EFFECT OF HIGH HYDROSTATIC PRESSURE AND THERMAL PROCESSING ON CRANBERRY JUICE

by

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ABSTRACT OF THE THESIS

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Cranberry is a native crop of North America. Many studies that have looked into the health benefits of consuming cranberry juice point to cranberry proanthocyanidins for the health promoting properties of the juice. Cranberry juice is usually sold as pasteurized juice; however, it is not known whether processing has any detrimental effect on the health promoting compounds. Anthocyanins, the major pigment compounds responsible for the color of cranberry juice are known to be unstable and sensitive to light, oxygen, high temperatures, and enzyme activity. Therefore, an alternative processing technology is needed to maintain color and nutraceuticals in cranberry juice. High hydrostatic pressure processing (HHPP) is a novel, non-thermal food processing method that destroys food borne bacteria while retaining thermally labile

compounds. It has been shown the HHPP can be used to produce high quality orange juice.

The objective of this research was to investigate the effect of thermal and high hydrostatic pressure processing on proanthocyanidins and anthocyanins in cranberry juice, immediately after processing and during storage, and to evaluate the impact of processing on the sensory attributes of the juice. During the study untreated cranberry juice was used as control. Proanthocyanidin content in the juice was analyzed by HPLC with UV/Fluorescence detection. Anthocyanin content was determined by pH differential method. Proanthocyanidin content was negatively affected by both pasteurization and high hydrostatic pressure treatments, and by storage time and temperature. Combination of higher pressure and longer time during HHPP was found to be the most detrimental process of for procyanidin retention. Anthocyanidin levels in the juice increased immediately after all treatments, but decreased in all samples during storage. However, no visual differences in color were observed after processing or during storage of the samples. Sensory evaluation of the processed and unprocessed juice samples showed no significant differences between the unprocessed and the processed samples.

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I would like to thank Ed Pappas and Ivy Koelliker for their help and collaboration on the analyses executed during this study. And to my labmates for their support during the time working together.

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1. INTRODUCTION

1.1 CRANBERRY

Cranberry, along with Blueberry and Concord grape, are recognized as the three native fruits of North America and are commercially grown in the United States. The American Cranberry (*Vaccinium macrocarpon*) is recognized by the United States Department of Agriculture (USDA) as the standard fruit variety for fresh cranberries and cranberry juice cocktail. Consumption of cranberry in the U.S. is about two pounds per capita, almost entirely in the form of juice. Only about 5% of cranberries are sold as fresh fruit (Geisler, 2007).

Cranberries are mostly found in the northeastern states of U.S, Massachusetts, New Jersey, Oregon, Wisconsin, and Washington; and in the provinces of British Columbia and Quebec in Canada. The other minor production regions include Delaware, Maine, Minnesota and Rhode Island in the U.S. and Nova Scotia and Ontario in Canada. Cranberry production of the United States for the last 5 years is shown in Table 1. In 2006 United States total production of cranberries was 6,899,000 barrels (100 lb per barrel) and the forecasted production for 2007 was almost the same, with 690 million pounds, according to The National Agricultural Statistics Service (NASS). The leading producer state in the U.S. is Wisconsin, with almost half of the domestic production, followed by Massachusetts, with about the third part of the US production and New Jersey in the third place.

Table 1: Cranberries Production by State and United States 2003-2006 and Forecast for 2007

State	Total Production (*Barrels*)				
	2003	2004	2005	2006	2007
WI	3,607,000	3,295,000	3.660,000	3,940,000	3,900,000
MA	1,406,000	1,808,000	1,423,000	1,895,000	1,800,000
NJ	480,000	402,000	533,OOO	485,000	520,000
OR	510,000	495,000	440,000	465,000	500,000
WA	190,000	170,000	187,000	114,000	180,000
US	6,193,000	6,170,000	6,243,000	6,899,000	6,900,000

[1] A barrel weighs 100 pounds
Source: National Agricultural Statistics Service (NASS). August 2007

According to the Agricultural Marketing Resource Center (Geisler, 2007) Massachusetts based Ocean Spray accounts for about 80 percent of raw cranberry utilization. Other handlers include Northland Cranberries Inc., Wisconsin; Decas Cranberry Products, Massachusetts; Clement Pappas & Company Inc., New Jersey; and Cliffstar Corporation, New York.

Cranberry crop production consists of three distinctive phases. During winter, from late December through mid-March bogs are flooded to protect the vines and buds from winter injury and provide a chilled dormancy period required to prepare the bogs for the coming growing season. As spring season arrives and the warm weather begins, the winter flood is removed so the vines slowly come out of dormancy and the growing season starts and extends from April to November.

During fall, from mid September through October cranberries are harvested. During the early stages of this phase cranberry fruits have not yet acquired their characteristic color, and these berries are used for white cranberry juice production. As the season advances fruits mature and develop their distinctive deep red color (Figure 1).

Figure 1: Cranberry fruit

Depending on the end consumption of cranberries, they can be harvested in two different ways. Fruits destined to the fresh market are dry harvested, using machines to rake the berries off the vines. Once they get to the receiving stations they are sorted by color and ability to bounce (soft berries will not bounce).

Most of the cranberry production is destined to industrial processing, which accounts for more than 85% of the crops. These cranberries are wet harvested (Figure 2) by flooding the bogs with six to ten inches of water. A harvester with a beater is driven through the beds to remove the fruit from the vines; so the fruit can float to the surface and be corralled and conveyed from the bed into a truck. Cranberries can float in water because they have air pockets inside, so growers use this characteristic to aid in the removal of the fruit form the vines. Cranberries are then taken to the receiving station for cleaning. They are usually frozen shortly after arriving at the station.

Figure 2: Wet harvest of cranberries

Fresh cranberries are usually consumed during Thanksgiving and the end of the year holiday season, mostly as cranberry sauce, which is considered as a staple

of the traditional Thanksgiving menu. The fruit is edible, but it is normally considered too sharp to be eaten alone, unlike other berries, because of its highly acidic taste. About 95% of the U.S. cranberry industrial production is made up of cranberry juice cocktail, or blends with other fruit juices (NASS). Other industrial products include sweetened dried cranberries, quick frozen and powdered cranberries. See Table 2 for more information on available industrial cranberry products,

Table 2: Types, Availability and Usage of Cranberries

Product	Description	Comments	Applications
Fresh	Whole, fresh cranberries	Available September – November	Bakery products, sauces
Frozen	Whole cranberries	Available year round	Bakery products, sauces, condiments, dairy products
Sliced	3/8” (10mm) thick	Available year round; individually quick frozen (IQF)	Bakery products, sauces, condiments, dairy products
Single Strength Juice	7.5° Brix	Direct expressed juice	Beverages, natural colorant
Concentrate	50° Brix, 14+1.5% titrable acidity	High colored, pure cranberry concentrate	Beverages, natural colorant, condiments, dairy products, confections
Puree	5.4 or 6.1° Brix	Well-colored, pure cranberry concentrate	Sauces beverages, bakery products
Sweetened Dried Cranberries*	Sugar-infused, dehydrated fruit	No artificial color, flavor or preservative; excellent color retention	Bakery products, cereals, trail mix, snack foods, dairy products, confections
Cranberry Powder	Spray-dried cranberry concentrate	Soluble, hygroscopic fruit; 90+ % solids	Nutraceuticals, confections, teas, beverages, colorants,

Source: Cape Cod Cranberry Growers Association

Cranberries are considered a healthy food because they are low in fat content and have zero cholesterol, sodium levels are also low. They can add nutritional value to a healthy diet due to their vitamin content, fiber and phytochemical content, which are believed to have beneficial health properties. Typical values for proximate composition of cranberries are shown in Table 3.

Table 3: Nutritional Composition of Raw Cranberries

Nutrient	Value per 100 grams
Energy	46 kcal
Water	85.6 g
Protein	0.4 g
Ash	0.2 g
Fat	0.2 g
Available Carbohydrates	12.2 g
Fiber, total dietary	4.6 g
Calcium Ca	8 mg
Magnesium Mg	6 mg
Phosphorous P	13 mg
Potassium K	85 mg
Sodium Na	2 mg
Vitamin C, total ascorbic acid	13.3 mg

Source: USDA – Nutrient data laboratory
http://www.nal.usda.gov/fnic/foodcomp/search/

1.1.1 Health Benefits of Cranberry Juice

Cranberry juice has been traditionally used as a natural remedy for the prevention and treatment of urinary tract infections, as well as other diseases

such as diarrhea or for the prevention of scurvy during overseas trips in the Colonial times. Nowadays cranberries are considered among the healthiest fruits due to its antioxidant properties and other health benefits linked to their phytochemical content, although several research studies have reported cranberries health benefits, there are also other researchers reporting that his evidence is not conclusive.

Since 1984, many studies, both in vivo and in vitro, have found that most of the health benefits attributed to consumption of cranberry juice are related to the anti-adhesion properties of the juice, which inhibit or prevent infecting bacteria from adhering to different body tissues. A large number of studies have been done on the effect of cranberry on the urinary tract, and there is evidence that cranberry consumption, in different forms such as juice and tablets, prevent and reduce the recurrence of urinary tract infections (Howell, 2005; Foo, 2000[A,B]; The Cranberry Institute, 2007). It was also found that the bacterial anti-adhesion mechanism worked for dental infections, reducing gum diseases and reducing certain oral pathogens as well as total bacterial count; delaying the formation of dental plaque (The Cranberry Institute, 2007; Weiss, 2004; Labrecque, 2006). In addition there are various studies showing evidence that relate consumption of cranberry products with reduction in stomach ulcers caused by infection with *Helicobacter pylori*, major cause of gastric and duodenal ulcers, by preventing its adhesion to the mucosal lining of the gastrointestinal tract suggesting that a combination of antibiotics and cranberry may improve the elimination of *H. pylori*

(The Cranberry Institute, 2007; Zhang, 2005). Because of its high polyphenolic antioxidant content, cranberry has been considered as a natural defense against atherosclerosis. Researchers have found that cranberry juice decreased total cholesterol and LDL cholesterol, and increased HDL cholesterol. It inhibited oxidation of LDL and promoted cardiovascular health. Consumption of cranberry juice has also been related to prevention of lung and respiratory infections, inhibition of tumor growth in lungs and colon, and growth of leukemia cells in vitro (The Cranberry Institute, 2007; Porter, 2001; Neto, 2007).

1.2 FLAVONOIDS

The importance of polyphenol compounds and its impact in human health has been extensively studied in the past few years, especially the subgroup called flavonoids, which are also the largest class of polyphenols. Flavonoids are plant derived compounds, responsible for most of the characteristic flavor, taste and color of fruits and vegetables. They also act as antimicrobials, photoreceptors and antioxidants; are involved in plant growth and reproduction, provide resistance to pathogens and predators and protect crops from diseases (Ross, 2002; Cheynier, 2005). They all have a common basic structure (C_6-C_3-C_6), consisting in two aromatic or phenolic rings (A and B) and an oxygenated heterocycle(C) as shown in Figure 3. Flavonoids are divided in six major subgroups depending on variations of the heterocycle C: flavones, flavonols, flavanones, flavanols (catechins and proanthocyanidins), anthocyanidins and isoflavones.

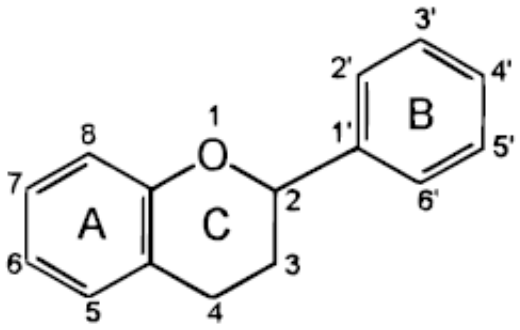

Figure 3: Basic flavonoid structure (Pietta, 2000)

When phenolic content of different fruits was analyzed by Folin-Ciocalteu test, cranberries total phenolic compound content was among the highest in the group. Cranberries contain four different classes of phenolic compounds, phenolic acids or hydroxycinnamic acids, anthocyanins, flavonols and flavanols (Vvedensaya, 2004). The three last compounds belong to the flavonoid subgroup.

Three individual flavonols have been identified in cranberries: quercetin, myricetin and kaempferol glycosides; with quercetin compounds contributing with up to 80% of the total flavonol content, ranging from 11 to 25mg/100g fresh fruit, one of the highest compared to other fruits (Vvedensaya, 2004[A,B]). Quercetin has been associated with many health benefits such as radical scavenging and inhibition of inflammation related processes in the body (Vvedensaya, 2004[A]).

Four major and two minor anthocyanins (Vvedenskaya, 2004[B]; Prior, 2001) can be found in cranberries. Presence of these compounds will be addressed later as a separate topic.

1.2.1 Proanthocyanidins (PACs)

As mentioned before most of the health benefits of cranberry juice consumptions have been related to its PACs content. PACs belong to the flavonoid subgroup called flavanols or flavan-3-ols. Monomeric units of these flavanols (catechins and gallocatechins, and their isomers) are characterized by the presence of an OH group attached to the carbon 3 of the basic flavonoid unit; and differ from each other depending on the substitution in the 3', 4' and 5' carbons (Ross, 2002; Pietta, 2000) as shown in Figure 4.

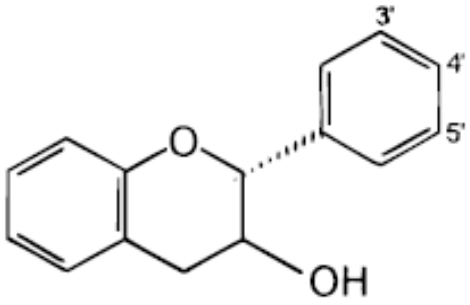

Figure 4: Basic flavanol unit

Proanthocyanidins, also called condensed tannins, are oligomers and polymers of the flavanol monomers. Depending on the type of monomer present, PAC can take different names; catechin and epicatechin polymers are called procyanidins

(Cheynier, 2005). The majority of PACs present in cranberries are monomers or polymers of (−) - epicatechin (Prior, 2001). In procyanidins, flavanol monomers can be either singly linked at the 4→6 or 4→8 positions, or doubly linked with a second interflavonoid bond formed by C-O oxidative coupling at the 2→ O→7 positions (Prior, 2001). Polymers containing exclusively single links are called B-type procyanidins, while the ones presenting at least one double link in their structure are called A-type procyanidins. Figure 5 shows the structure of procyanidin dimers linked by either A or B linkages. In cranberries, data indicated that both A-type and B-type oligomers were present; and in the case of A-type PACs only one double linkage per oligomers was observed (Prior, 2001).

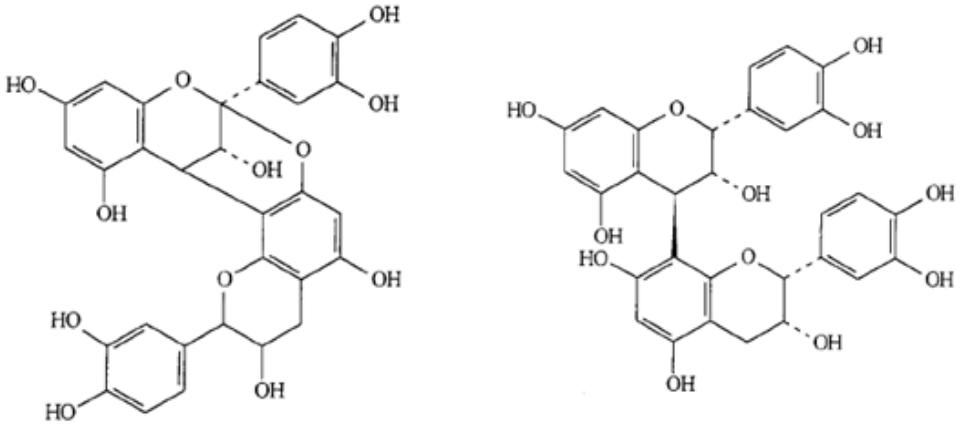

Figure 5: Structures of singly linked (B type) and doubly linked (A type) procyanidin dimers (Foo, 2000 [A])

Most of the previously mentioned health benefits associated with consumption of cranberry products have been related to the presence of proanthocyanidins in

the fruit. Among these benefits, one that has only been attributed to cranberry juice consumption is the inhibition of microbial adhesion to several body tissues. It is believed that the presence of proanthocyanidins with A type links, are responsible for this unique property of cranberries (Howell, 2005; Foo, 2000[A,B]; Prior, 2001). Howell et al (2005) concluded that consumption of other proanthocyanidin containing products, such as grape and apple juice, dark chocolate and green tea did not result in urinary bacterial anti-adhesion, possibly due to the absence of the A-type linkage in the proanthocyanidins in these products.

Most commercial cranberry products available for consumption undergo some kind of thermal processing; therefore investigating the impact that processing conditions may have on the phenolic and procyanidin content is of great interest, considering the increasing evidence on the role that they may play in human health. To date there is very little and contradictory information on how thermal processing affects procyanidin levels in food products. Asami et al (2003) found that processing peaches above 213°F for less than 10 minutes decreased procyanidin levels as much as 100%, and if they were processed at or less than 213°F for 40 minutes, no significant changes were found in total phenolic level. On the downside, the samples processed at lower temperature presented the highest loss of total phenolics after 3 months of storage at room temperature. Additional studies on thermal processing of canned peaches at 220°F for 10 minutes resulted in reduction of all procyanidin oligomers. Storage of the

peaches for 3 months resulted in a decrease of all procyanidin monomers and oligomers. The loss percentage increased with the degree of polymerization (Hong, 2004).

Lower temperature thermal treatments appear to have no significant impact on the polyphenolic content of fruit juice, while most of the loss compare to whole berries occurred during the primary steps of processing including thawing, crushing, depectinization and pressing of the fruits (Lee, 2002; Skrede, 2000). Contradictory results were found when processing grape juice, where pasteurization at 85°C increased the concentration of most of the procyanidins analyzed in hot pressed juice, while catechin concentration of the same juice decreased after the thermal treatment. These results were attributed to polymerization and depolymerization reactions (Fuleki, 2003).

1.2.2 Anthocyanins

Cranberry is known to have high concentration of anthocyanins; its quantitation is used by the juice industry as an important characteristic when measuring the quality of the fruit, because of their contribution to appearance, taste and nutritional benefits of the berry (Vvedenskaya, 2004[B]; Lee, 2002). Anthocyanins are water soluble pigments responsible for red and blue color of fruits and vegetables. They are one of the most broadly distributed pigment group in the plant world. Collectively they belong to the flavonoid category of phenolic compounds. Similarly to most polyphenolic compounds they are considered a

good source of antioxidants, widely known for their benefits in human health. Anthocyanin pigments are extensively degraded during processing and storage, causing serious problems to food processors, mostly because of the impact on color quality of the products (Skrede, 2000).

The basic structure of anthocyanin pigments is called flavylium cation. Different anthocyanidins (free and unbound form of anthocyanin) are derived from this basic structure, with each anthocyanidin identified based on hydroxy and methoxy groups attached to the basic molecule. Twenty naturally occurring aglycones (molecules without a sugar molecule attached) have been identified, but only six are important in produce, with structural differences mostly found in the substitution pattern of the 3', 4' or 5' carbons (Fennema, 2008; Watson, 1997). Table 4 lists the six major naturally occurring anthocyanidins in nature.

Table 4: Major Anthocyanidins in Nature

Anthocyanidin	**3'**	**4'**	**5'**
Pelargonidin	H	OH	H
Cyanidin	OH	OH	H
Peonidin	OCH_3	OH	H
Delphinidin	OH	OH	OH
Petunidin	OCH_3	OH	OH
Malvinidin	OCH_3	OH	OCH_3

Source: Watson (1997)

Anthocyanins (glycoside form of anthocyanidins) present a sugar moiety attached to the carbon 3 of the anthocyanidin molecule. Glucose is the most commonly found sugar, but rhamnose, galactose, xylose, arabinose and fructose can also be present (Watson, 1997). Figure 6 shows the general structure of anthocyanins.

