumer juice questionnaire. Panelists were provided with water, a ballot and napkin. Four samples were presented simultaneously in random order. The panelists were asked to taste the samples in the order presented and to cleanse their palates with water between sampling. Panelists were asked to rate the degree of liking/disliking and intensity levels for each attribute for a given sample using a nine-point hedonic scale with nine structural levels ranging from 9 "like extremely" through 5 "neither like nor dislike" to 1 "dislike extremely". The intensity levels ranged from 9 "high" through 5 "moderate" to 1 "low". Evaluation of the samples was based on the following attributes: overall acceptability, blueberry flavor intensity, blueberry flavor liking, sweetness intensity, sweetness liking, overall flavor liking, overall thickness/mouthfeel liking, and overall appearance liking.

STATISTICAL ANALYSIS

Analyses of data were analyzed using ANOVA to determine differences between treatment means. Means were separated by Fisher's Least Significant Difference ($p<0.005$) using SAS 8.2 (Cary, NC).

RESULTS AND DISCUSSION

Physiochemical Characteristics

The pH of the pasteurized juices and titratable acidity were measured before and after the addition of apple juice (Table 1 and Table 2). The total soluble sugars as indicated by ^{o}Brix for the experimental blueberry juices were approximately 11.0 ^{o}Brix. After the addition of the 100% apple juice made from concentrate the total soluble sugars increased to approximately 13.0 ^{o}Brix. In addition, the pH and titratable acidity increased among the different treatments. In comparison, the commercial blueberry juice blend had a pH of 3.32 with a titratable acidity of 0.39% citric acid and 14.0 ^{o}Brix.

Table 1. Physiochemical Characteristics of 100% Blueberry Juice

Treatment	pH	%Titratable Acidity	^{o}Brix
Cold press	3.44±0.02	0.32±0.01	11.0
Hot press 43^{o}C	3.46±0.01	0.41±0.01	11.3
Hot press 75^{o}C	3.48±0.00	0.42±0.004	11.2

Table 2. Physiochemical Characteristics of Blueberry Juice Containing 20% Apple Juice.

Treatment	pH	%Titratable Acidity	^{o}Brix
Cold press	3.47±0.01	0.36±0.01	13.1
Hot press 43^{o}C	3.48±0.02	0.43±0.004	13.0
Hot press 75^{o}C	3.49±0.00	0.44±0.01	13.1

Consumer Profile

Beverage consumption is influenced by many factors such as gender, age, and the nutritional and sensory qualities of the product. Fruit and berry juices rich in anthocyanins such as blueberry juice have become attractive to consumers due to the relative high levels of antioxidants and the potential health benefits. Panelists participating in the study were 35% males and 63% females. Seventy-five percent were within the age group of 18-35 years and 19% where between the ages of 36-55 years, thus the remaining 5% were grouped in the 56-65 age brackets. Of the consumer panelists, 87% were the primary household shopper. When consumers were asked about their consumption of fruit beverages, approximately 68% consumed fruit beverages regularly. Juice beverages are readily available on the market and sold as products of 100% juice, juice concentrates, cocktails, and juice drinks in a wide variety of flavors and blends. A poll of panelists' consumption of fruit beverage product types is shown in Figure 1. Some common factors that influence consumers of their intent to purchase fruit beverages were appearance, availability of the product, price, health and flavor of the product (Figure 2). The top three influential factors were price, flavor, and health polling at 77%, 63%, and 60% respectively.

The development of juice products is an important segment of the fruit industry as it affects local farmers, food processors and consumers. By consumers becoming increasingly aware of the potential health benefits with increased consumption of fruits and berries rich in anthocyanins and other phytochemicals, there is a need to effectively process fruits to maximize the amount of nutrients and phytochemicals retained in the final product. In addition, producing a quality product with minimum losses in sensory characteristics and that is economically viable will be beneficial to local farmers and food processors.