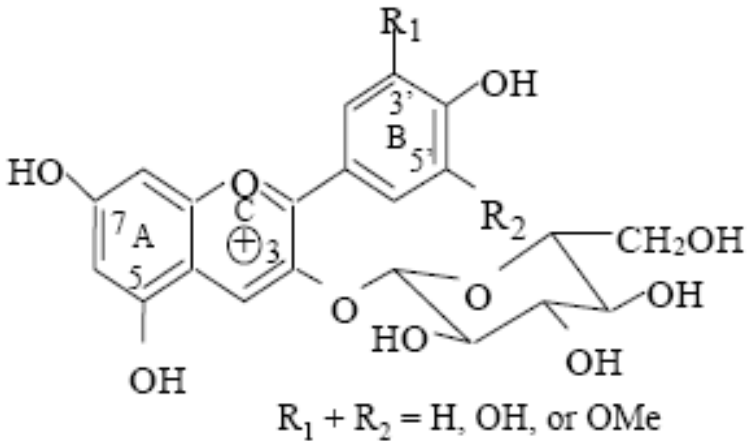

Figure 6: General structure of anthocyanins (Wrolstad, 2005)

Anthocyanin pigments are found mostly in the peel and outer layers of the cranberry fruit. Six different types of anthocyanins have been identified in cranberries, with four of them accounting for the majority of the total pigment content, responsible for giving the fruit its characteristic red color. These anthocyanins are cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside; about 55% of them are cyanidins. Two minor anthocyanins are also present in cranberries; they are cyanidin-3-glucoside and peonidin-3-glucoside (Vvedenskaya, 2004[B]; Prior, 2001).

Anthocyanin Stability

Color of fruit products is considered one of the most important quality attributes as it is closely related to freshness perception by consumers. Several factors influence and affect the stability of anthocyanin pigments in fruits. Among the most important factors are the structure of the anthocyanins, pH of the solution temperature, oxygen, presence of other substances and enzyme activity.

Structure

Substitutions on the flavan nucleus have an effect on the stability of the anthocyanin pigment. The number and location of the hydroxy and methoxy groups in the pigment molecule affect its color properties. Increasing number of hydroxyl group will shift the color spectra from orange to blue, with increasing number of methoxy groups reversing this trend. Anthocyanins containing more methoxy groups and sugars are more stable than those containing more hydroxy groups because in the last case equilibrium of the system becomes more complex (Fennema, 2008; Watson, 1997).

pH

In water solutions different structural forms of anthocyanins coexist in a dynamic equilibrium, which may be disrupted by a change in the parameters of the system. In a slightly acidic pH at room temperature the following four species are present: the red flavylium cation (AH+), the blue quinonoidal base (A), the

colorless pseudobase or carbinol (B), and the colorless chalcone(C) (Fennema, 2008).

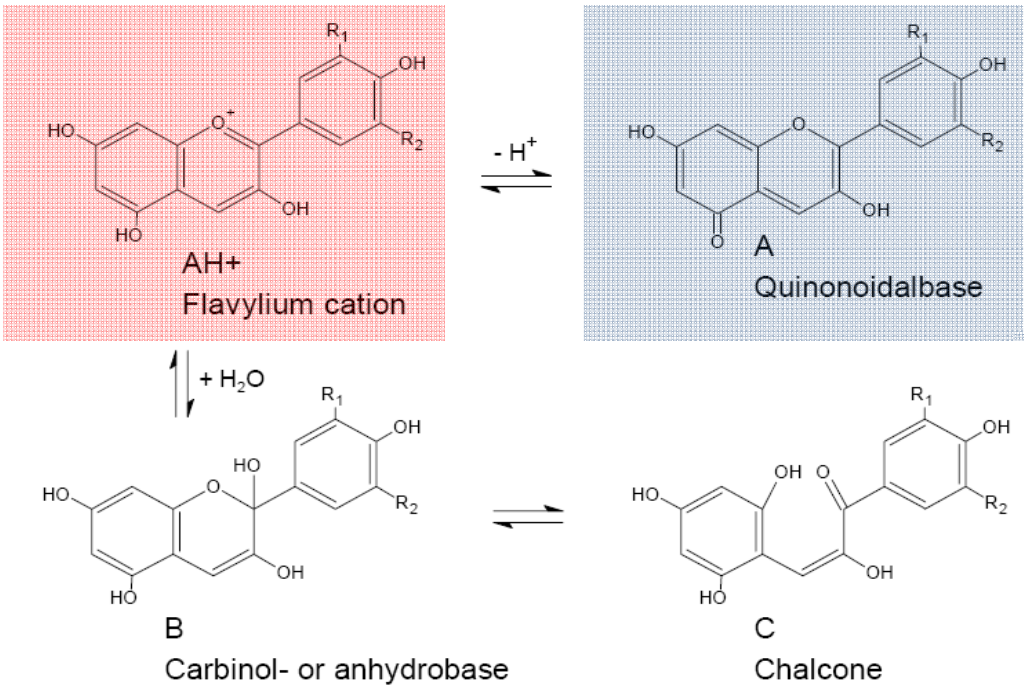

Figure 7 : Structural forms of anthocyanin (Fennema, 2008)

Acidity of the system as expressed by pH has a dominant influence on the color of the anthocyanin pigment. In a lower range of pH up to 3, flavylium cation is mostly responsible for the color, along with some contribution from the quinonoidal base. With an increase of pH a large bathochromic shift occurs and the quinonoidal base increasingly influences the color. At high pH values the colorless structures are dominant causing the system to lose its color. Flavylium cation is the most stable form of anthocyanins; therefore low pH levels are preferable for anthocyanin retention (Carlson, 2003). At pH 1, cranberry anthocyanins present their highest absorbance level; with increasing pH causing

decrease in the absorption spectra, becoming significantly low around pH 4.5, where the colorless forms are dominant (Figure 8).

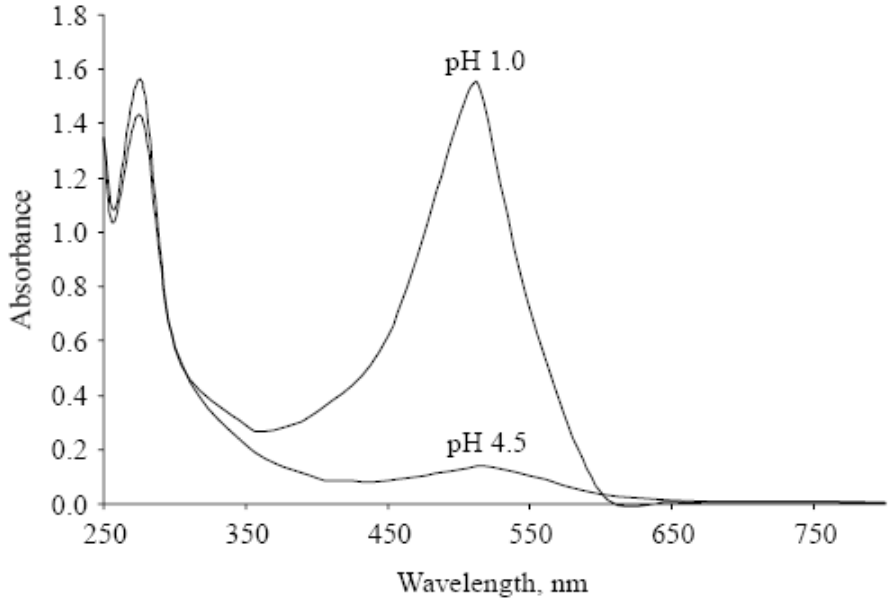

Figure 8 Absorption spectra of anthocyanins (Wrolstad, 2005)

Temperature and light

Processing temperature significantly influences the degradation of anthocyanins, especially in presence of oxygen. The rate of degradation increases with increasing temperature, while freezing had little effect on anthocyanin levels. Boiling temperatures of cranberry juice (100°C) had the greatest effect on anthocyanin destruction (Watson, 1997; Carlson, 2003). Storage temperature had negative effects on anthocyanin retention. Accelerated pigment destruction was caused by storage at 20-25°C, while samples stored at lower temperatures

showed better pigment retention, with increased retention when using freezing temperatures (Watson, 1997).

Light also influences the stability of anthocyanins. Photochemical degradation may be caused by excitation of the flavylium cation, generally having the same products caused by thermal degradation. Anthocyanins containing more methoxy groups or sugars are more stable than those containing more hydroxy groups (Fennema, 2008).

Ascorbic acid and other chemicals

Numerous chemicals used in processing of foods containing fruits may interact with anthocyanins. Ascorbic acid is frequently added to fruit juices to increase its nutritional value. However, anthocyanins and ascorbic acid cause mutual degradation, involving a condensation mechanism (Watson, 1997). Oxygen acts in a synergistic way with ascorbic acid in the degradation of anthocyanins, possibly by the formation of an intermediate molecule of hydrogen peroxide, known to cause anthocyanin bleaching (Watson, 1997).

Sulfites and sulfur dioxide added for preservation purposes are known to cause loss of color of anthocyanins. However under some circumstances the color may be restored (Fennema, 2008).

Metal complexation

Anthocyanins are known to form complexes with metals during food processing when using metal equipment or by addition of salts. Metals may change the color of anthocyanins. But complexes of metals with anthocyanins also exist in nature and contribute to a wide array of colors. Molecules of anthocyanins may form complexes with molecules of other compounds as well. This phenomenon is known as co-pigmentation and may involve flavonoids, amino acids, proteins, pectin, carbohydrates and polyphenols (Fennema, 2008).

1.3 HIGH HYDROSTATIC PRESSURE PROCESSING

Producing foods that can be associated to good health and wellness is of strong interest for the food industry. Consumer demand for foods of high quality in terms of flavor and appearance has lead to the development of non thermal technologies for food processing. High hydrostatic pressure processing (HHPP), along with irradiation, pulsed electric field, and UV light processing are the emerging non-thermal technologies focused on substituting heat processing of foods, which can produce undesirable changes in food products, such as changes in color, flavor or texture.

The first attempts of using high pressures to processed food date back to 1899, when Bert Hite pressurized milk and reported increased shelf life as well as changes in the physical properties of milk, but it wasn't until late in the 1980's that food industry started commercially using high pressure processing and

taking advantage of its benefits (Hendrickx, 2001). Today high pressure processed food products can be found in the market, some examples are guacamole, fruit juices, sliced ham and oysters.

During HHPP pressures up to 1000 MPa are applied to food, liquids or solids, although pressures between 400 and 700 MPa are more commonly used and processing is often done at room temperature. Products to be pressurized are placed inside a vessel which is then filled with a fluid called the pressure transmitting medium. Water is used for this purpose in most cases.

Pressure can be applied either by direct compression, reducing the volume inside the vessel using a piston; or by indirect compression, where an intensifier pump is used to apply pressure on the pressurizing medium until the desired pressure is achieved. Indirect compression is the commonly used method used in food applications. Once the working pressure is reached the pump is stopped and the pressure is maintained until the desired holding time has elapsed.

A small increase in temperature occurs during HHPP due to the compression applied to the transmitting fluid. For water the increase is about 3°C for every 100 MPa of applied pressure (Guerrero-Beltran, 2005; San Martin, 2002).

After the processing of the products is achieved by maintaining the desired pressure for the desired period of time, the depressurization of the system takes

place, which takes just a few seconds. The vessel is then opened and the product unloaded. After depressurization, temperature of the system returns to its starting point. Industrial high pressure processing is commonly a batch process, although continuous systems can be found for other specific purposes (San Martin, 2002). Figure 9 shows the typical graph of a high hydrostatic pressure process.

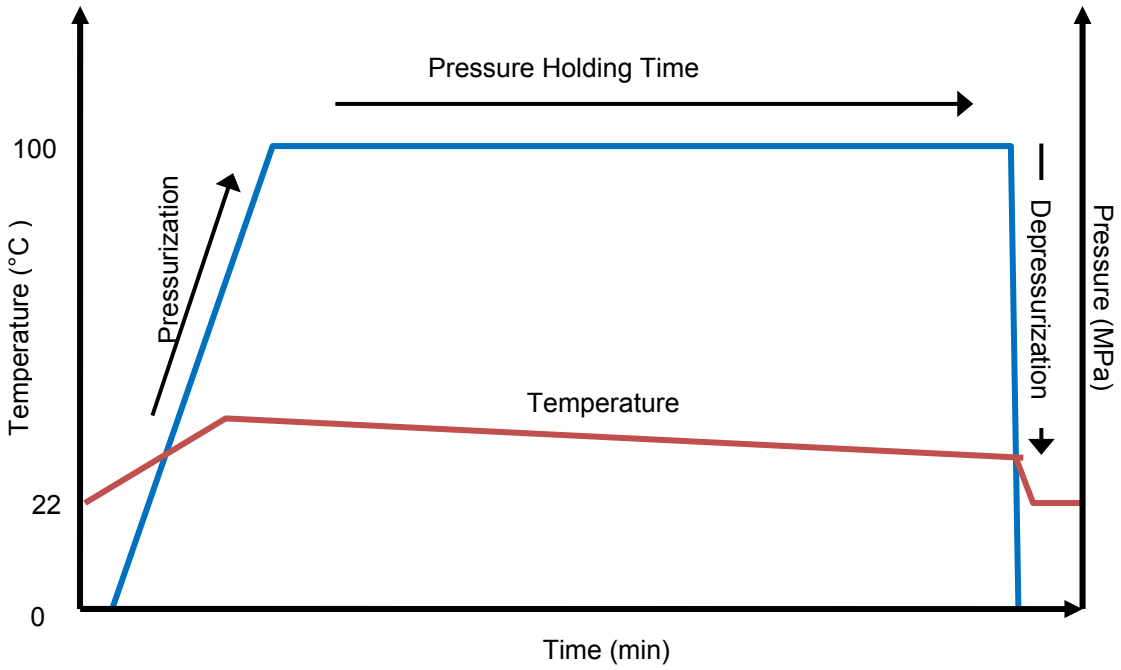

Figure 9: Schematic of variation of pressure and temperature during a typical HHPP run

It is important to point out that during HHPP the pressure is isostatic, so the process is independent of the size of shape and the product, which can be a limiting factor during conventional thermal treatments.

1.3.1 HHPP Effect on Microorganisms

Food safety is one of the main concerns of the food industry. HHPP has proved to be an effective method to inactivate most microorganisms. The extent of microbial inactivation depends on several factors, such as process pressure, time and temperature; type of microorganism, species, and food composition. An important food property to consider is the pH of the product, given that at low pH microbial inactivation is enhanced during HHPP treatment and can also help inhibit outgrowth of surviving cells after pressure treatment (Hendrickx, 2001).

Two main factors have been described as responsible for microbial inactivation due to high pressures: protein denaturation and cell injury. At pressures between 300~600 MPa, vegetative cells are sensitive to pressure and therefore can be inactivated with the use of high pressure which increases the permeability of the cell membrane leading to microbial death. Changes in the cell membrane may occur due to denaturation of proteins and enzymes which are vital for the survival of the microorganism. Also changes in the cell volume due to pressurization/depressurization can lead to cell injury and disruption, causing leakage of the cell content and cell death.

Although vegetative cells, including yeasts and molds can usually be inactivated at room temperature, spores are highly resistant to HHPP. They are capable of surviving pressures as high as 1200 MPa. Because of this a combination of high pressure and moderate temperature is needed in order to inactivate spores.

1.3.2 HHPP Effect on Food Components

Small molecules, namely amino acids, vitamins and flavor compounds generally remain unaffected by high pressure, while the structure of large molecules such as proteins, enzymes, polysaccharides and nucleic acids can be altered by the high pressure. These differences could be explained by the fact that high pressure is likely to affect interactions where a reduction in volume is possible, for example in non-covalent bonds (hydrogen bonds, hydrophobic interactions, etc). Covalent bonds remain unaltered, which is the reason why small molecules like vitamins, color, and flavor compounds are not affected by high pressure. This phenomena might change when very high pressures (>800 MPa) are applied to the food. Retention of vitamins can be reduced (San Martin, 2002) and formation of new flavor compounds can be promoted, while these changes do not appear at lower pressures (Lambert, 1999; Sumitani, 1994). Since many fruit and vegetable products contain large amounts of flavor and color compounds, susceptible to thermal degradation, HHPP can be a good alternative to thermal treatments, as demonstrated by several research studies (San Martin, 2002; Lambert, 1999; Rastogi, 2007).

1.3.3 HHPP Effect on Enzymes

Quality preservation of food products during shelf life is one of the principal concerns of the food industry. Besides microbial deterioration, enzyme activity is responsible for most of the quality losses occurring during storage. Therefore

elimination or inactivation of enzymes is of high importance during food processing to ensure retention of food characteristics.

Enzymes can be successfully inactivated by thermal processing, but the heat applied to the food can lead to loss of nutritional and sensory attributes, equally important for the consumer. In contrast to thermal processing, HHPP usually causes little to no change to color, flavor or nutritional content (Rastogi, 2007). Some pressure resistant enzymes may not be inactivated by pressure alone, or they may require very high pressures that it won't be economically feasible. Several authors have suggested that the use of HHPP in combination with a moderately higher temperature (Hendrickx, 2001) or certain other pretreatments (San Martin, 2002) that may be required to achieve complete enzymatic inactivation. Effect of high pressure treatments on enzymes varies and depends on different factors, such as type and origin of the enzyme, nature of the substrate, applied pressure, holding time or processing time and temperature (Hendrickx, 2001; San Martin, 2002).

The use of high pressures can also lead to different results. For example changes in enzymes can be reversible or irreversible, enzyme inactivation can be complete, partial or the enzyme can become activated by the pressure applied. These differences can be explained or be a result of several changes occurred during pressurization. The changes in the conformation of the protein structure (the active site or protein denaturation), or changes in the volume of the food

system that may affect enzyme-substrate interaction or the reaction mechanisms, may induce changes in the catalytic rate. Pressure induced damage of cell membranes also facilitates enzyme-substrate contact. Another proposed explanation is that the changes in the macromolecular substrate after pressurization may leave cell membranes more susceptible to the enzymatic action due to unfolding of proteins or gelatinization of starches (Hendrickx, 2001; San Martin, 2002; Rastogi, 2007). Different combinations of pressure treatments and its effects on enzymes are summarized in Table 5.

The effect of partial inactivation/activation of enzymes by HHPP has been demonstrated in several studies, and it can have either beneficial or damaging effects on the shelf life of a product. Gimenez et al (2001) studied the effect of HHPP on jams and found that even though traditionally processed jams (heat processed) showed lower anthocyanin content than HHPP samples after processing, during storage traditional jam was more stable than all the HHPP samples. Lower storage temperatures seamed to show a protective effect on pigment degradation. Sumitani et al (1994) evaluated the formation of benzaldehyde in peaches after high pressure processing and thermal treatment and showed that the formation of enzymatic benzaldehyde and alcohols by disruption of fruit tissues was present in HPPP and in unprocessed crushed peaches, and that an increase in benzaldehyde during storage was caused by remaining enzymes after high pressure treatment.

Table 5 : Effects of HHPP on Enzyme Activity

Product	Effect	Enzyme/Pressure
Orange juice	Complete inactivation No cloud loss for 90 days Economically viable	PE 700 MPa x 1 min PME - >500 MPa
Tomato puree	Rheology improvements High inactivation rates	PME - >700 MPa
Strawberry puree	Optimal inactivation Some activation 60% inactivation	POD – 230 MPa/43°C 250-400 MPa PPO – 250 MPa
Mushroom whole	High stability, Increased activity 7%reduction Complete inactivation	PPO 400 MPa 600 MPax10min >800 MPa
Banana puree	Increased effects with blanching pretreatment, <5% residual activity Complete inactivation with 0.5% citric acid solution	PPO – 689 MPax10 min 400MPax15 min

Sources: Guerrero-Beltran (2005); San Martin (2002); Rastogi (2007)

1.4 HYPOTHESIS

HHP treated cranberry juice samples will have better retention of procyanidins, anthocyanins and color compared to thermally treated and untreated cranberry juice. HHP treated cranberry juice taste will be more similar to unprocessed juice than thermally treated cranberry juice .

1.5 OBJECTIVES

- To process the cranberry juice using HHP and thermal processing
- To study the stability of proanthocyanidins, anthocyanins and color in cranberry juice after thermal and HHP treatment and during storage, and compared it to unprocessed juice.
- To study the correlation between anthocyanin content and color.
- To perform sensory evaluation of HHPP and thermally processed cranberry juice comparing them to unprocessed cranberry juice.

1.6 RATIONALE

This study addresses important issues related to the effect of juice processing by current thermal processing and High Hydrostatic Pressure Processing (HHPP) on the most important health and sensory attributes of cranberry juice.

Understanding the effect of both processing technologies on key components of cranberry juice that are related to the juice most important attributes will help in maintaining the quality as close as possible to the fresh cranberry juice. Results of this study will be useful for cranberry juice and other juice processors to manufacture high quality and microbiologically safe fruit juices.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Cranberry Juice

Fresh unprocessed clarified cranberry juice was procured from a cranberry juice processor located in New Jersey. Juice was a mix or Stevens and Ben Lear varieties, with 8°Brix and pH of 2.3. Untreated juice was used as control for all the analyses performed in the study.

2.1.2 Pouches

Pouches used to process and store cranberry juice, were obtained from the Food Manufacturing Technology Facility (FMT), Piscataway, NJ, of Rutgers University in New Jersey.

Specifications for the pouches are as follows:

- Capacity: min 5 oz , max 8 oz
- Oxygen transmission rate (OTR): max. 0.06 cc/m^2/24 hrs/atm
- Water vapor transmission rate (WVTR): max 0.01 gr/m^2/24 hrs
- Material structure: from inside to outside, 0.003 to 0.004 inch thick polyolefin, 0.00035 to 0.0007 inch thick aluminum foil, 0.0006 inch thick biaxially oriented polyamide-type 6, and 0.0005 inch thick polyester
- Temperature range: able to withstand commercial sterilization

2.1.3 Reagents

- Potassium chloride, ACS reagent 99% (Sigma Aldrich Inc., MO)
- Sodium acetate (Sigma-Aldrich Inc., MO)

2.2 PROCESSING EQUIPMENT AND PROCEDURES

2.2.1 Sample Preparation

Pouches were filled with approximately 130 ml of cranberry juice, and then heat sealed at 125°C using a foot sealer, model AIE 402CH (American Int'l Electric Inc, Whittier, CA). For control and HHPP samples the pouches were filled, heat sealed and processed, while for thermal processing they were processed, filled and finally sealed. Figure 10 shows a filled pouch before processing and storage.

Figure 10: Sealed pouch filled with cranberry juice

2.2.2 High Hydrostatic Pressure Processing

A 10 liter High Hydrostatic Pressure Unit (Elmhurst Research Inc, Albany NY.) (Figure 11) was used for processing the cranberry juice samples. The vessel of the high hydrostatic pressure unit has an internal diameter of 127 mm, its length is 800 mm and the wall thickness is 145 mm. The maximum working pressure of the high pressure unit is 690 MPa or 100,000 psi, which can be reached in less than 3 minutes, using a 20 HP pump. Starting pressure of the process is at atmospheric pressure (0.1MPa = 14.7psi = 1 atm). Pressure transmitting fluid used is filtered tap water. Maximum depressurization time is less than 10 seconds.

The operation of high pressure process used in our experiments was monitored using a tabletop PC, where pressure, temperature and time data were logged using Labview 7® software (National Instruments, Texas). Temperature inside the vessel was measured using a type K thermocouple. To load the pouches, the vessel was tilted horizontally and the top closure was removed pneumatically. Once filled with the pouches, the closure was closed, the vessel returned to the vertical position and filled with water. Once the desired parameters (operating pressure and hold time) were set in the control module, the vessel was pressurized using a 20 HP high pressure pump, and the pressure held for the preset time. The vessel was then depressurized, tilted horizontal, the water emptied and the top closure removed for unloading the samples.

Figure 11: High Hydrostatic Pressure Unit

Three different processing conditions were evaluated using pressures and time combinations ranging between 278 MPa and 551 MPa, for 5 to 15 minutes. Initially, the statistic design of experiments was thought to be a central composite design, using 5 and 15 minutes, and 278 MPa and 551 MPa, as initial points. Low pressure level was determined by results obtained in preliminary

experiments, and high pressure was determined considering the maximum working pressure of the high hydrostatic pressure unit (586 MPa). The full design of experiment would have needed at least 12 time/pressure combinations for processing conditions to be evaluated. The decision to not follow the full design was taken because the number of samples to be analyzed would have been too many given the available resources. Also, processing of the samples at 278 MPa for 5 minutes was discarded because during preliminary experiments, processing of cranberry juice at that pressure/time combination showed to be not as effective in retaining the procyanidin content of the samples when analyzed by HPLC, as processing samples at the same pressure for longer time (15 minutes). The final design of the study consisted of 3 extreme processing conditions. For each condition 20 pouches were processed in the same batch. Table 6 shows the parameters for the three different HHPP treatments. Letter C was reserved for control (unprocessed) samples.

Table 6: HHPP Treatments

Treatment	Pressure (MPa)	Time (min)
HHPP A	278	15
HHPP B	551	15
HHPP D	551	5

Figure 12 shows the processing graph of HHPP treatment B. The blue line represents the pressure (MPa) and the pink line represents temperature (°C) during the treatment.

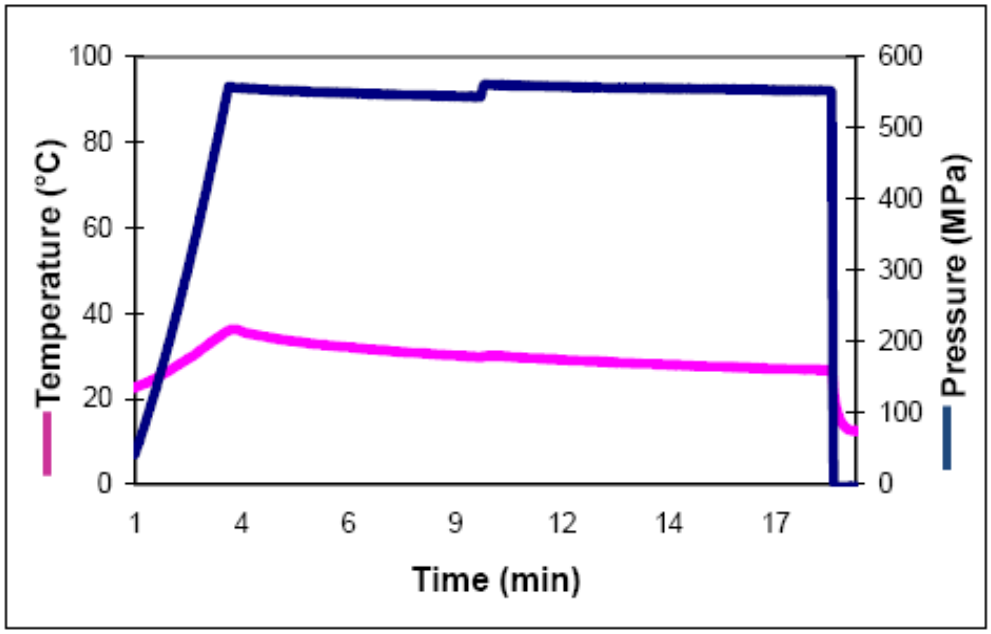

Figure 12: Actual variation of pressure and temperature during the HHPP treatment B

2.2.3 Thermal Processing

Samples were individually pasteurized at 90°C for 90 seconds, (Lee, 2002). For pasteurizing the juice,130 ml of cranberry juice was filled in a stainless steel vase (175 mL capacity) and covered with a lid holding a 6 inch type T thermocouple connected to the data acquisition system which consisted of a high speed USB career NI USB9162 (National Instruments, TX) connected to a laptop computer.

Temperature and time data was logged using Labview 7® (National Instruments, TX). The vase containing the juice was placed on a water bath at 95°C until the desired thermal processing was achieved. Pasteurized juice was poured in a pouch which was heat sealed and cooled under tap water. Figure 13 shows the set up used for thermal processing of the samples.

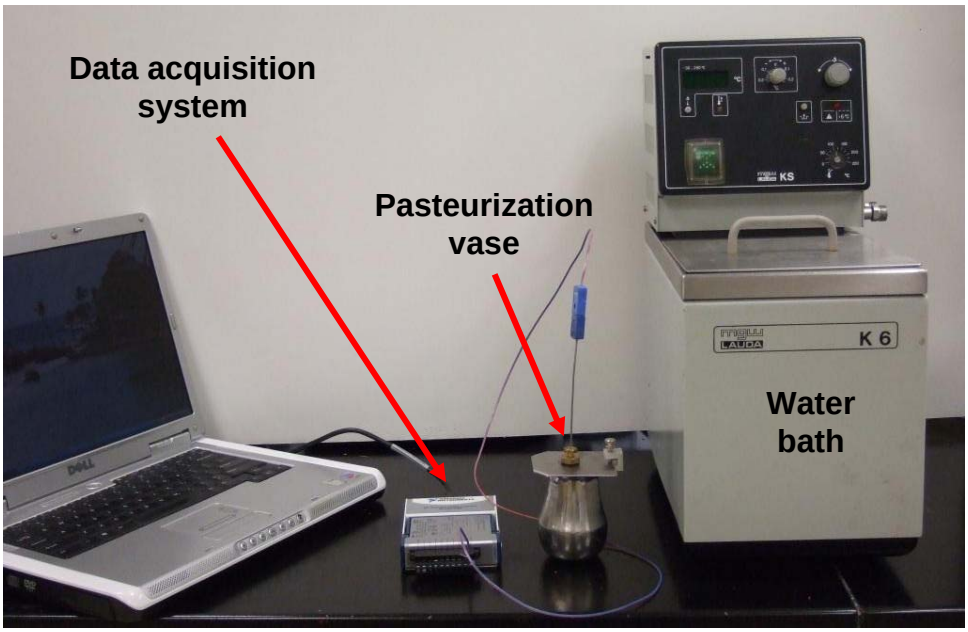

Figure 13: Thermal processing set up used for pasteurization of cranberry juice

Figure 14 shows a typical temperature vs. time variation during the pasteurization of cranberry juice in the steel container. Temperature came up to 90°C, and is then it was maintained for 90 seconds, to be finally cooled down.

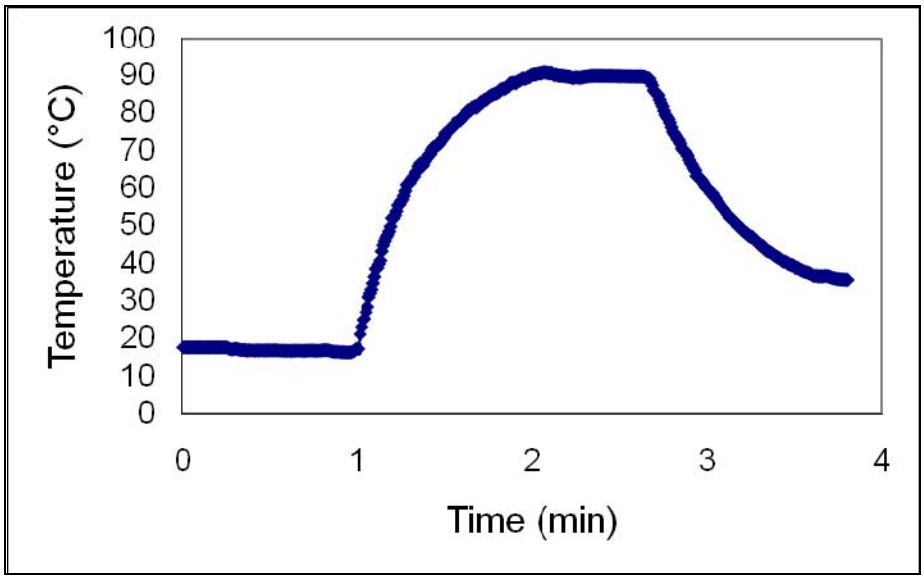

Figure 14: A typical temperature vs. time variation during the pasteurization of cranberry juice in the steel container shown in Figure 13

2.2.4 Storage Studies

Selected storage temperatures are the standard accepted practice for shelf life.

a. Storage at 22°C

Cranberry juice samples in pouches were stored at room temperature with the thermostat set at 22°C. Samples were analyzed on the first day and on a weekly basis, over one month.

b. Storage at 37°C

Samples were stored in a temperature controlled room set at 37°C. Samples were analyzed on day 0, day 2, day 4, day 8, day 16, and day 30. The set up for this part of the study was made based on preliminary experiments performed in our laboratory, where results showed that the majority of changes

in proanthocyanidin and anthocyanin content of processed and unprocessed cranberry juice occurred during the first two weeks of storage at 37°C.

2.3 ANALYTICAL METHODS

2.3.1 Proanthocyanidins (PAC)

Cranberry juice samples were analyzed following the methodology explained by Kelm, et al (2006), performed using normal phase HPLC with flourescense detection (FLD). A diol column was used as the stationary phase. The binary mobile phase consisted of (A) CH_3CN:HOAc (98:2 v/v) and (B) CH_3OH:H_2O:HOAc (95:3:2 v/v/v). Compounds were eluted according to degree of polymerization and characterized as flavan-3-ol monomers and procyanidins. Quantification was done following the methodology by Adamson (1999.) Results were expressed as mg of proanthocyanidin per gram of cranberry juice.

2.3.2 Total Monomeric Anthocyanin (TMA)

Total monomeric anthocyanin values were evaluated following the pH differential method by Giusti and Wrolstad (2001). Each sample was analyzed in triplicates. Dilution factor (DF) for all the analyses was determined according to the method by Giusti and Wrolstad (2001). The best readings were obtained by adding 8 ml of buffer to 1 ml of cranberry juice. For our experiments a dilution factor of 9 was used.

One liter of each potassium chloride buffer (pH 1) and sodium acetate buffer (pH 4.5) were prepared and stored in volumetric flasks at room temperature. pH of both solutions was adjusted prior to each use. Two dilutions of the sample were prepared, one with potassium chloride buffer and the other with sodium acetate buffer, using the previously determined DF. Dilutions were allowed to equilibrate for at least 15 minutes, but less than 1 hour.

Absorbance of each dilution was measure at 520nm and 700nm, using 1 cm (pathlength) cuvettes. The equipment used was UV-Vis spectrophotometer CARY 50 Bio (Varian Inc, CA).

Absorbance of each dilution was calculated as follows:

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

Total monomeric anthocyanin concentration (expressed as cyaniding-3-glucosidase equivalents) in the original sample was calculated using the formula:

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

MW: molecular weight of cyaniding-3-glucoside (449.2 g mol^{-1})

ε: molar extinction coefficient of cyaniding-3-glucoside (26900 L cm^{-1} mol^{-1})

l: cuvette pathlength (1 cm)

DF: 9

Results were expressed as mg of cyanidin-3-glucoside equivalent per liter of cranberry juice.

2.3.3 Color

Color of the samples was measured in undiluted samples of cranberry juice using a Mini Scan Hunter lab colorimeter (Hunter Associates Laboratories Inc., Reston, VA). D65/10° observer angle, calibrated with a reflectance standard X=79.8, Y=84.7, Z=88.5; port size 1.25”; UV filter was not used.

For each color measurement, juice sample from the pouch were poured in the vase. A plastic dark ring was previously placed inside the vase to prevent transmission of the light; samples were covered with a metallic lid. Vase was placed in the colorimeter and covered with a dark cover. Each sample was measured in triplicate.

Results were obtained in CIEL*a*b* system and later converted to L*CH system using the standard formulas (Wrolstad, 2005):

- Lightness: $L^* = L^*$ 0 (black) 100 (white)
- Chroma (saturation): $C = (a^{*2}+b^{*2})^{1/2}$
- Hue (color itself): $h = \arctan b^*/a^*$ 0° (bluish-red)
  90° (yellow)
  180° (green)
  270° (blue)

2.4 SENSORY EVALUATION

The principal objective in performing sensory evaluation was to determine if the treatments, pasteurization and HHPP, had any effect on the flavor of the juice as compared to the unprocessed cranberry juice. In order to present the participants with a familiar product, cranberry juice cocktail was prepared for sensory evaluation. Fresh clarified cranberry juice was diluted with water in a 30/70 (cranberry juice/water) proportion. Table sugar was added to the dilution until it reached 10° Brix. The cranberry juice cocktail was then high pressure processed or pasteurized, and unprocessed cranberry juice cocktail was used as control.

Test selected for evaluation of the samples was the Difference –from-Control Test (Carr, 1999). For this test one sample is designated the “control”, “reference” or “standard”, and all other samples are evaluated with respect to how different each is from the control. In our case the untreated juice was the “control” sample. Pasteurized samples and HHPP sample A (processed at 278 MPa for 15 minutes) were the test samples. HHPP A sample were selected because from all three HHPP treatments it was overall the most successful, and provide the best procyanidin retention of all treatments.

During the test, the subjects were presented three pairs of samples:

- Control vs “blind” control
- Control vs pasteurized juice
- Control vs HHPP A juice

50 subjects were recruited to take the test; all of them were untrained panelists. Sensory evaluation test was done at the sensory evaluation laboratory of the Food Science Department of Rutgers University, under the supervision of Dr. Beverly Tepper. Subjects were asked to determine the degree of difference between the control and the samples, and that some of the test samples may be the same as control.

At the beginning of the test the evaluation worksheet was presented to the panelists and they were instructed to follow the instructions. Samples were presented in pairs, with one sample labeled as control and the other with a three digit number. All three sample pairs were evaluated during the same session. Panelists were encouraged to comment on their opinion.

Figure 15 shows the ballot used for the test to analyze the data. The numerical scale shown below was used to quantify the choices given to the participants in the verbal category scale:

1= no difference
2= very slight difference
3= slight/moderate difference
4= moderate difference
5= moderate/large difference
6= large difference
7= very large difference

DIFFERENCE FROM CONTROL TEST

Date: 02- 19 -2008
Code of test sample:
Type of sample: Cranberry juice cocktail

Instructions:

1. You have received two samples, a control sample and a test sample labeled with a 3 - digit number
2. Taste the sample marked " Control" first
3. Taste the sample marked with the three digit code
4. Asses the overall sensory difference between the two samples using the scale below
5. Mark the scale to indicate the size of the overall difference

_____ No difference
_____ Very slight difference
_____ Slight/moderate difference
_____ Moderate difference
_____ Moderate/large difference
_____ Large difference
_____ Very large difference

REMEMBER THAT A DUPLICATE CONTROL IS THE SAMPLE SOME OF THE TIME

COMMMENTS: _____
_____
_____

Figure 15: Sensory evaluation ballot

3. RESULTS AND DISCUSSION

In this chapter results from the analyses performed on the unprocessed and processed cranberry juice will be presented and discussed. Changes in proanthocyanidin, anthocyanin and color of the cranberry juice samples immediately after processing and during storage were evaluated and compared with control samples. Results from sensory evaluation of the cranberry juice cocktail are also presented and discussed. Means and standard deviations of the results are shown on each table. Standard error bars are shown on the figures.

3.1 PROANTHOCYANIDINS (PAC)

A sample fluorescence response curve from proanthocyanidin analyses is shown in Figure 16, the curve shown is from a sample processed by HHPP treatment A (278 MPa for 15 minutes), stored for one week at 22°C. All the other samples showed similar graphs. Mass spectral data indicate the samples contained primarily flavan-3-ol monomers, dimers and trimers, with lower levels of proanthocyanidin tetramers and pentamers. No higher oligomers were found in any of the samples, or if present, the concentration might have been too low to be detected.

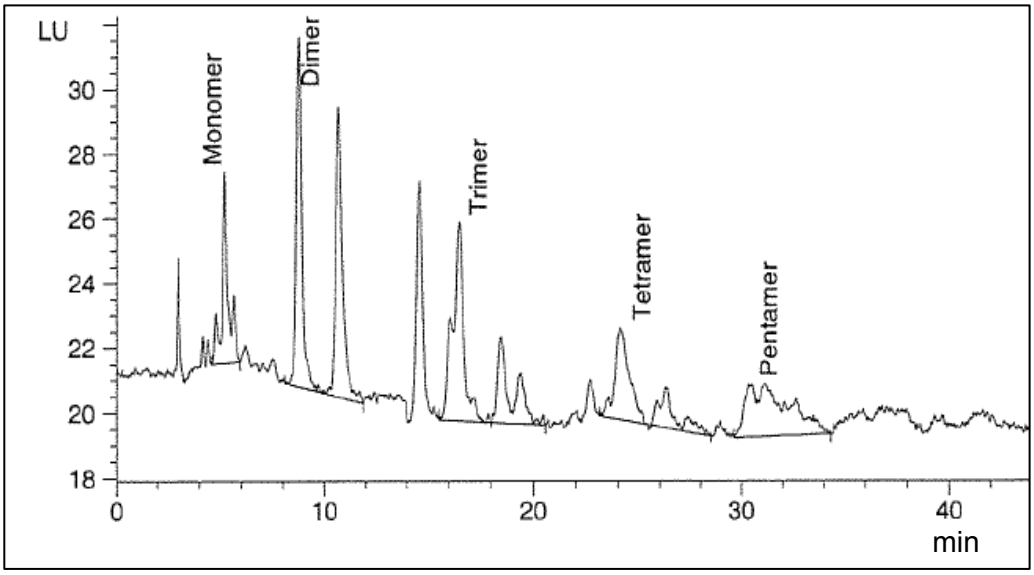

Figure 16: Chromatogram of cranberry juice, sample HHPP A, after one week of storage at 22°C, showing various procyanidins

These results are comparable with previously reported by Prior et al (2001), where mass spectral data from two cranberry juice samples showed that monomer, dimers and trimers were the primary procyanidins, with only trace levels of tetramers and a notable absence of higher oligomers.