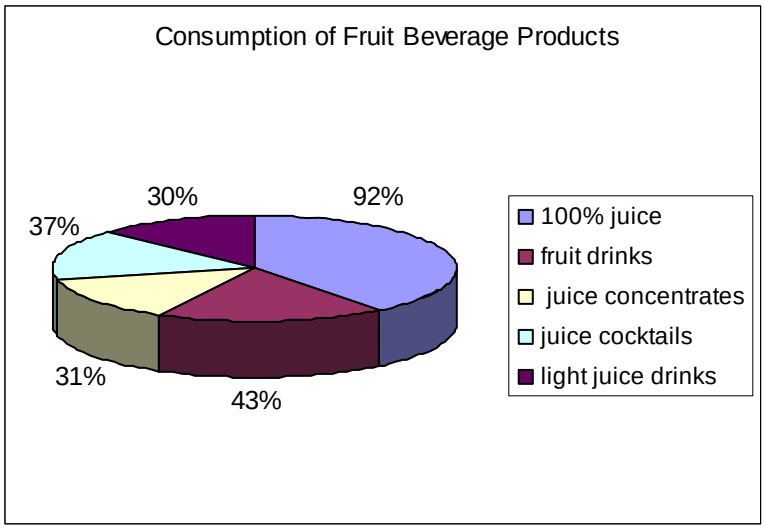


Figure 1. Demographic data of the consumption of fruit juice beverage product types.

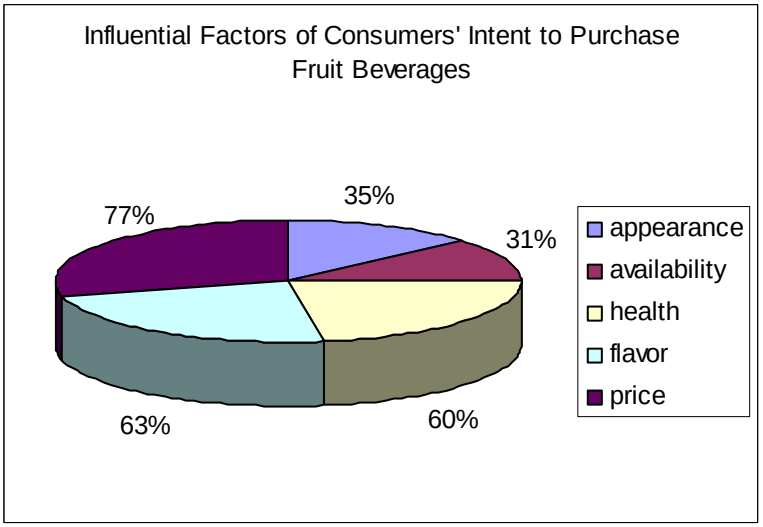


Figure 2. Demographic data of influential factors of consumers' intent to purchase fruit juice beverages.

Consumer Acceptability

This study was conducted to evaluate the acceptability of three experimental blueberry juice blends and one commercial blueberry juice product. The mean hedonic

ratings from the consumer acceptability test for the blueberry juice blends are summarized in Table 3.

Table 3. Mean Hedonic Ratings from Consumer Acceptability Test

Sensory Attributes	CP	HP_1	HP_2	Commercial Juice
Overall Acceptability	4.78^{c}	6.05^{a}	6.33^{a}	5.30^{b}
Blueberry Flavor Intensity	5.13^{b}	6.32^{a}	6.33^{a}	5.07^{b}
Blueberry Flavor Liking	4.56^{c}	6.03^{a}	6.07^{a}	5.30^{b}
Sweetness Intensity	5.06^{b}	5.60^{a}	5.77^{a}	5.53^{a}
Sweetness Liking	5.14^{c}	5.72^{b}	6.35^{a}	5.52^{bc}
Overall Flavor Liking	4.51^{c}	5.93^{a}	6.32^{a}	5.23^{b}
Overall Thickness/Mouthfeel	5.25^{b}	6.39^{a}	6.43^{a}	5.68^{b}
Overall Appearance	5.70^{b}	6.94^{a}	7.16^{a}	4.14^{c}

1. CP, HP_1, HP_2 represents the pasteurized juices prepared from berries subjected to cold press, hot press 43°C, and hot press 75°C processing methods respectively.
2. Statistical analysis ANOVA and Fisher's LSD was performed using SAS 8.2. Mean values in a row followed by different letters are significantly different ($p<0.05$).

Treatments 1, 2, and 3 represent juices extracted from cold press and hot press berries processed at temperatures of, 22°C, 43°C and 75°C respectively whereas treatment 4 corresponds to the commercial juice product. The heat extracted juice at 75°C had the highest consumer acceptability rating as well as the highest ratings across all attributes compared to the other juices. There were no significant differences in sensory attributes between the heat extracted juice at 43°C and 75°C in sensory attributes, except for sweetness liking. The average intensity of the blueberry flavor was rated relatively the same for both treatments. Though there were no significant differences in the overall acceptability and overall appearance, the mean hedonic rating for the heat extracted juice at 75°C was slightly higher for acceptability and appearance than the juice extracted at 43°C. However, the cold press juice at 22°C was significantly different from the heat extracted juices, treatments 2 and 3. The addition of heat caused an increase in the development and release of flavor compounds present in the skin, thus increasing the flavor intensity of the blueberry juice.