PAC content of all samples was evaluated at day 0 of the study and compared to unprocessed control cranberry juice to determine the effect of each treatment on PAC content of the cranberry juice tested. Proanthocyanidin content of the samples stored at 22°C was also evaluated during one month period, with analyses performed at the first week and fourth week of storage. Data was quantified according to the methodology followed by Adamson, 1999. Table 7

shows the results from the samples stored at 22°C. Tables 8 and 9 show the results of statistical analyses of the data, analyzed, it can be said that both processing treatments and storage time have a significant effect on PAC content of the cranberry juice.

Table 7: PAC Changes During Storage at 22°C

Storage 22°C (weeks)	**PAC's (mg/g cranberry juice)**				
	Untreated (Control)	**Pasteurized**	**High pressure treatment**		
			A	**B**	**D**
		90°C , 90 s	278MPa,15min	551MPa,15min	551 MPa,5min
0	$0.131\pm0.001^{a}{}_{a}$	$0.122\pm0.003^{a}{}_{b}$	$0.127\pm0.001^{a}{}_{a}$	$0.112\pm0.001^{a}{}_{c}$	$0.121\pm0.002^{a}{}_{b}$
1	$0.124\pm0.004^{a}{}_{a}$	$0.115\pm0.006^{b}{}_{b}$	$0.125\pm0.002^{a}{}_{a}$	$0.113\pm0.001^{a}{}_{b}$	$0.117\pm0.003^{a}{}_{b}$
4	$0.130\pm0.002^{a}{}_{a}$	$0.110\pm0.005^{b}{}_{b,c}$	$0.114\pm0.005^{b}{}_{b}$	$0.109\pm0.003^{a}{}_{b,c}$	$0.106\pm0.001^{b}{}_{c}$

^{a,b,c} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 8: PAC TWO WAY ANOVA - Storage at 22°C

Source	DF	SS	MS	F value	Pr > F
Model	14	0.00239	0.00017	17.66	<0.0001
Error	28	0.0027	0.00000965		
Corrected total	42	0.002657			
Process	4	0.00143	0.000357	37.0	<0.0001
Storage	2	0.00057	0.000283	29.3	<0.0001
Process*storage	8	0.00041	0.000052	5.34	0.0004

R-square	Coeff Var	Root MSE	PAC mean
0.8982	2.6384	0.003107	0.1177

Table 9: PAC DUNCAN TEST - PROCESS - Storage at 22°C

Duncan grouping	Mean	Process
A	0.128	Control
B	0.116	Pasteurized
C	0.121	HHPP A
D	0.111	HHPP B
B	0.114	HHPP D

PAC content was affected by both pasteurization and pressurization. Significant differences were found between control and thermally treated samples, HHPP B and HHPP D samples immediately after processing. HHPP A samples lost only 3% of PAC immediately after processing, showing no significant differences with control samples on day 0, at the same time HHPP A samples showed significant differences with HHPP B samples, the last ones had a 14.5% lost of PAC immediately after processing; these results show that different pressures had different effects on proanthocyanidin levels, with increasing pressures having a direct effect on PAC degradation. Processing time also had a similar impact as that of pressure used. Samples pressurized at 551 MPa for 5 minutes had PAC loss of 7.6%, significantly lower than samples pressurized at the same pressure but longer time. Pasteurization of cranberry juice also decreased the content of PAC in the juice immediately after processing, with losses up to 6.8%, likely by thermal degradation. The effect of pasteurization was similar to pressurization at 278 MPa, but direct comparison between the two processes is challenging due to the difference in physicochemical changes occurred during each one (Talcott, 2003).

Although little is known about the fate of procyanidins after processing, especially after HHPP, previous studies have reported negatives effects on PAC content after food processing, either by thermal or HHPP (Prior, 2001; Asami, 2003; Hong, 2004; Skrede, 2000; Fuleki, 2003; Talcott, 2003; Spanos, 1992; Del Pozo-Insfran, 2007). According to Kalt (2005), other processing steps, such as

maceration and separation, also lower antioxidants present in fresh fruit and produce by oxidation, leaching and other reactions. Effects of pasteurization include reactions such as thermal degradation and polymerization (reflected in a decrease in PAC levels) and depolymerization (increasing the total PAC content) of flavanols (Fuleki, 2003).

Use of higher pressures, 400-600 MPa (Talcott, 2003; Del Pozo-Insfran, 2007), had detrimental effects on juice quality, reducing the antioxidant capacity in grape juice. Authors suggested that autoxidation reactions were responsible for antioxidant losses that may have been catalyzed by enzymatic activity. PPO activity increased up to 3-fold from the control juices after pressurization under 400-550 MPa (Del Pozo-Insfran, 2007), indicating that losses on PAC content of cranberry juice may be due to activation of PPO activity during HHPP and residual activity present after processing. In addition to increased PPO activity, a reduction in volume of the cranberry juice system during HHPP may facilitate oxidative reactions, and according to Le Chatelier principle increase reaction rates (Del Pozo-Insfran, 2007), causing the drastic reductions in PAC content observed in HHPP samples. More studies are needed to confirm the degree of activation of PPO during HHPP. Figure 17 shows the trend of the changes of PAC content in the samples during storage at 22°C.

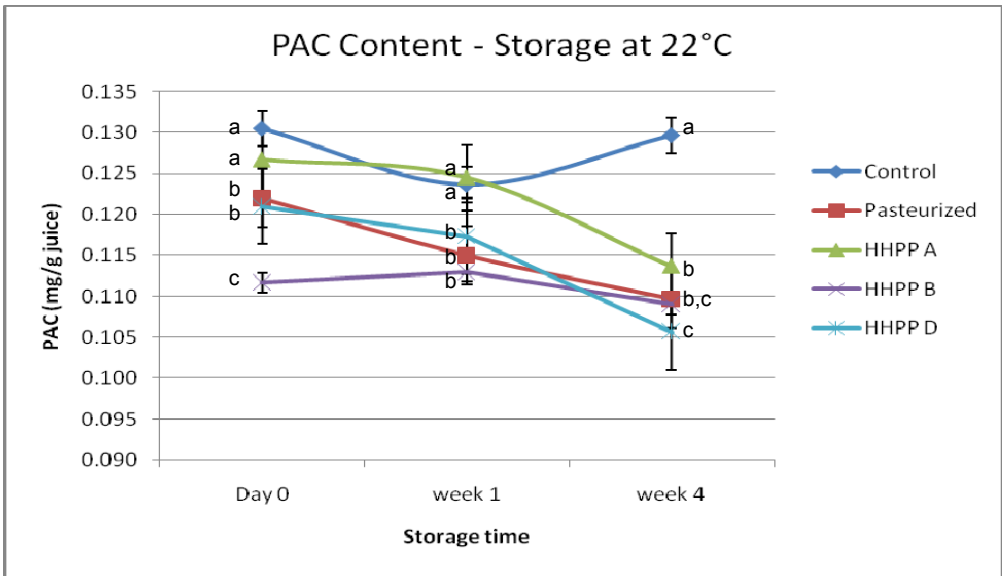

Figure 17: PAC concentration during storage at 22°C

Significant overall differences were found between control and all processed samples, and between the samples. There was no significant difference between the pasteurized and HHPP D samples (see Table 9). Control samples had the highest PAC content at the end of the storage, and although HHPP A had the second highest final PAC content, there was no significant difference between this sample and pasteurized and HHPP B samples on the last day of storage at 22°C.

During storage at 22°C, control or untreated samples presented a different behavior than all the other samples. Results after the first week showed a decrease of PAC content of the samples, as it was expected; but PAC content level was higher after the four week of storage. This could be explained by polymerization and depolymerization reactions that often occur with flavanol

units. Although samples HHPP B (551 MPa for 15 minutes), had the greatest loss after processing, no significant differences were found within this sample during the month of storage, and the final PAC content was even higher that HHPP D and almost the same than pasteurized samples, but no significant differences were found between these three samples. This may indicate that even if the PPO activity was increased during pressurization, the final inactivation might have been higher than treatments A (lower pressure) and treatment D (lower time), this is a conjecture at this time and further analyses are required to determine enzyme activity on all the samples. Thermally treated samples also had significant PAC losses during storage. In this case the degradation occurred at a more constant rate, while for HHPP samples A and D, the PAC content remained more stable until the first week and rapidly declined during the following weeks.

Results from storage of cranberry juice at 37°C are presented in Table 10, showing the changes in the PAC content, evaluated during one month, with analyses done at days 2, 4, 8, 16 and 30 of storage. Tables 11 and 12 show the results from the statistical analyses of the data. Results are plotted in Figure 18, showing the PAC concentration during storage at 37°C.

Storage of the samples at 37°C also caused losses in the PAC content of all samples, but degradation occurred at a faster rate, as expected, resulting in lower final values than those from the samples stored at 22°C.

Table 10: PAC Change During Storage at 37°C

Storage 22°C (days)	PAC's (mg/g cranberry juice)				
	Untreated (Control)	Pasteurized	High pressure treatment		
			A	B	D
		90°C , 90 s	278MPa,15min	551MPa,15min	551 MPa,5min
0	$0.131\pm0.001^{a}{}_{a}$	$0.122\pm0.003^{a}{}_{b}$	$0.127\pm0.001^{a}{}_{a}$	$0.112\pm0.001^{a,b}{}_{c}$	$0.121\pm0.002^{a}{}_{b}$
2	$0.125\pm0.^{001a,b}{}_{a}$	$0.120\pm0.005^{a}{}_{a,b}$	$0.119\pm0.003^{b,c}{}_{a,b}$	$0.114\pm0.001^{a}{}_{b}$	$0.118\pm0.001^{a}{}_{a,b}$
4	$0.122\pm0.001^{b}{}_{a}$	$0.115\pm0.007^{a}{}_{a,b}$	$0.120\pm0.006^{a,b,c}{}_{a,b}$	$0.112\pm0.002^{a,b}{}_{b}$	$0.118\pm0.004^{a}{}_{a,b}$
8	$0.124\pm0.002^{a,b}{}_{a}$	$0.106\pm0.003^{b}{}_{b}$	$0.124\pm0.006^{a,b}{}_{a}$	$0.107\pm 0.003^{a,b,c}{}_{b}$	$0.117\pm0.009^{a}{}_{a}$
16	$0.120\pm0.001^{b}{}_{a}$	$0.104\pm0.010^{b}{}_{b}$	$0.115\pm0.004^{c,d}{}_{a,c}$	$0.108\pm0.003^{b,c}{}_{b,c}$	$0.107\pm0.003^{b}{}_{b,c}$
30	$0.119\pm0.006^{b}{}_{a}$	$0.096\pm0.005^{c}{}_{b}$	$0.109\pm0.005^{d}{}_{c}$	$0.101\pm0.010^{c}{}_{b,d}$	$0.105\pm0.004^{b}{}_{c,d}$

^{a,b,c,d} Different superscript letters in the same treatment indicate significant difference (P<0.05)
${}_{a,b,c,d}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 11: PAC TWO WAY ANOVA - Storage at 37°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	29	0.0056	0.000195	9.82	<0.0001
Error	57	0.00112	0.00002		
Corrected total	86	0.00678			

R-square	Coeff Var	Root MSE	PAC mean
0.83	3.8696	0.00445	0.115

Table 12: PAC DUNCAN TEST - PROCESS – Storage at 37°C

Duncan grouping	Mean	Process
A	0.123	Control
B	0.111	Pasteurized
C	0.119	HHPP A
B	0.109	HHPP B
D	0.114	HHPP D

At the higher storage temperature, pasteurized samples showed the highest percentage of PAC loss, and also had the lowest value of PAC content at the end of storage, but their final value showed no significant difference with HHPP B samples final PAC value. Untreated samples had the highest final PAC content, significantly different from all the other samples, followed by samples treated by HHPP A and HHPP D. As previously reported (Asami, 2003; Hong, 2004; Spanos, 1992) storage conditions affect levels of polyphenolics, and can cause complete degradation of procyanidins after a long storage at higher temperatures. During storage at 37°C, only pasteurized and HHPP B samples showed no significant overall difference between them, all other samples showed overall differences between each other (see Table 12).

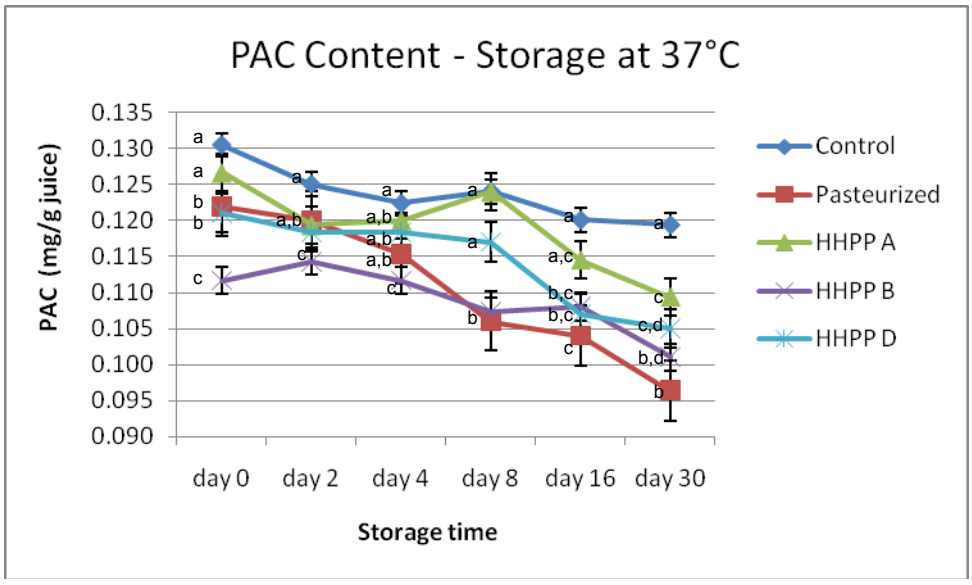

Figure 18: PAC concentration during storage at 37°C

3.2 TOTAL MONOMERIC ANTHOCYANIN (TMA)

TMA content increased in all cranberry juice samples after thermal and HPP processing, all of them showing significant differences with TMA content of control (unprocessed) samples. Although processing at 551 MPa for 5 minutes gave the highest increase of all the samples (10.5% increase), no significant differences were found between all of the HHPP samples. Pasteurized samples showed the least TMA increase with only 4.8% above control, but it showed no significant differences with TMA content in HHPP B samples immediately after processing. Increase in the TMA content may indicate that pigment-sugar bonds, or pigment-pigment bond (polymeric anthocyanins) are broken by processing of the cranberry juice, resulting in an increase of monomeric anthocyanin units that will react with the buffers and result in higher absorbance values. Table 13 shows the values of TMA in the samples during storage at 22°C. Tables 14 and 15 show the results from the statistical analysis of the samples stored at 22°C.

Table 13: TMA Change - Storage at 22°C

Storage	**TMA (mg/L cranberry juice)**				
22°C	**Untreated**	**Pasteurized**	**High pressure treatment**		
(weeks)	**(Control)**		**A**	**B**	**D**
		90°C , 90 s	278MPa,15min	551MPa,15min	551MPa,5min
0	$79.4\pm4.93^{a}_{a}$	$83.2\pm1.11^{a}_{b}$	$86.5\pm0.71^{a}_{c}$	$85.4\pm0.21^{a}_{b,c}$	$87.7\pm1.14^{a}_{c}$
1	$75.2\pm1.58^{b}_{a,b}$	$77.6\pm1.58^{b}_{a}$	$72.9\pm0.80^{b}_{b}$	$75.2\pm0.88^{b}_{a,b}$	$76.0\pm0.22^{b}_{a}$
2	$63.9\pm1.66^{c}_{a}$	$70.0\pm1.03^{c}_{b}$	$67.4\pm0.99^{c}_{c}$	$69.4\pm0.17^{c}_{b,c}$	$69.6\pm1.11^{c}_{c}$
3	$57.6\pm2.43^{d}_{a}$	$64.3\pm0.96^{d}_{b}$	$64.7\pm0.43^{d}_{b}$	$65.8\pm0.70^{d}_{b}$	$64.5\pm1.89^{d}_{b}$
4	$51.4\pm0.86^{e}_{a}$	$54.8\pm0.68^{e}_{b}$	$51.6\pm0.96^{e}_{a}$	$56.9\pm1.23^{e}_{b}$	$57.0\pm0.93^{e}_{b}$

^{a,b,c,d,e} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 14: TMA TWO WAY ANOVA –- Storage at 22°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	24	8351.76	347.99	156.77	<0.0001
Error	50	110.988	2.219		
Corrected total	74	8462.749			

R-square	Coeff Var	Root MSE	TMA mean
0.986885	2.1551	1.48988	69.1313

Table 15: TMA DUNCAN TEST – PROCESS - Storage at 22°C

Duncan grouping	Mean	Process
A	65.50	Control
B	69.99	Pasteurized
C	68.63	HHPP A
B	70.55	HHPP B
B	70.97	HHPP D

TMA content of all samples decreased steadily through the whole storage time under 22°C. After one week of storage untreated samples presented the best anthocyanin retention with only 5.3% loss, followed by pasteurized samples at 6.7%. At the same time HHPP samples showed TMA losses higher than control and pasteurized samples, with HHPP at 278 MPa been the worst with 15.7% TMA loss, but TMA content value of all samples after one week of storage showed no significant difference between them and control samples. For the remaining storage time the control samples had the lowest TMA levels every week, with the final pigment loss of 35.3%. HHPP A samples had the second lowest final TMA content, but showed no significant differences with unprocessed samples. at the end of the storage. HHPP B had one of the highest final TMA

content and the lowest percentage loss at 33.4%. Pasteurized samples levels, both in TMA content and loss percentage were very similar to HHPP B samples. Pressurization at 551MPa for 5 minutes, HHPP D, had the highest final TMA content of all samples, although almost identical to the one from HHPP B, and these three samples showed no significant difference between them.

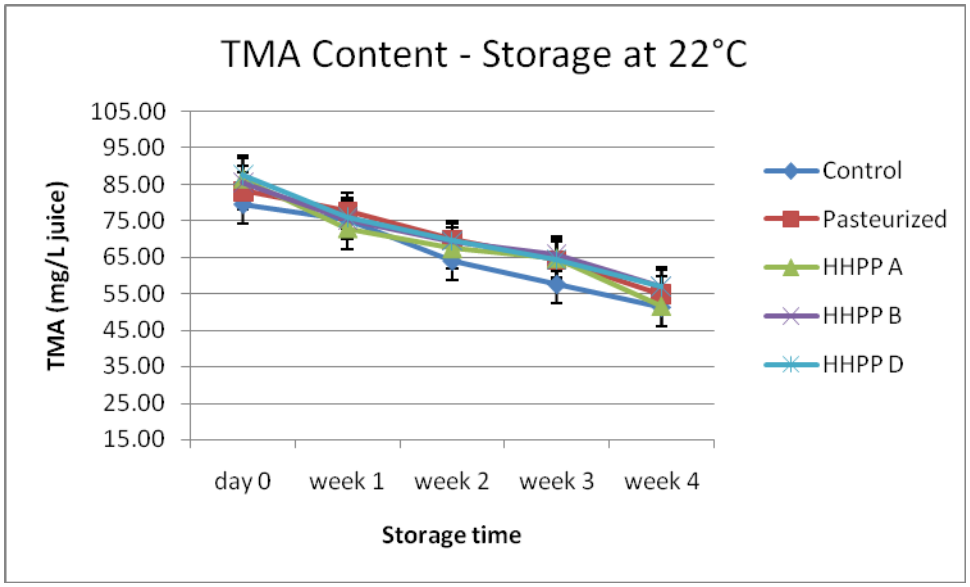

Figure 19: TMA changes during storage at 22°C

Table 16 shows the TMA content during storage at 37°C. As expected, due to higher storage temperatures, loss of anthocyanins during storage at 37°C was greater than pigment losses at storage at 22°C. One of the reasons may be increased enzyme activity, given that optimum temperature for polyphenol oxidase activity is close to 37°C. Showing similar trends as during lower temperature storage, HHPP D had the highest TMA content and the lowest percentage loss at week 4 of the study followed by HHPP B and pasteurized

samples, both having the same pigment content, with no significant difference between the three. Control samples had the second lowest TMA content, and the least effective treatment being HHPP A with the lowest anthocyanin content and the highest percentage loss (71.9%) at the end of the storage. Tables 17 and 18 show the statistical analyses of the samples. Control and HHPP A samples showed no significant overall differences in TMA content throughout the storage at 37°C. With Pasteurized and HHPP B samples being statistically similar in their overall TMA content during the storage at 37°C. Two way anova analyses of TMA content of the samples during storage under 22°C and 37°C showed that both processing treatments and storage time have an effect on TMA content of the cranberry juice.

Table 16: TMA Change - Storage at 37°C

Storage	**TMA (mg/L of cranberry juice)**				
at 37°C	**Untreated**	**Pasteurized**	**High pressure treatment**		
(days)	**(Control)**		**A**	**B**	**D**
		90°C , 90 s	278MPa,15m	551MPa,15m	551MPa,5min
0	$79.4\pm4.93^{a}_{a}$	$83.2\pm1.11^{a}_{b}$	$86.5\pm0.71^{a}_{b}$	$85.4\pm0.21^{a}_{b}$	$87.7\pm1.14^{a}_{b}$
2	$73.8\pm2.45^{b}_{a}$	$76.1\pm2.30^{b}_{a}$	$68.9\pm2.03^{b}_{b}$	$75.4\pm2.18^{b}_{a}$	$74.0\pm2.02^{b}_{a}$
4	$67.0\pm3.08^{c}_{a,c}$	$71.0\pm1.15^{c}_{b}$	$63.8\pm1.52^{c}_{a}$	$67.3\pm1.19^{c}_{c}$	$70.7\pm0.44^{c}_{b}$
8	$51.6\pm0.97^{d}_{a}$	$59.7\pm2.58^{d}_{b}$	$51.2\pm0.77^{d}_{a}$	$54.1\pm0.34^{d}_{a}$	$53.7\pm0.42^{d}_{a}$
16	$37.8\pm1.67^{e}_{a}$	$45.7\pm0.69^{e}_{b}$	$38.0\pm2.07^{e}_{a}$	$44.3\pm1.81^{e}_{b}$	$43.1\pm0.17^{e}_{b}$
30	$25.9\pm0.88^{\dagger}_{a}$	$27.7\pm2.95^{\dagger}_{a,c}$	$22.3\pm2.71^{\dagger}_{b}$	$27.7\pm4.57^{\dagger}_{a,c}$	$30.1\pm1.27^{\dagger}_{c}$

^{a,b,c,d,e,†} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 17: TMA TWO WAY ANOVA - Storage at 37°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	29	35204.35	1213.94	291.22	<0.0001
Error	60	250.101	4.16		
Corrected total	89	35454.46			

R-square	Coeff Var	Root MSE	TMA mean
0.9929	3.5136	2.04168	58.107

Table 18: TMA DUNCAN TEST – PROCESS - Storage at 37°C

Duncan grouping	Mean	Process
A	55.91	Control
B	60.57	Pasteurized
A	55.12	HHPP A
C D	59.04	HHPP B
B D	59.88	HHPP D

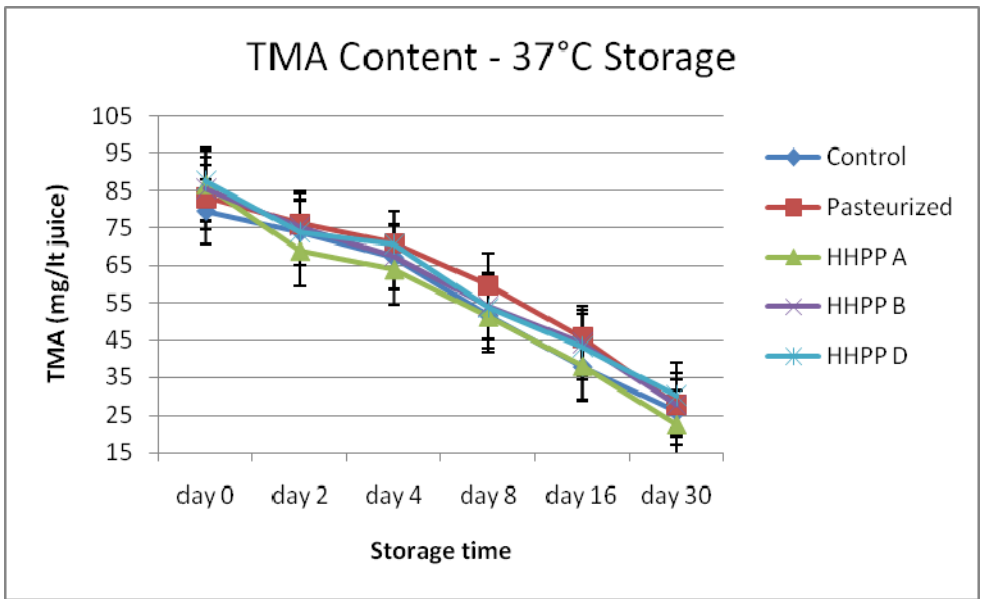

Figure 20: TMA changes during storage at 37°C

Similar to results obtained on previous research, storage temperatures negatively affected anthocyanin stability, showing a rapidly increase in pigment degradation as storage temperatures increased (Gimenez, 2001; Kouniaki, 2004; Suthanthangjai, 2005). To date it has not been possible to determine specific high hydrostatic pressure processing parameters that will be equally effective for all products given that inactivation requirement varies greatly depending on the product and its characteristics, as well as for each enzyme. It is probable that a processing condition that may seem optimal in preserving one specific characteristic of a product could be detrimental to another desirable quality factor within the same product.

3.3 COLOR

To track visual changes of the samples after processing and during storage digital pictures were taken on each evaluation day of storage at 22°C and 37°C. Figure 21 shows the picture of the samples on day 0. No visual differences were detected between the untreated sample and processed samples. Figures 22 and 23 show pictures taken after one month of storage at 22°C and 37°C, respectively. As can be seen, there are no visual differences between the untreated and treated samples, and no differences were observed between samples stored at 22°C and 37°C.

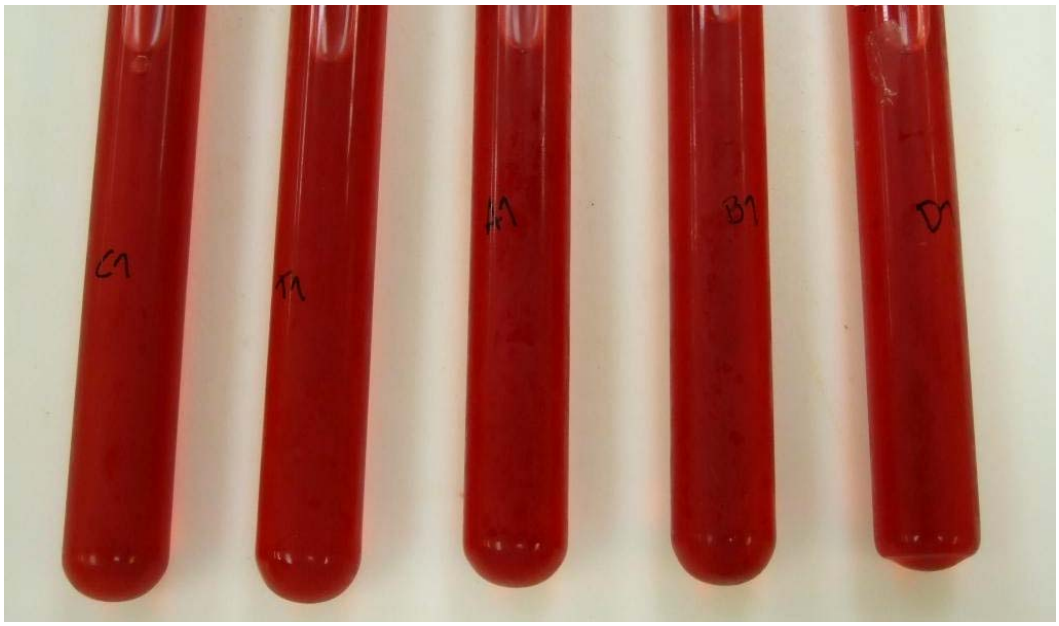

Figure 21: Cranberry juice samples at day 0. From left to right: Control, Pasteurized, HHPP A, HHPP B and HHPP D

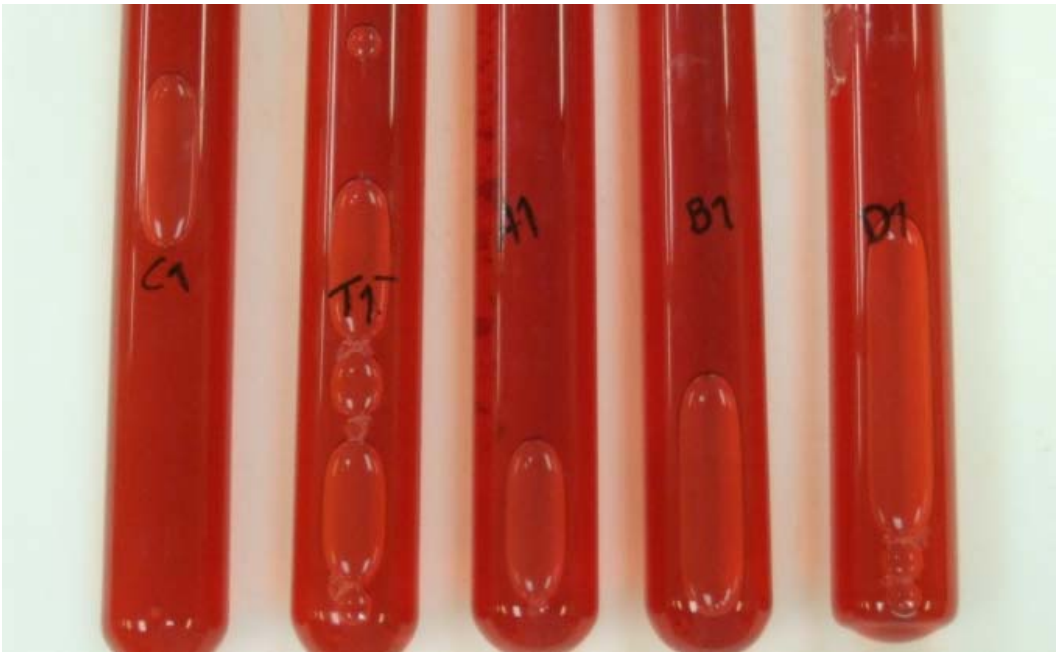

Figure 22: Cranberry juice samples at week 4, storage at 22°C. From left to right: Control, Pasteurized, HHPP A, HHPP B and HHPP D

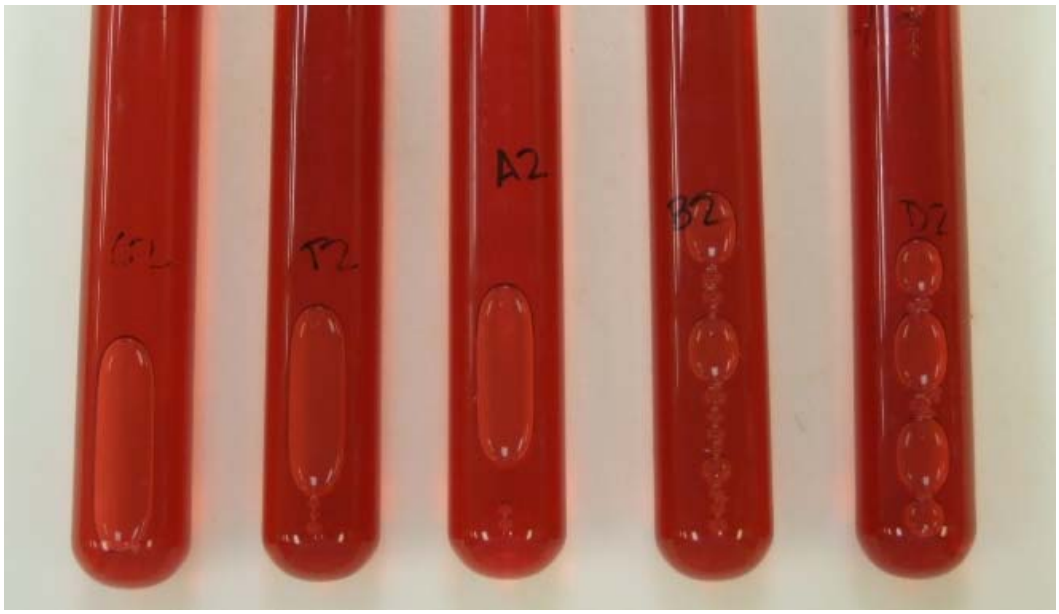

Figure 23: Cranberry juice samples at day 30, storage at 37°C. From left to right: Control, Pasteurized, HHPP A, HHPP B and HHPP D

Figure 24 shows cranberry juice samples from the preliminary experiments of the present study. The sample at the left was processed by HHPP at 227 MPa for 15 minutes and the sample at the right was unprocessed juice used as control. For this study samples were stored in polyethylene bags that did not provide a good oxygen barrier. Samples were kept at room temperature for one month. As can be seen, the untreated sample showed significant color change and became significantly darker than the sample that was high pressure processed. The study showed the protective effect of HHPP on the color of cranberry juice when the package used is not a good light and oxygen barrier. The purpose of using a different packaging material for the present study was to isolate the effect of storage temperature on the samples and not confound with the effects of light or

oxygen so as to have a better understanding of the changes that occur on fruit products during storage.

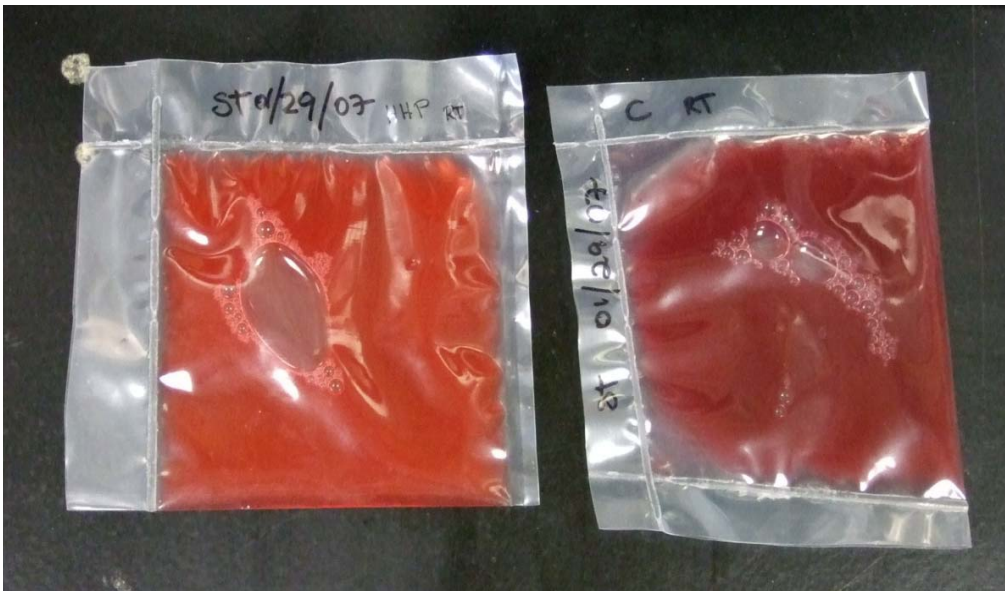

Figure 24: Results from a preliminary study on cranberry juice stored at room temperature for one month, HHPP (left) vs control (right)

Figure 25 shows two samples from the same preliminary study. In this case the samples were stored under refrigeration temperature (3°C) using polyethylene bags. After one month of storage both samples maintained the color that they had on day 0. HHPP sample from figure 24 had the same color than the samples stored under refrigeration, showing that HHPP had a protective effect on the color of the cranberry juice sample stored at room temperature when compared to the control (unprocessed sample).

Figure 25: Results from a preliminary study on cranberry juice stored at refrigeration temperature for one month, HHPP (left) vs control (right)

In addition to the TMA analysis, the color of the juice samples was also measured by colorimetry using a Hunterlab colorimeter. Results expressed in L*Ch system and their statistical analysis of variance can be found in Tables 19-24 for samples stored at 22°C, and Figures 28-30 show the same result on graphs. Tables 25-30 for samples stored at 37°C, Figures 31-33 also show the results from storage of the samples at 37°C. Although significant statistical differences between the samples were found by two-way ANOVA for each day and during storage under both temperatures used in this study, the changes in all three color characteristics of the samples were small when compared with the maximum values of each one of the color values (L*, Hue and Chroma).

Results of color measurement of the cranberry juice samples at week 4 of storage of at 22°C were not considered for the evaluation. At first view, results from week 4 of storage appear not to correlate with the rest of the data for the storage at 22°C. After statistical analysis of the data it was decided not to include them for the evaluation of the color characteristics of the cranberry juice samples, because when a regression line was obtained with the results from all 4 week it had a regression coefficient of 29.7%. Also the analysis was repeated using the data from the first three weeks of storage, and a 93.9 regression coefficient was obtained, and since the results form week 4 did not fell on the 95% confidence interval curve, the y could be considered as not accurate. Graphs of the statistical evaluation can be found in Figure 26 and Figure 27.

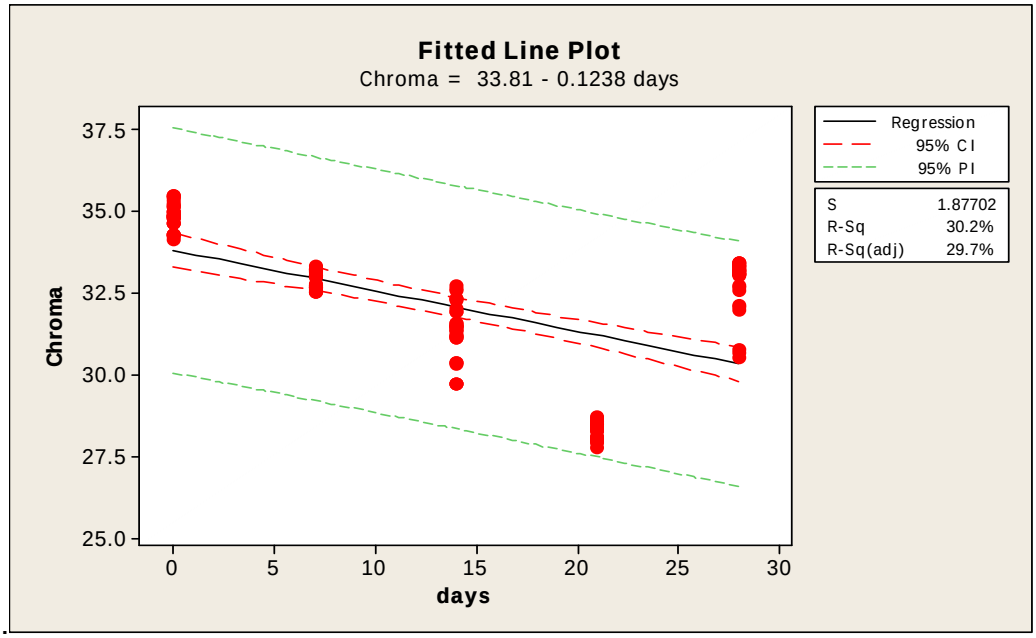

Figure 26: Statistical analysis to determine if results from color evaluation by on week 4 should be excluded from the final data

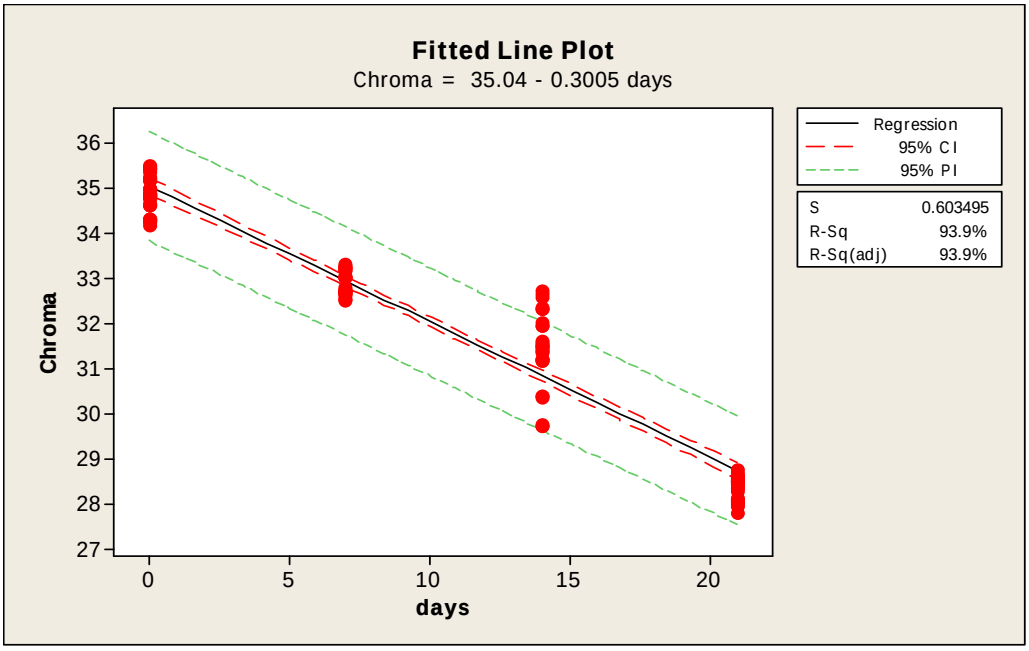

Figure 27: Graph showing chroma values of all samples including the results from week 4, excluding week 3