The increase in phenolic content may have been due to the increased permeability of the cell membranes within the skin during the maceration of the blueberries using heat at elevated temperatures (Spanos et. al, 1990; Kalt et. al, 2000; Rossi et. al, 2003). Phenolic compounds widely distributed in fruits and berries can be affected by post harvest storage conditions and food processing techniques such as various thermal treatments, pressing, enzyme treatment, and fermentation (Tomás-Barberán and Espín, 2001). Furthermore, processing can also enhance the degradation of phenolic compounds if oxidative enzymes (i.e. polyphenoloxidase and peroxidase) are not inactivated, thus leading to chemical changes causing undesirable sensory characteristics and loss of quality within the final product (Tomás-Barberán and Espín, 2001).

The experimental juices varied in color prior to the addition of apple juice. Through observation and color index measurements the cold press juice was lighter in color than the hot press juices, which were richer in color resembling a deep purplish color. The commercial product was rated in the "neither like nor dislike category" (5.07-5.68) for all attributes except for overall appearance which received a low rating of 4.14. Though the blueberry flavor liking for the commercial product was rated in the "neither like nor dislike category" with a mean score of 5.30, it was considered more favorable in blueberry flavor than the cold press juice, but least favorable compared to the hot press juices.

One of the key attributes evaluated was the overall appearance of the juices. The appearance of the blueberry juices as it relates to color and clarity may have contributed to the lower ratings, thus affecting the consumers' behavior towards the acceptability of the products. The commercial product was a blueberry juice blend with apple and grape juice concentrates. The brownish color that was noted by consumers may have been due to the

percentage at which the apple juice concentrate was added to the product. Also, heating and storage conditions (i.e. at room temperature 22^{o}C) can be additional factors contributing to browning due to potential overheating of the product resulting in color loss and accumulation of polymerized anthocyanins resulting in detrimental effects on flavor and color characteristics of the juice. Several studies have investigated the stability and enhancement of berry juice color using copigmentation fortification by phenolic acids to improve and stabilize juice color during storage (Talcott et al., 2003; Rein and Heinonen, 2004). Main et al. (2001) investigated the quality and stability of blueberry juice blends formulated at 70%, 50%, and 25% blueberry with apple, grape, and cranberry juice using trained panelists. The following sensory attributes were evaluated using an unstructured 15cm line scale: intensity of blueberry flavor, oxidized flavor, body, astringency, and blueberry color. In the study, blueberry juice blended with apple and cranberry juice cocktail overwhelmed the blueberry characteristics as opposed to the grape juice blends (Main et al., 2001). Roberts et al. (2004) evaluated the utilization of dried apple pomace as a press aid to improve the quality of strawberry, raspberry and blueberry juices. A flavor analysis profile was completed on the various juices. Among the juices, blueberry had very few volatile aroma compounds identified compared to strawberry and raspberry juices. When the juice was pressed with dried apple pomace, more aroma compounds were identified (Roberts et al., 2004). Sensory evaluation showed differences between juices pressed with conventional press aids, rice hulls and paper than those pressed with dried apple pomace. It was concluded from the flavor analyses that the differences could have been due to off-flavors from the conventional press aids used in making berry juices (Roberts et al., 2004).

Sims and Morris (1987) evaluated the flavor and color of grape juices from French-American hybrid cultivars. The panelists rated the quality of the flavor and color of the juice samples from 1 to 9, with 1=very poor, 5=acceptable, and 9=excellent. In the red cultivar "Chancellor" grape juice, the heat extracted juices at 60^{o}C were rated higher for flavor and color than the immediate cold press juices. The mean ratings for flavor and color for the initial heat extracted juice was 6.2 and 7.6 respectively. After five months of storage at 37^{o}C, the heat extracted juices did not show any significant browning and maintained a superior flavor with ratings of 6.4 and 7.6 for flavor and color respectively (Sims and Morris, 1987). In contrast, the immediate cold press grape juices were rated relatively poor in sensory attributes before and after five months of storage at 37^{o}C with scores of 5.8 and 4.9 for the acceptability of color and flavor respectively. After five months of storage, the sensory qualities were rated lower in acceptance for flavor and color with mean ratings of 4.8 and 3.4 respectively, however the results did not differ significantly from the initial sensory evaluation (Sims and Morris, 1987).