Table 19: Lightness - Storage at 22°C

Storage at 22°C (week)	Lightness				
	Untreated (Control)	Pasteurized	High pressure treatment		
			A	B	D
		90°C , 90 s	278 MPa,15 min	551 MPa, 15 min	551 MPa, 5 min
0	$15.3\pm0.02^{a}{}_{a}$	$15.7\pm0.08^{a}{}_{b}$	$15.6\pm0.13^{a}{}_{c}$	$15.4\pm0.04^{a}{}_{d}$	$15.4\pm0.04^{a}{}_{d}$
1	$14.2\pm0.04^{b}{}_{a}$	$14.6\pm0.15^{b}{}_{b}$	$14.4\pm0.07^{b}{}_{a}$	$14.5\pm0.22^{b}{}_{b}$	$14.6\pm0.04^{b}{}_{b}$
2	$13.3\pm0.13^{c}{}_{a}$	$14.5\pm0.11^{b}{}_{b}$	$14.0\pm0.07^{c}{}_{c}$	$13.9\pm 0.08^{c}{}_{c}$	$13.9\pm0.05^{c}{}_{c}$
3	$13.0\pm0.25^{d}{}_{a}$	$13.1\pm0.07^{c}{}_{b}$	$12.8\pm0.09^{d}{}_{c}$	$13.0\pm0.13^{d}{}_{a}$	$13\pm0.13^{d}{}_{a}$

^{a,b,c,d} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c,d}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 20:Two-way ANOVA - Lightness, storage at 22°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	19	105.27	5.541	429.29	<0.0001
Error	100	1.291	0.0129		
Corrected total	119	106.565			

R-square	Coeff. Var	Root MSE	Lightness mean
0.988	0.799	0.114	14.2

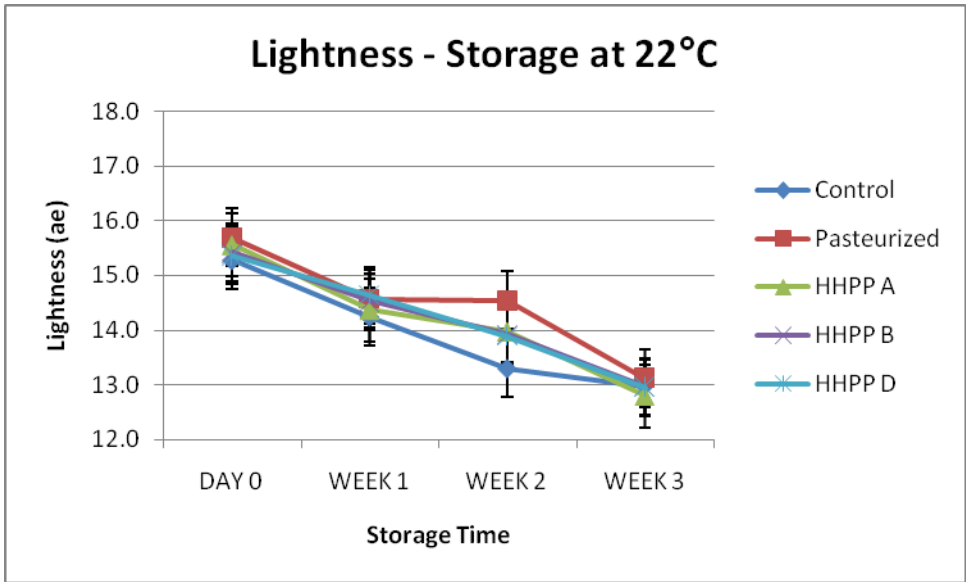

Figure 28: Lightness – cranberry juice samples stored at 22°C

Table 21: Chroma - Storage at 22°C

Storage at 22°C (week)	CHROMA				
	Untreated (Control)	Pasteurized	High Pressure Treatment		
			A	B	D
		90°C , 90 s	278 MPa , 15 min	551 MPa, 15 min	551 MPa, 5 min
0	$34.3\pm0.06^{a}{}_{a}$	$35.2\pm0.16^{a}{}_{b}$	$34.9\pm0.20^{a}{}_{b}$	$34.7\pm0.02^{a}{}_{c}$	$34.7\pm0.10^{a}{}_{c}$
1	$32.6\pm0.13^{b}{}_{a}$	$32.8\pm0.22^{b}{}_{a,b}$	$32.9\pm0.20^{b}{}_{b}$	$33.0\pm0.30^{b}{}_{b}$	$33.2\pm0.03^{b}{}_{d}$
2	$30.1\pm0.35^{c}{}_{a}$	$32.5\pm0.19^{c}{}_{b}$	$31.8\pm0.22^{c}{}_{c}$	$31.4\pm0.17^{c}{}_{d}$	$31.3\pm0.09^{c}{}_{d}$
3	$28.3\pm0.40^{d}{}_{a}$	$28.5\pm0.13^{d}{}_{b}$	$28.5\pm0.11^{d}{}_{b}$	$28.5\pm0.11^{d}{}_{b}$	$28.1\pm0.04^{d}{}_{a}$

^{a,b,c,d} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c,d}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 22: Two-way ANOVA - Chroma, storage at 22°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	19	702.778	36.988	1010.15	<0.0001
Error	100	3.662	0.0366		
Corrected total	119	706.439			

R-square	Coeff Var	Root MSE	Chroma mean
0.995	0.6	0.191	31.884

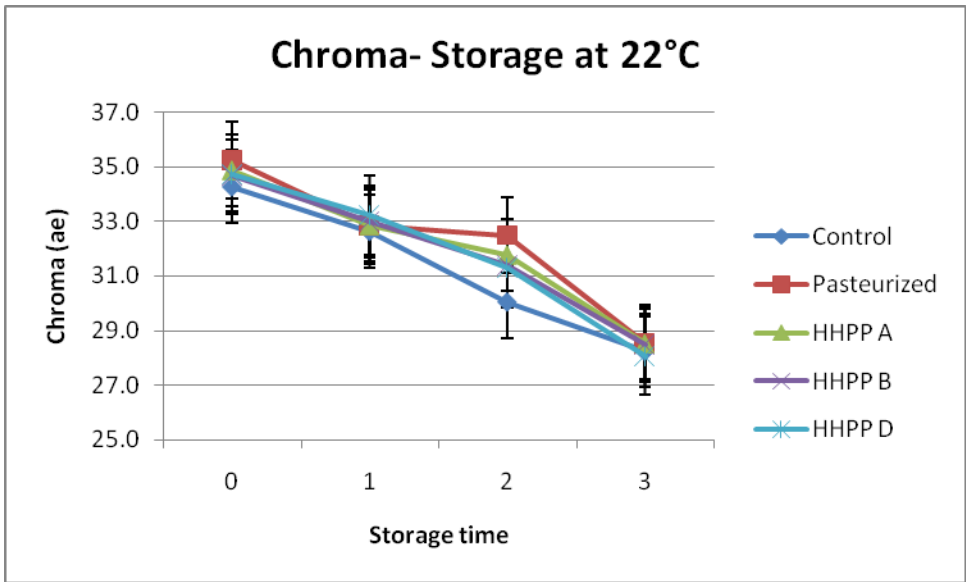

Figure 29: Chroma - cranberry juice samples stored at 22°C

Table 23: Hue - Storage at 22°C

Storage at 22°C	HUE				
	Untreated (Control)	Pasteurized	High Pressure Treatment		
			A	B	D
(week)		90°C , 90 s	278 MPa , 15 min	551 MPa, 15 min	551 MPa, 5 min
0	$17.2\pm0.01^{a}{}_{a}$	$17.0\pm0.07^{a,c}{}_{a}$	$17.1\pm0.06^{a}{}_{a}$	$17.1\pm0.05^{a}{}_{a}$	$17.2\pm0.03^{a,c}{}_{a}$
1	$16.8\pm0.05^{b}{}_{a}$	$17.1\pm0.05^{a,b}{}_{b}$	$16.9\pm0.07^{b}{}_{a}$	$17.0\pm0.14^{b}{}_{a,b}$	$17.0\pm0.06^{a,b}{}_{a,b}$
2	$16.8\pm0.12^{c}{}_{a}$	$17.2\pm0.16^{b}{}_{b}$	$16.9\pm0.07^{b}{}_{a}$	$17.1\pm0.04^{a,c}{}_{b}$	$17.1\pm0.08^{b}{}_{b}$
4	$17.1\pm0.17^{d}{}_{a}$	$17.6\pm0.12^{c}{}_{b}$	$17.0\pm0.15^{c}{}_{c}$	$17.4\pm0.14^{b,c}{}_{b}$	$17.6\pm0.12^{c}{}_{b}$

^{a,b,c,d} Different superscript letters in the same treatment indicate significant difference (P<0.05)
$_{a,b,c,}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 24: Two-way ANOVA - Hue, storage at 22°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	19	5.182	0.305	15.99	<0.0001
Error	100	1.193	0.019		
Corrected total	119	7.725			

R-square	Coeff Var	Root MSE	Hue mean
0.752	0.816	0.1383	16.948

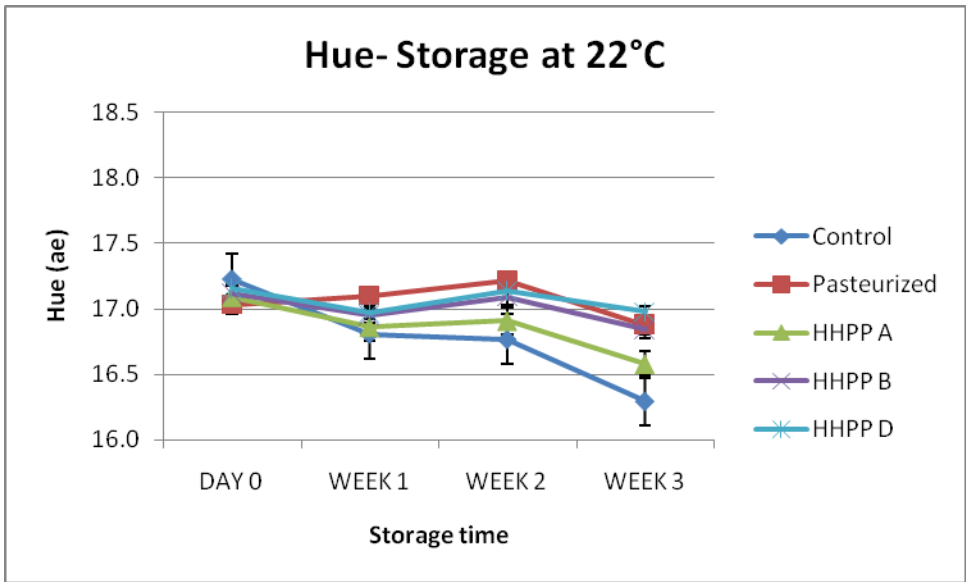

Figure 30: Hue - cranberry juice samples stored at 22°C

Table 25: Lightness - Storage at 37°C:

Storage at 37°C (days)	Lightness				
	Untreated (Control)	Pasteurized	High Pressure Treatment		
			A	B	D
		90°C , 90 s	278 MPa ,15 min	551 MPa, 15 min	551 MPa, 5 min
0	$15.3\pm0.02^{a}{}_{a}$	$15.7\pm0.08^{a}{}_{b,c}$	$15.6\pm0.13^{a}{}_{b,d}$	$15.4\pm0.04^{a}{}_{a,d}$	$15.4\pm0.04^{a}{}_{a,c}$
2	$13.7\pm0.29^{b}{}_{a}$	$13.9\pm0.18^{b}{}_{b}$	$13.9\pm0.04^{b}{}_{a,b}$	$13.9\pm0.06^{b}{}_{b}$	$13.9\pm0.16^{b}{}_{b}$
4	$15.4\pm0.09^{a}{}_{a}$	$15.8\pm0.07^{a}{}_{b}$	$15.7\pm0.12^{c}{}_{b}$	$15.8\pm0.17^{c}{}_{b,c}$	$15.6\pm0.09^{c}{}_{c}$
8	$16.2\pm0.11^{c}{}_{a}$	$15.4\pm0.14^{c}{}_{b}$	$15.7\pm0.03^{a,c}{}_{c}$	$15.6\pm0.03^{c,e}{}_{c}$	$15.9\pm0.07^{d}{}_{d}$
16	$15.3\pm0.04^{a}{}_{a}$	$15.1\pm0.15^{d}{}_{b}$	$15.2\pm0.18^{d}{}_{b}$	$14.8\pm0.14^{d}{}_{c}$	$14.8\pm0.02^{e}{}_{c}$
30	$17.3\pm0.13^{d}{}_{a}$	$16.1\pm0.20^{e}{}_{b}$	$17.5\pm0.17^{e}{}_{a}$	$15.6\pm0.15^{e}{}_{c}$	$15.9\pm0.03^{d}{}_{d}$

^{a,b,c,d,e} Different superscript letters in the same treatment indicate significant difference (P<0.05)

$_{a,b,c,d}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 26: Two-way ANOVA - Lightness, storage at 37°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	29	138.7	4.782	293.24	<0.0001
Error	150	2.44	0.0163		
Corrected total	179	141.14			

R-square	Coeff Var	Root MSE	Lightness mean
0.982667	0.83	0.1277	15.379

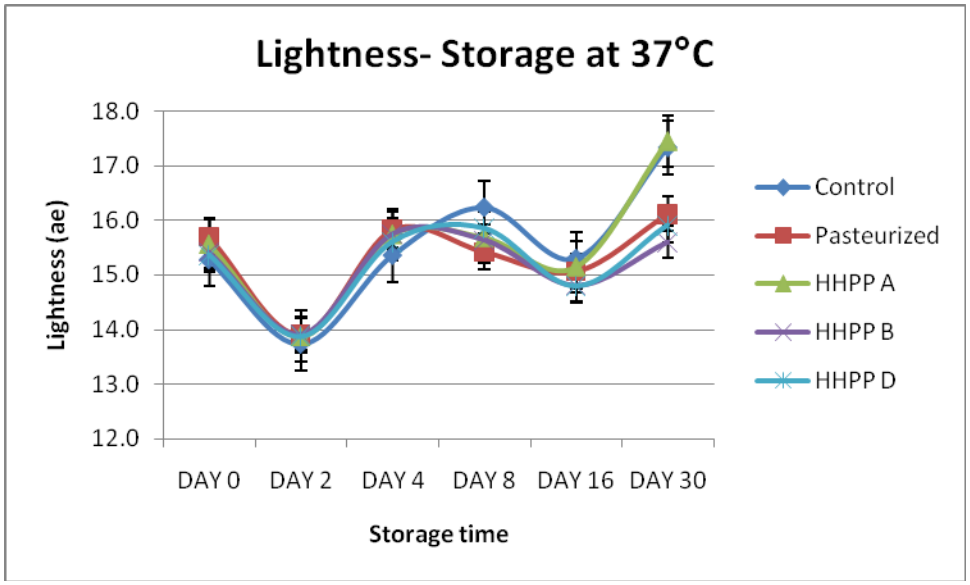

Figure 31: Lightness - cranberry juice samples stored at 37°C

Table 27: Chroma- Storage at 37°C

Storage at 37°C	CHROMA				
	Untreated (Control)	Pasteurized	High Pressure Treatment		
			A	B	D
(days)		90°C , 90 s	278 MPa, 15 min	551 MPa, 15 min	551 MPa, 5 min
0	$34.3\pm0.06^{a}{}_{a}$	$35.3\pm0.16^{a}{}_{b}$	$35.2\pm0.2^{a}{}_{b,c}$	$34.9\pm0.02^{a}{}_{c,d}$	$34.7\pm0.01^{a}{}_{d}$
2	$30.4\pm0.89^{b}{}_{a}$	$30.5\pm0.5^{b}{}_{a}$	$30.9\pm0.10^{b}{}_{b}$	$31.2\pm0.16^{b}{}_{b}$	$31.2\pm0.33^{b}{}_{b}$
4	$34.7\pm0.13^{c}{}_{a}$	$35.0\pm0.32^{a}{}_{a,b}$	$35.1\pm0.12^{a}{}_{a,b}$	$35.1\pm0.27^{a}{}_{b}$	$34.8\pm0.11^{a}{}_{a,b}$
8	$35.2\pm0.16^{d}{}_{a}$	$34.1\pm0.02^{c}{}_{b}$	$34.4\pm0.03^{c}{}_{c}$	$33.9\pm0.06^{c}{}_{b,c}$	$34.0\pm0.15^{c}{}_{b,c}$
16	$33.1\pm0.04^{e}{}_{a}$	$32.6\pm0.03^{d}{}_{b}$	$33.1\pm0.07^{d}{}_{c}$	$32.3\pm0.28^{d}{}_{b,c}$	$32.2\pm0.10^{d}{}_{b,c}$
30	$35.1\pm0.13^{c,d}{}_{a}$	$31.7\pm0.17^{d}{}_{b}$	$34.8\pm0.04^{e}{}_{c}$	$32.6\pm0.49^{d}{}_{d}$	$33.1\pm0.29^{e}{}_{e}$

^{a,b,c,d,e} Different superscript letters in the same treatment indicate significant difference (P<0.05)

$_{a,b,c,d,e}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 28:Two-way ANOVA - Chroma, storage at 37°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	29	443.466	15.29	152.49	<0.0001
Error	150	15.0425	0.1		<0.0001
Corrected total	179	458.508			<0.0001

R-square	Coeff Var	Root MSE	Chroma mean
0.9671	0.946	0.3166	33.466

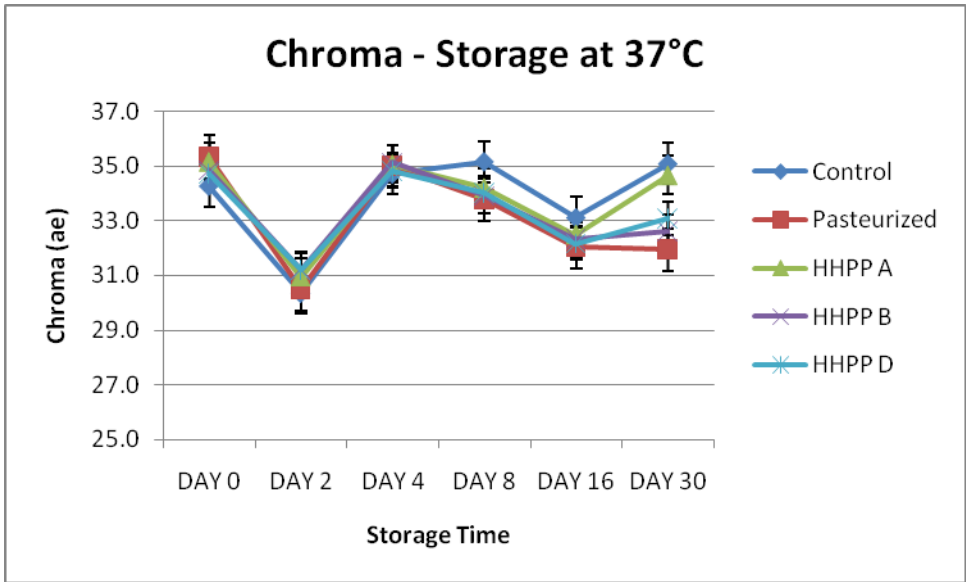

Figure 32: Chroma - cranberry juice samples stored at 37°C

Table 29: Hue - Storage at 37°C

Storage at 37°C	HUE				
	Untreated (Control)	Pasteurized	High Pressure Treatment		
			A	B	D
(days)		90°C , 90 s	278 MPa ,15 min	551 MPa,15 min	551 MPa, 5 min
0	$17.2\pm0.04^{a,d}{}_{a}$	$17.0\pm0.07^{a}{}_{a}$	$17.1\pm0.06^{a}{}_{a}$	$17.1\pm0.05^{a}{}_{a}$	$17.2\pm0.03^{a}{}_{a}$
2	$16.7\pm0.15^{b}{}_{a}$	$17.0\pm0.15^{a}{}_{b}$	$17.1\pm0.14^{a}{}_{b}$	$17.1\pm0.13^{a}{}_{b}$	$17.1\pm0.06^{a}{}_{b}$
4	$17.2\pm0.12^{a}{}_{a}$	$17.4\pm0.31^{b}{}_{b}$	$17.2\pm0.19^{a}{}_{a,b}$	$17.2\pm0.21^{a,b}{}_{a,b}$	$17.2\pm0.17^{a}{}_{a,b}$
8	$17.7\pm0.16^{c}{}_{a}$	$17.7\pm0.06^{c}{}_{a,b}$	$17.7\pm0.13^{b}{}_{a,b}$	$17.9\pm0.08^{c}{}_{b,c}$	$18.1\pm0.03^{b}{}_{c}$
16	$17.4\pm0.19^{d}{}_{a}$	$17.9\pm0.42^{c,d}{}_{b}$	$17.6\pm0.18^{c}{}_{a}$	$17.4\pm0.20^{b}{}_{a}$	$17.6\pm0.12^{c}{}_{a}$
30	$17.7\pm0.13^{c}{}_{a}$	$18.0\pm0.33^{d}{}_{b}$	$17.8\pm0.07^{b}{}_{a}$	$17.2\pm0.21^{a,b}{}_{c}$	$17.5\pm0.09^{c}{}_{d}$

^{a,b,c,d} Different superscript letters in the same treatment indicate significant difference (P<0.05)

$_{a,b,c,d}$ Different subscript letters in the same day of storage indicate significant difference (P<0.05)

Table 30: Two-way ANOVA - Hue, storage at 37°C

Source	DF	SS	MS	F value	Pr > F
Process*storage	29	20.455	0.705	23.96	<0.0001
Error	150	4.4166	0.02944		<0.0001
Corrected total	179	24.872			<0.0001

R-square	Coef. Var.	Root MSE	Hue mean
0.822	0.9865	0.171	17.39

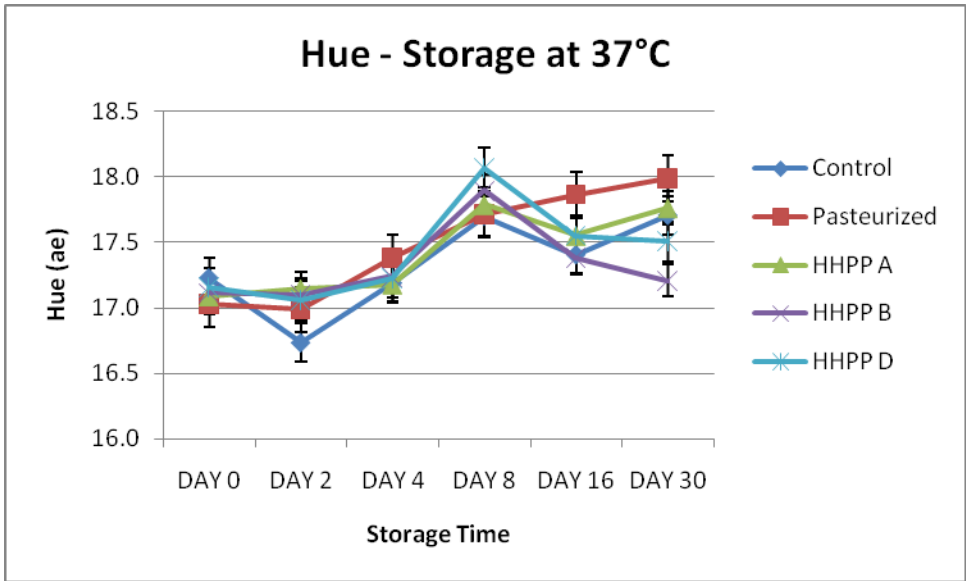

Figure 33: Hue - cranberry juice samples stored at 37°C

3.4 SENSORY EVALUATION

Single strength cranberry juice is highly acidic and tart to be consumed as is and commercially available cranberry juice is sold as cranberry juice cocktail, which contain at least 27% of cranberry juice, water and sugar added to reach 10 °Brix. In order to provide participants a product which they were familiar with, cranberry juice cocktail was prepared for the sensory evaluation session as explained in the methodology.

Results from the sensory evaluation test of cranberry juice are summarized in Table 31. The table shows the average value of difference from control on a 1-7 scale, with 0 showing “no difference” between samples and 7 showing ”very large difference” between samples. All three samples listed in the table were compared to an unprocessed sample used as control. As per the methodology (Carr, 1999), one of the samples to be compared with the control was a blind control, and this samples was also an unprocessed sample.

Table 31: Summary of Sensory Evaluation Results

Groups	*Count*	*Sum*	*Average*	*Variance*
HHPP	50	154	3.08	2.32
Thermal	50	157	3.14	1.63
Blind Control	50	127	2.54	1.64

Statistical analysis of the sensory evaluation data showed no significant difference between the three test samples (Table 32). Since the blind control was the same unprocessed juice as the control sample, and participants still found some difference between the two; therefore any difference between the processed samples and the control, may be explained by individual perception of the participants and not real difference between the samples.

Table 32: ANOVA of Sensory Evaluation Results

Sample	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.92	2	5.46	2.93	0.06	3.06
Within Groups	274.12	147	1.86			
Total	285.04	149				

Given the results from the sensory evaluation of the samples it can be said that processing of cranberry juice by thermal treatment at 90°C for 90 seconds, and by high hydrostatic pressure processing at 278 MPa for 15 minutes did not affect the sensory quality of the juice, and that the color and flavor of the juice did not change when compared to untreated juice.

4. CONCLUSIONS

The following conclusions could be drawn from this research:

- High hydrostatic pressure processing at 278 MPa for 15 minutes could be used as an alternative processing method to pasteurization of cranberry juice. Samples processed under these conditions showed better proanthocyanidin retention than pasteurized samples, and after one month of storage under 22°C and 37°C, the samples showed no visual differences in color. Also, sensory evaluation of the samples showed no differences between the two samples.

- High hydrostatic pressure processing may not lead to complete enzyme inactivation. The results from this study were similar to those from published studies, where changes in the sensory characteristics of samples processed by high pressure processing were correlated to partial inactivation of polyphenol oxidase.

- Cranberry proanthocyanidins were affected by HHPP. Higher pressure and longer processing time led to increased loss of PAC. Pasteurization of cranberry juice also caused losses in PAC content of the juice. Storage of cranberry juice also caused loss of PAC in all the samples evaluated in this study.

- Total monomeric anthocyanin content of cranberry juice increased immediately after thermal and HHP processing; TMA content was negatively affected by storage conditions such as time and temperature. Anthocyanin pigment values decreased after a month of storage, and samples stored at 37°C had lower TMA values than samples stored at 22°C.

- No visual differences in color were observed between untreated and treated samples immediately after HHPP and thermal processing. After one month of storage at 22°C and 37°C, therefore visual aspect of the juice cannot be correlated with anthocyanin loss.

- Processing of cranberry juice by pasteurization or HHPP at 278 MPa for 15 minutes doesn't affect the color and flavor of the juice, as shown by the results of the sensory evaluation.

- Use of HHPP did not show any overall benefits over the use of conventional thermal processing of cranberry juice.

5. FUTURE WORK

The present study left several questions about the effect of HHPP on cranberry juice and the causes that led to the results obtained. In order to complement the results showed in this work it would be recommended to do the follow up work suggested next:

- Evaluate the changes in enzyme activity and enzyme kinetics before and after processing; and during storage of the cranberry juice. Results from enzyme analysis should help explain changes in total monomeric anthocyanin. The most important enzyme to be analyzed would be polyphenol oxidase.

- Use of different packaging materials to evaluate the effect of oxygen and light on the pigment content and color of the juice.

- Measure changes in individual procyanidin polymers quantities to see if polymerization and/or depolymerization reactions are causing the variations found in total procyanidin content of the cranberry juice samples.

6. REFERENCES

U.S. Department of Agriculture: Nutrient Data Laboratory - Nutrient Database. http://www.nal.usda.gov.

Cape Cod Cranberry Growers' Association. http://www.cranberries.org.

(NASS), National Agricultural Statistics Service. http://www.nass.usda.gov.

Adamson, G., Lazarus, A. 1999. HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *Journal of Agriculature and Food Chemistry.* Vol. 47, pp. 4184-4188.

Asami, D., Hong, Y., Barrett, D., Mitchell, A. 2003. Processing-induced changes in total phenolics and procyanidins in clingstone peaches. *Journal of the Science of Food and Agriculture.* Vol. 83, pp. 56-63.

Carlson, J. 2003. Processing effects on the antioxidant activities of blueberry juices. *Thesis.* Raleigh, North Carolina, U.S. : North Carolina State University.

Carr, M. C. 1999. *Sensory Evaluation Techniques.* 3rd. Boca Raton : CRC Press LLC, pp. 86-94.

Cheynier, V. 2005. Polyphenols in foods are more complex than often thought. *The American Journal of Clinical Nutrition.* Vol. 81, pp. 223s-229s.

Del Pozo-Insfran, D., Del Follo-Martinez, A., Talcott, S.T., Brenes, C. 2007. Stability of copigmented anthocyanins and ascorbic acid in muscadine grape juice processed by high hydrostatic pressure. *Journal of Food Science.* Vol. 72, pp. S247-S253.

Fennema, O. 2008. *Fennema's Food Chemistry.* Fourth. s.l. : Marcel Dekker Inc.

Foo, L., Lu, Y.. Howell, A., Vorsa, N. (A). 2000. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fiambriated Escherichia coli in vitro. *Phytochemistry.* Vol. 54, pp. 173-181.

Foo, L., Lu, Y., Howell, A., Vorsa, N (B). 2000. A type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fiambriated Escherichia coli. *Journal of Natural Products.*Vol. 63, pp. 1225-1228.

Fuleki, T., Ricardo-Da-Silva, J.M. 2003. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *Journal of Agricultural and Food Chemistry.* Vol. 51, pp. 640-646.

Geisler, M. 2007. *Ag. Marketing Resource Center.* http://www.agmrc.com/agmrc/commodity/fruits/cranberries/cranberriesprofile.htm

Gimenez, J. Kadja, P. Margomenou, L., Pigott, J., Zabetakis, I. 2001. A study on the colour and sensory attributes of high hydrostatic pressure jams as compared with traditional jams. *J. Sci Food Agric.* Vol. 81, pp. 1228-1234.

Giusti, M., Wrolstad, R.E. 2001. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. *Current Protocols in Food Analytical Chemistry.* New York : Wiley, Unit F1.2.1-13.

Guerrero-Beltran, J., Barbosa-Canovas, G., Swanson, B. 2005. High hydrostatic pressure processing of fruit and vegetable products. *Food Reviews International.* Vol. 21, pp. 411-425.

Hendrickx, N., Knorr, D. 2001. *Ultra high pressure treatment of foods.* NY : Plenum Publishers.

Hong, Y., Barrett, D., Mitchell, A. 2004. Liquid chromatography.mass spectrometry investigation of the impact of thermal processing and storage on peach procyanidins. *Journal of Agricultural and Food Chemistry.* Vol. 52, pp. 2366-2371.

Howell, A., Reed, J., Krueger, C., Winterbottom, R., Cunningham, D., Leathy, M. 2005. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry.* Vol. 66, pp. 2281-2291.

Kalt, W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of Food Science.* Vol. 70, 1, pp. R11-R19.

Kelm, M., et al. 2006. High-Performance Liquid Chromatography Separation and Purification of Cacao (*Theobroma cacao L.)* Procyanidins According to Degree of Polymerization Using a Diol Stationary Phase. Vol. 54, pp. 1571-1576.

Kouniaki, S., Kajda, P., Zabetakis, I. 2004. The effect of high hydrostatic pressure on anthocyanins and ascorbic acid in blackcurrants (Ribes nigrum). *Flavour and Fragrance Journal.* Vol. 19, pp. 281-286.

Labrecque, J., Bodet, C., Chandad, F., Grenier, D. 2006. Effects of a high molecular weight cranberry fraction on growth, biofilm formation and adherence of Porphyromonas gingivalis. *Journal of Antimicrobial Chemotherapy.* Vol. 58, pp. 439-443.

Lambert, Y., Demazeau, G., Largeteau, A., Bouvier, J-M. 1999. Changes in aromatic volatile composition of strawberry after high pressure treatment. *Food Chemistry.* Vol. 67, pp. 7-16.

Lee, J., Durst, R.W., Wrolstad, R.E. 2002. Impact of juice processing on blueberry anthocyanins and polyphenolics: comparison of two pretreatments. *Journal of Food Science.* Vol. 67, 5, pp. 1660-1667.

Neto, C. 2007. Cranberry and its phytochemicals: a review of in vitro anticancer studies. *The Journal of Nutrition.* Vol. 137, pp. 186-193.

Pietta, G. 2000. Flavonoids as antioxidants. *Journal of Natural Products.* Vol. 63, pp. 1035-1042.

Porter, M., Krueger, C., Wiebe, D., Cunningham, D., Reed, J. 2001. Cranberry proanthocyanidins associate with low density lipoprotein and inhibit in vitro CU2+ -induced oxidation. *Journal of Food Science and Agriculture.* Vol. 81, pp. 1306-1313.

Prior, R., Lazarus, S., Cao, G., Muccitelli, H., Hammerstone, J. 2001. Identification of procyanidins and anthocyanins in blueberries and cranberries (Vaccinium Spp.) using high-performance liquid chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry.* Vol. 49, pp. 1270-1276.

Rastogi, N.K., Raghavarao, S.M.S., Balasubramaniam, V.M., Niranjan, K. Knorr, D. 2007. Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition.* Vol. 47, pp. 69-112.

Ross, J., Kasum, C. 2002. Dietary flavonoids: bioavailability,metabolic effects, and safety. *Annual Reviews on Nutrition.* Vol. 22, pp. 19-34.

San Martin, M.F., Barbosa-Canovas, G., Swanson, B. 2002. Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition.* Vol. 42, 6, pp. 627-645.

Skrede, G., Wrolstad, R.E., Durst, R.W. 2000. Changes in anthocyanins and polyphenolics during juice processing of highblush blueberries (Vaccinium corymbosum L.). *Journal of Food Science.* Vol. 65, 2, pp. 357-364.

Spanos, G., Wrolstad, R. 1992. Phenolics of apple, pear, and white grape juices and their changes with processing and storage - A review. *Journal of Agricultural and Food Chemistry.* Vol. 40, 9, pp. 1478-1487.

Sumitani, H., Suekane, S., Nakatani, A., Tatsuka, K. 1994. Changes in composition of volatile compounds in high pressure treated peach. *J. Agric. Food Chem.* Vol. 42, pp. 785-790.

Suthanthangjai, W., Kadja, P., Zabetakis, I. 2005. The effect of high hydrostatic pressure on the anthocyanins of raspberry (Rubus idaeus). *Food Chemistry.* Vol. 90, pp. 193-197.

Talcott, S., Brenes, C., Pires, D., Del Pozo-Insfran, D. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *Journal of Agricultural and Food Chemistry.* Vol. 51, 4, pp. 957-963.

The Cranberry Institute. 2007. http://www.cranberryinstitute.org

Vvedensaya, I., Rosen, R., Guido, J., Russell, D., Mills, K., Vorsa, V. (A). 2004. Characterization of flavonols in cranberry (Vaccinium macrocarpon) powder. *Journal of Agricultural and Food Chemistry.* Vol. 52, pp. 188-195.

Vvedenskaya, I., Vorsa, N. (B). 2004. Flavonoid composition over fruit development and maturation in American cranberry, Vaccinium macrocarpon Ait. *Plant Science.* Vol. 167, pp. 1043-1054.

Watson, D.J. 1997. Chemical characterization of wild maine cranberries. *Thesis.* Maine, U.S. : University of Maine.

Weiss, E., Kozlovsky, A., Steinberg, D., Lev-Dor, R., Ronit Bar Ness Greenstein, Feldman, M., Sharon, N., Ofek, I. 2004. A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits in vitro adhesion to hydroxyapatite. *FEMS Microbiology Letters.* Vol. 232, pp. 89-92.

Wrolstad, R. Durst, R., Lee, J. 2005. Tracking color and pigments changes in anthocyanin products. *Trends in Food Science and Technology.* Vol. 16, pp. 423-428.

Zhang, L., Ma, J., Pan, K., Liang W. Go, V., Chen, J., You, W. 2005. Efficacy of cranberry juice on Helicobacter pylori infection: a double blind, randomized placebo controlled trial. *Helicobacter.* Vol. 10, 2, pp. 139-145.

7. APPENDIX

7.1 Proanthocyanidins (PAC)

Table 33: Proanthocyanidin Content (mg/g juice), storage at 22°C

	day 0	week 1	week 4
Sample C1	0.174*	0.119	0.128
Sample C2	0.130	0.127	0.130
Sample C3	0.131	0.125	0.131
Sample T1	0.125	0.108	0.107
Sample T2	0.121	0.119	0.107
Sample T3	0.120	0.118	0.115
Sample A1	0.128	0.149*	0.108
Sample A2	0.126	0.123	0.117
Sample A3	0.126	0.126	0.116
Sample B1	0.112	0.112	0.112
Sample B2	0.112	0.113	0.107
Sample B3	0.111	0.114	0.108
Sample D1	0.122	0.114	0.107
Sample D2	0.122	0.118	0.105
Sample D3	0.119	0.120	0.105

*results came from samples of smaller size and were not considered for the final results

Table 34: Proanthocyanidin Content (mg/g juice), storage at 37°C

	day 0	day 2	day 4	day 8	day 16	day 30
Sample C1	0.174*	0.125	0.123	0.125	0.121	0.123
Sample C2	0.130	0.126	0.121	0.125	0.119	0.113
Sample C3	0.131	0.124	0.123	0.122	0.120	0.122
Sample T1	0.125	0.119	0.111	0.107	0.114	0.092
Sample T2	0.121	0.116	0.123	0.108	0.094	0.101
Sample T3	0.120	0.125	0.112	0.103	0.104	0.096
Sample A1	0.128	0.118	0.115	0.138*	0.112	0.112
Sample A2	0.126	0.117	0.126	0.128	0.117	0.112
Sample A3	0.126	0.123	0.119	0.120	0.132*	0.104
Sample B1	0.112	0.115	0.113	0.107	0.105	0.094
Sample B2	0.112	0.115	0.109	0.105	0.110	0.112
Sample B3	0.111	0.113	0.113	0.110	0.109	0.097
Sample D1	0.122	0.118	0.115	0.121	0.110	0.106
Sample D2	0.122	0.122	0.123	0.107	0.106	0.101
Sample D3	0.119	0.115	0.117	0.123	0.105	0.108

*results came from sample of smaller size and were not considered for the final results

7.2 Total Monomeric Anthocyanin (TMA)

Table 35: TMA Content (mg/L juice), Day 0

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.6111	0.0609	0.5502	9	82.69
Sample C2	0.5527	0.0621	0.4906	9	73.73
Sample C3	0.6087	0.0646	0.5441	9	81.77
Sample T1	0.6214	0.0691	0.5523	9	83.01
Sample T2	0.6127	0.0655	0.5472	9	82.24
Sample T3	0.6271	0.0654	0.5617	9	84.42
Sample A1	0.6429	0.0641	0.5788	9	86.99
Sample A2	0.6499	0.0798	0.5701	9	85.68
Sample A3	0.6454	0.0678	0.5776	9	86.81
Sample B1	0.6361	0.0662	0.5699	9	85.65
Sample B2	0.6353	0.0671	0.5682	9	85.39
Sample B3	0.6377	0.0706	0.5671	9	85.23
Sample D1	0.6461	0.0667	0.5794	9	87.08
Sample D2	0.6474	0.0685	0.5789	9	87.00
Sample D3	0.6456	0.0533	0.5923	9	89.02

Table 36 Percentage Loss of TMA, Storage at 22°C

Storage	**TMA % Loss**				
22°C	**Untreated**	**Pasteurized**	**High pressure treatment**		
(weeks)	**(Control)**		**A**	**B**	**D**
		90°C , 90 s	278MPa,15m	551MPa,15m	551 MPa,5min
0	0.0	0.0	0.0	0.0	0.0
1	5.3	6.7	15.7	12.0	13.3
2	19.5	15.9	22.1	18.7	20.6
3	27.5	22.7	25.2	22.9	26.4
4	35.3	34.1	40.3	33.4	35.0

Table 37: TMA Content (mg/L juice), Storage at 22°C, Week 1

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.572	0.077	0.495	9	74.439
Sample C2	0.590	0.078	0.513	9	77.024
Sample C3	0.571	0.077	0.494	9	74.168
Sample T1	0.587	0.078	0.509	9	76.513
Sample T2	0.607	0.078	0.529	9	79.428
Sample T3	0.589	0.078	0.512	9	76.903
Sample A1	0.563	0.075	0.487	9	73.251
Sample A2	0.566	0.078	0.489	9	73.447
Sample A3	0.556	0.077	0.479	9	71.974
Sample B1	0.579	0.079	0.500	9	75.130
Sample B2	0.576	0.082	0.495	9	74.363
Sample B3	0.587	0.081	0.507	9	76.122
Sample D1	0.589	0.081	0.507	9	76.227
Sample D2	0.590	0.083	0.506	9	76.107
Sample D3	0.588	0.084	0.504	9	75.806