The processing methods of different hybrid cultivars of grapes which included the cultivar Chancellor, a red grape, had an effect on the sensory qualities of the juice (Sims and Morris, 1987). Similar results were shown in our study with the consumer acceptance of blueberry juice flavor and overall appearance of the cold press blueberry juice rated lower than the hot press blueberry juices. This may have been due to light color of the juice as opposed to the deep rich blue-purplish color as observed in the hot press juice. In the hot press juices, more of the anthocyanin pigments and other phenolic compounds were extracted from the berries thus having an effect on flavor and color intensities of the final juice product.

CONCLUSION

This study showed that heat extracted juices was readily accepted by consumers more than the cold processing method. There were no significant differences between the heat extracted juice at 43^{o}C and 75^{o}C across all sensory attributes, except for sweetness liking. Thermally processed blueberry juice yielded a product with relatively high levels of antioxidants and a deep rich blue-purplish color which was appealing to consumers. Additional sensory evaluation studies are needed to fully develop and market an acceptable product produced from North Carolina blueberries. Future studies should include evaluating the acceptability of pure blueberry juice. In addition, another area of focus to consider is the effects of high hydrostatic pressure on the quality and sensory properties of blueberry juices compared to blueberry juices prepared from traditional thermal processing methods evaluated during various storage intervals at room temperature.

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Appendices

Informed Consent for Consumer Test

Sensory Evaluation of Blueberry Juices

This research study will evaluate consumer acceptability of blueberry juices. The data obtained from your participation in this research study will be kept confidential and shall be maintained so that no data may be linked to you individually. The data will be stored in the Department of Food Science and access shall be made to the investigators and advisors only. Your participation in this research study is voluntary and you are free to withdraw at any time without penalty.

Print Name_____

Signature_____ Date_____

Blueberry Juice Consumer Questionnaire

Instructions: Please answer the following questions**.**

1. Gender ____ Male ____Female

2. Age
____18 25
____26-35
____36-45
____46-55
____56-55
____over 65

3. Do you do most of the shopping for your household? ____yes ____no

4. How often do you consume fruit beverages?
____Do not drink these types of beverages
____Daily
____Once a week
____More than once a week
____Once a month
____2-4 times per month

5. What type(s) of fruit beverages do you purchase? (Check all that apply)
____100% juice ____juice cocktails
____fruit drinks ____light juice drinks
____juice concentrates ____Other (Please specify)_____

6. What brands(s) of fruit beverages do you purchase? (Check all that apply)
____Tropicana ____Minute Maid
____Ocean Spray ____Dole
____Store Brand ____Other (Please specify)_____

7. What influences you when purchasing fruit beverages?
____Appearance ____Availability ____Health ____Flavor
____Price ____Taste

Blueberry Juice Consumer Ballot

Instructions: Taste each sample as indicated by the number on the cup, and circle your response for the questions below. **Please rinse your mouth between samples.**

Sample_____

Overall Acceptability								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Thickness/Mouthfeel Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Appearance Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Please make any additional comments:

Likes

_____
_____
_____

Dislikes

_____
_____
_____

Overall which sample did you prefer?

_____105 _____321 _____518 _____217

Blueberry Juice Consumer Ballot

Instructions: Taste each sample as indicated by the number on the cup, and circle your response for the questions below. **Please rinse your mouth between samples.**

Sample_____

Overall Acceptability								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Thickness/Mouthfeel Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Appearance Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Please make any additional comments:

Likes

_____
_____
_____

Dislikes

_____
_____
_____

Overall which sample did you prefer?

_____105 _____321 _____518 _____217

Blueberry Juice Consumer Ballot

Instructions: Taste each sample as indicated by the number on the cup, and circle your response for the questions below. **Please rinse your mouth between samples.**

Sample_____

Overall Acceptability								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Thickness/Mouthfeel Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Appearance Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Please make any additional comments:

Likes

_____
_____
_____

Dislikes

_____
_____
_____

Overall which sample did you prefer?

_____105 _____321 _____518 _____217

Blueberry Juice Consumer Ballot

Instructions: Taste each sample as indicated by the number on the cup, and circle your response for the questions below. **Please rinse your mouth between samples.**

Sample_____

Overall Acceptability								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Blueberry Flavor Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Intensity								
1	2	3	4	5	6	7	8	9
Low				Moderate				High

Sweetness Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Flavor Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Thickness/Mouthfeel Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Overall Appearance Liking								
1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor dislike				Like Extremely

Please make any additional comments:

Likes

_____
_____
_____

Dislikes

_____
_____
_____

Overall which sample did you prefer?

_____105 _____321 _____518 _____217