Table 38: TMA Content (mg/L juice), Storage at 22°C, Week 2

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.508	0.075	0.433	9	65.000
Sample C2	0.488	0.075	0.413	9	62.040
Sample C3	0.506	0.074	0.431	9	64.805
Sample T1	0.548	0.078	0.471	9	70.726
Sample T2	0.549	0.080	0.469	9	70.426
Sample T3	0.540	0.083	0.458	9	68.803
Sample A1	0.517	0.076	0.441	9	66.308
Sample A2	0.531	0.080	0.450	9	67.691
Sample A3	0.532	0.078	0.454	9	68.232
Sample B1	0.540	0.079	0.461	9	69.269
Sample B2	0.541	0.078	0.463	9	69.599
Sample B3	0.548	0.087	0.462	9	69.359
Sample D1	0.541	0.082	0.459	9	68.953
Sample D2	0.553	0.081	0.472	9	70.877
Sample D3	0.555	0.096	0.459	9	68.953

Table 39: TMA Content (mg/L juice), Storage at 22°C, Week 3

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.445	0.075	0.370	9	55.622
Sample C2	0.456	0.078	0.378	9	56.863
Sample C3	0.478	0.077	0.401	9	60.313
Sample T1	0.503	0.082	0.421	9	63.232
Sample T2	0.512	0.079	0.433	9	65.083
Sample T3	0.511	0.081	0.430	9	64.594
Sample A1	0.504	0.075	0.428	9	64.354
Sample A2	0.508	0.078	0.430	9	64.623
Sample A3	0.511	0.078	0.434	9	65.197
Sample B1	0.515	0.083	0.433	9	65.010
Sample B2	0.521	0.081	0.441	9	66.215
Sample B3	0.528	0.087	0.441	9	66.238
Sample D1	0.521	0.084	0.437	9	65.694
Sample D2	0.525	0.089	0.436	9	65.492
Sample D3	0.515	0.100	0.415	9	62.324

Table 40: TMA Content (mg/L juice), Storage at 22°C, Week 4

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.407	0.071	0.336	9	50.520
Sample C2	0.419	0.071	0.348	9	52.235
Sample C3	0.410	0.069	0.342	9	51.365
Sample T1	0.442	0.083	0.360	9	54.062
Sample T2	0.450	0.082	0.368	9	55.335
Sample T3	0.449	0.083	0.367	9	55.110
Sample A1	0.424	0.073	0.351	9	52.692
Sample A2	0.420	0.077	0.342	9	51.435
Sample A3	0.416	0.078	0.338	9	50.805
Sample B1	0.459	0.084	0.376	9	56.451
Sample B2	0.453	0.081	0.373	9	56.000
Sample B3	0.475	0.087	0.388	9	58.318
Sample D1	0.458	0.086	0.372	9	55.953
Sample D2	0.464	0.082	0.382	9	57.452
Sample D3	0.470	0.086	0.384	9	57.663

Table 41: Percentage Loss of TMA, Storage at 37°C

Storage at 37°C (days)	TMA % Loss				
	Untreated (Control)	Pasteurized	High pressure treatment		
			A	B	D
		90°C , 90 s	278MPa,15m	551MPa,15m	551MPa,5min
0	0.00	0.00	0.00	0.00	0.00
2	7.09	8.52	20.37	11.77	15.69
4	15.65	14.64	26.20	21.16	19.32
8	35.01	28.32	40.80	36.62	38.82
16	52.35	45.03	56.03	48.15	50.81
30	67.38	65.16	71.87	65.17	62.06

Table 42: TMA Content (mg/L juice), Storage at 37°C, Day 2

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.566	0.092	0.473	9	71.12
Sample C2	0.575	0.081	0.494	9	74.24
Sample C3	0.572	0.066	0.505	9	75.96
Sample T1	0.561	0.068	0.493	9	74.06
Sample T2	0.573	0.069	0.504	9	75.72
Sample T3	0.589	0.066	0.523	9	78.62
Sample A1	0.537	0.071	0.466	9	70.05
Sample A2	0.523	0.080	0.443	9	66.53
Sample A3	0.530	0.064	0.466	9	70.04
Sample B1	0.568	0.082	0.486	9	73.07
Sample B2	0.580	0.065	0.515	9	77.40
Sample B3	0.571	0.067	0.503	9	75.64
Sample D1	0.559	0.082	0.477	9	71.69
Sample D2	0.569	0.073	0.496	9	74.56
Sample D3	0.570	0.067	0.503	9	75.58

Table 43: TMA Content (mg/L juice), Storage at 37°C, Day 4

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.516	0.078	0.439	9	65.902
Sample C2	0.548	0.079	0.469	9	70.441
Sample C3	0.505	0.075	0.430	9	64.565
Sample T1	0.549	0.078	0.470	9	70.681
Sample T2	0.560	0.079	0.481	9	72.320
Sample T3	0.552	0.085	0.467	9	70.110
Sample A1	0.509	0.076	0.433	9	65.106
Sample A2	0.486	0.073	0.414	9	62.145
Sample A3	0.501	0.074	0.427	9	64.234
Sample B1	0.538	0.081	0.457	9	68.713
Sample B2	0.527	0.085	0.443	9	66.503
Sample B3	0.524	0.079	0.445	9	66.819
Sample D1	0.550	0.076	0.474	9	71.252
Sample D2	0.549	0.080	0.469	9	70.441
Sample D3	0.550	0.081	0.470	9	70.561

Table 44: TMA Content (mg/L juice), Storage at 37°C, Day 8

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.413	0.065	0.348	9	52.361
Sample C2	0.399	0.062	0.336	9	50.512
Sample C3	0.409	0.063	0.346	9	51.925
Sample T1	0.453	0.072	0.381	9	57.245
Sample T2	0.487	0.072	0.415	9	62.370
Sample T3	0.467	0.073	0.395	9	59.350
Sample A1	0.404	0.066	0.338	9	50.783
Sample A2	0.415	0.068	0.347	9	52.091
Sample A3	0.403	0.065	0.338	9	50.738
Sample B1	0.431	0.074	0.358	9	53.774
Sample B2	0.435	0.073	0.362	9	54.450
Sample B3	0.436	0.076	0.361	9	54.210
Sample D1	0.429	0.073	0.356	9	53.488
Sample D2	0.431	0.076	0.355	9	53.338
Sample D3	0.435	0.074	0.360	9	54.134

Table 45: TMA Content (mg/L juice), Storage at 37°C, Day 16

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.311	0.063	0.248	9	37.272
Sample C2	0.306	0.063	0.243	9	36.520
Sample C3	0.325	0.061	0.264	9	39.707
Sample T1	0.376	0.073	0.304	9	45.658
Sample T2	0.383	0.073	0.309	9	46.485
Sample T3	0.374	0.074	0.300	9	45.102
Sample A1	0.326	0.061	0.265	9	39.872
Sample A2	0.304	0.066	0.238	9	35.784
Sample A3	0.319	0.063	0.256	9	38.444
Sample B1	0.363	0.076	0.287	9	43.163
Sample B2	0.362	0.074	0.288	9	43.329
Sample B3	0.385	0.077	0.309	9	46.379
Sample D1	0.367	0.079	0.288	9	43.253
Sample D2	0.362	0.075	0.288	9	43.223
Sample D3	0.369	0.083	0.286	9	42.953

Table 46: TMA Content (mg/L juice), Storage at 37°C, Day 30

	pH 1.0 A 520nm - 700nm	pH 4.5 A 520nm - 700nm	A	DF	TMA
Sample C1	0.232	0.053	0.179	9	26.883
Sample C2	0.222	0.052	0.170	9	25.593
Sample C3	0.223	0.055	0.168	9	25.213
Sample T1	0.234	0.073	0.161	9	24.262
Sample T2	0.267	0.072	0.195	9	29.286
Sample T3	0.268	0.072	0.196	9	29.441
Sample A1	0.191	0.051	0.140	9	20.994
Sample A2	0.228	0.058	0.169	9	25.454
Sample A3	0.192	0.055	0.137	9	20.556
Sample B1	0.210	0.061	0.149	9	22.416
Sample B2	0.271	0.065	0.205	9	30.856
Sample B3	0.269	0.072	0.198	9	29.693
Sample D1	0.266	0.068	0.197	9	29.668
Sample D2	0.279	0.069	0.210	9	31.557
Sample D3	0.266	0.072	0.194	9	29.135

7.3 Color

Table 47: L, a, and b values - storage at 22°C

	DAY 0			WEEK 1			WEEK 2			WEEK 3			WEEK 4		
	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
C1	15.28	32.76	10.13	14.28	31.37	9.45	13.17	28.49	8.53	13.29	27.45	7.89	14.81	29.24	8.9
	15.31	32.63	10.12	14.27	31.33	9.48	13.19	28.45	8.61	13.21	27.56	7.9	14.79	29.44	8.99
	15.29	32.75	10.19	14.29	31.4	9.44	13.16	28.51	8.55	13.08	27.4	8.23	14.8	29.37	9
C2	15.29	32.73	10.13	14.21	31.13	9.42	13.42	29.09	8.78	12.76	26.69	7.68	14.90	31.57	9.85
	15.28	32.79	10.14	14.21	31.16	9.39	13.41	29.04	8.84	12.77	26.82	7.82	14.90	31.58	9.84
	15.24	32.78	10.17	14.2	31.13	9.44	13.39	29.13	8.70	12.73	26.79	8.02	14.89	31.62	9.79
T1	15.77	33.95	10.33	14.7	31.58	9.72	14.64	31.11	9.73	13.2	27.25	8.39	15.32	31.72	10.04
	15.79	33.88	10.39	14.72	31.57	9.73	14.63	31.31	9.56	13.2	27.34	8.21	15.36	31.73	10.07
	15.75	33.96	10.37	14.71	31.57	9.74	14.66	31.16	9.73	13.13	27.49	8.38	15.35	31.81	9.96
T2	15.65	33.61	10.35	14.43	31.17	9.55	14.43	30.91	9.50	13.06	27.27	8.22	15.33	31.63	10.09
	15.63	33.63	10.31	14.44	31.24	9.57	14.44	30.84	9.61	13.04	27.30	8.23	15.31	31.67	10.12
	15.61	33.7	10.31	14.44	31.2	9.59	14.44	30.91	9.54	13.07	27.13	8.23	15.33	31.63	10.02
A1	15.71	33.66	10.37	14.31	31.3	9.47	14.01	30.66	9.24	12.86	27.42	8.19	14.84	31.62	9.73
	15.68	33.87	10.42	14.32	31.27	9.48	14.06	30.57	9.28	12.86	27.43	8.08	14.85	31.63	9.69
	15.64	33.8	10.4	14.29	31.24	9.52	14.04	30.58	9.29	12.87	27.35	8.14	14.84	31.7	9.59
A2	15.47	33.45	10.29	14.43	31.65	9.56	13.89	30.22	9.18	12.7	27.27	8.18	14.49	30.62	9.26
	15.47	33.46	10.21	14.42	31.63	9.52	13.93	30.23	9.21	12.79	27.17	8.08	14.46	30.72	9.33
	15.42	33.46	10.27	14.44	31.6	9.59	13.92	30.17	9.21	12.68	27.43	8.14	14.47	30.7	9.49
B1	15.37	33.32	10.25	14.29	31.33	9.46	13.97	30.08	9.27	13.07	27.20	8.25	15.15	31.96	9.91
	15.41	33.34	10.25	14.33	31.3	9.47	13.99	30.11	9.21	13.03	27.29	8.1	15.15	31.91	10.03
	15.39	33.3	10.25	14.33	31.32	9.46	13.96	30.1	9.24	13.11	27.02	8.3	15.17	31.91	9.96
B2	15.47	33.35	10.22	14.73	31.78	9.78	13.94	30.08	9.27	12.83	27.38	8.25	15	31.09	9.82
	15.46	33.34	10.31	14.72	31.7	9.72	13.82	29.78	9.15	12.85	27.27	8.27	14.97	31.21	9.92
	15.42	33.31	10.26	14.72	31.86	9.78	13.81	29.79	9.15	12.84	27.28	8.28	15	31.19	9.80
D1	15.42	33.25	10.23	14.7	31.74	9.71	13.94	29.94	9.29	13.08	26.82	8.19	15.32	31.84	9.96
	15.4	33.28	10.27	14.67	31.81	9.64	13.93	29.98	9.25	13.07	26.81	8.13	15.31	31.79	10.12
	15.38	33.27	10.26	14.66	31.75	9.72	13.95	30.01	9.2	13.06	26.73	8.46	15.33	31.8	10.13
D2	15.35	33.09	10.24	14.62	31.75	9.68	13.85	29.81	9.21	12.82	26.89	8.22	15.17	31.6	10.03
	15.36	33.11	10.21	14.61	31.82	9.72	13.83	29.84	9.18	12.83	26.88	8.14	15.15	31.63	10.08
	15.32	33.09	10.21	14.58	31.8	9.67	13.85	29.83	9.14	12.85	26.94	7.99	15.14	31.65	10.08

Table 48: L, a, and b values - Storage at 37°C

	DAY 0			DAY 2			DAY 4			DAY 8			DAY 16			DAY 30		
	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
C1	15.28	32.76	10.13	14.24	27.83	8.37	15.43	33.42	10.24	16.33	33.65	10.67	15.29	31.73	9.74	17.45	33.42	10.81
	15.31	32.63	10.12	13.94	28.22	8.53	15.46	33.12	10.27	16.37	33.42	10.84	15.38	31.62	9.93	17.46	33.61	10.67
	15.29	32.75	10.19	13.52	29.14	8.85	15.43	33.21	10.24	16.3	33.76	10.72	15.29	31.58	9.85	17.44	33.54	10.7
C2	15.29	32.73	10.13	13.62	29.68	8.97	15.28	33.22	10.20	16.14	33.37	10.7	15.28	31.59	9.98	17.21	33.33	10.57
	15.28	32.79	10.14	13.58	29.79	8.86	15.25	33.09	10.29	16.13	33.44	10.59	15.29	31.58	9.9	17.21	33.37	10.68
	15.24	32.78	10.17	13.52	29.8	8.84	15.31	33.00	10.30	16.14	33.4	10.6	15.28	31.55	10.0	17.21	33.31	10.57
T1	15.77	33.95	10.33	14.00	28.82	8.83	15.89	33.64	10.62	15.55	32.51	10.34	14.91	31.06	9.79	16.31	29.91	9.96
	15.79	33.88	10.39	14.05	28.88	8.66	15.83	33.65	10.55	15.55	32.54	10.35	14.96	31.04	9.74	16.31	30.00	9.87
	15.75	33.96	10.37	14.12	28.52	8.78	15.85	33.64	10.61	15.55	32.5	10.37	14.94	31.07	9.83	16.27	30.29	9.83
T2	15.65	33.61	10.35	13.71	29.56	9.02	15.91	33.04	10.10	15.31	31.75	10.2	15.19	29.92	9.8	15.93	30.70	9.82
	15.63	33.63	10.31	13.73	29.58	9.04	15.74	33.31	10.19	15.3	31.84	10.16	15.16	30	9.78	15.9	30.82	9.71
	15.61	33.7	10.31	13.77	29.62	9.09	15.77	33.09	10.61	15.3	31.89	10.19	15.24	29.89	9.98	16.01	30.56	9.95
A1	15.71	33.66	10.37	13.82	29.76	9.06	15.93	33.45	10.28	15.72	32.8	10.45	15.31	31.6	9.97	17.60	33.17	10.61
	15.68	33.87	10.42	13.86	29.57	9.11	15.81	33.56	10.25	15.72	32.86	10.42	15.33	31.43	10.1	17.61	33.11	10.54
	15.64	33.8	10.4	13.83	29.59	9.2	15.71	33.54	10.32	15.71	32.78	10.48	15.31	31.49	9.85	17.61	33.15	10.65
A2	15.47	33.45	10.29	13.91	29.53	9.08	15.77	33.56	10.29	15.67	32.33	10.45	14.98	30.43	9.57	17.3	32.85	10.49
	15.47	33.46	10.21	13.91	29.46	9.18	15.68	33.64	10.54	15.66	32.32	10.44	14.96	30.53	9.59	17.32	32.86	10.52
	15.42	33.46	10.27	13.91	29.50	9.06	15.58	33.30	10.44	15.65	32.34	10.42	15.01	30.44	9.66	17.27	32.98	10.62
B1	15.37	33.32	10.25	13.94	30.02	9.17	15.9	33.33	10.44	15.67	32.2	10.4	14.94	31.1	9.75	15.75	30.6	9.61
	15.41	33.34	10.25	14	29.86	9.21	15.71	33.67	10.47	15.65	32.24	10.44	14.92	31.06	9.78	15.73	30.78	9.56
	15.39	33.3	10.25	13.93	30.02	9.2	15.43	33.89	10.60	15.66	32.21	10.45	14.96	31.04	9.88	15.74	30.71	9.63
B2	15.47	33.35	10.22	13.95	29.6	9.21	15.89	33.17	10.29	15.65	32.3	10.44	14.68	30.71	9.47	15.47	31.54	9.72
	15.46	33.34	10.31	13.87	29.84	9.08	15.83	33.54	10.45	15.63	32.37	10.37	14.7	30.54	9.57	15.5	31.49	9.67
	15.42	33.31	10.26	13.83	29.72	9.18	15.74	33.73	10.19	15.59	32.35	10.4	14.68	30.63	9.46	15.43	31.76	9.64
D1	15.42	33.25	10.23	14.07	30.14	9.24	15.52	33.19	10.16	15.88	32.51	10.59	14.8	30.77	9.73	15.89	31.43	9.87
	15.4	33.28	10.27	14.03	30.06	9.28	15.53	33.19	10.2	15.90	32.47	10.59	14.81	30.77	9.68	15.96	31.16	9.81
	15.38	33.27	10.26	13.98	30.17	9.25	15.51	33.21	10.22	15.95	32.5	10.57	14.82	30.72	9.82	15.9	31.37	9.99
D2	15.35	33.09	10.24	13.75	29.55	9.05	15.68	33.38	10.37	15.78	32.25	10.52	14.8	30.6	9.58	15.86	31.83	9.99
	15.36	33.11	10.21	13.77	29.52	9.03	15.7	33.33	10.44	15.78	32.22	10.53	14.82	30.54	9.71	15.92	31.68	9.98
	15.32	33.09	10.21	13.71	29.6	9.05	15.69	33.31	10.46	15.81	32.23	10.51	14.76	30.65	9.66	15.92	31.85	10.04

7.4 Sensory evaluation

Table 49: Results from Sensory Evaluation Study

Subject	HHPP	Thermal	Control	Comments
1	5	3	1	contol#2bitter.control#3 disagreeasbly sour
2	4	3	4	
3	3	1	1	GOOD
4	3	4	4	
5	7	5	6	In each case the control seemed weaker, watered down, sample more bitter
6	2	4	3	In all three tastings, the sample tasted more acidic than the control.
7	2	1	3	
8	2	2	1	
9	2	3	2	First sample was slightly sweet; second sample was the most tart
10	2	4	4	
11	2	3	2	
12	2	3	1	
13	2	2	1	
14	3	4	3	not sweet
15	2	1	2	384 tastes less flavor (bitter) than control.
16	3	3	1	
17	2	1	4	
18	6	4	2	
19	2	4	1	Sample 163 tasted slightly metallic.
20	3	4	2	
21	2	4	1	
22	5	3	5	
23	1	2	2	perhaps opaque containers to prevent visual assessment. all were tasty.
24	3	3	3	slight difference in sweetness for all samples
25	2	2	1	maybe some differences in acidity. the samples tasted and smelled similar
26	3	3	2	
27	2	2	2	
28	2	2	4	
29	2	3	2	
30	5	5	2	
31	7	2	4	
32	2	4	3	
33	2	3	5	

34	4	4	4	
35	4	2	3	the taste a bit of sweet to me
36	5	2	4	
37	3	4	2	
38	4	3	1	
39	3	2	3	Treatment samples were more robust in flavor than control.
40	2	3	2	the samples all tasted fairly similar... some seemed to be slightly sweeter
41	3	2	3	noticed only slight differences, tasted like watered down cranberry juic
42	1	4	3	
43	6	2	2	The water was too bitter
44	4	4	2	
45	2	6	4	
46	3	2	2	
47	6	5	2	
48	1	6	4	The second sample tasted more watered down to me, the third was more sour
49	4	6	1	163 tasted sweeter then any other one
50	2	3	1	