THE EFFECTS OF HIGH-PRESSURE THOTTLING VERSUS THERMAL PASTEURIZATION ON A BLUEBERRY-WHEY BEVERAGE

by

DAVID C. PECK

(Under the Direction of Romeo T. Toledo)

ABSTRACT

Continuous high-pressure throttling is an emerging technology which can produce products of superior quality as compared to traditional thermal processing techniques. The effects of high-pressure throttling were compared to those of conventional thermal pasteurization using a blueberry-whey beverage. Taste, viscosity, particle size, sedimentation, pH and color were examined. Sensory analysis consisted of a triangle difference test 4 days after processing and two two-tailed preference tests, 5 and 35 days after processing. Viscosity was determined with a strain controlled rheometer, particle size by laser light scattering, pH by meter and color by chroma meter and was measured at approximately the same interval as the sensory analysis. Sedimentation was determined by centrifugation at the conclusion of the study. Sensory analysis show a difference in flavor (p=0.01) at day 4 but no clear preference at day 5. At day 35, the high-pressure processed product was deemed superior in flavor (p=0.01). Viscosity and pH were statistically the same between processes and storage times. Clear differences in color, particle size and sedimentation were observed.

INDEX WORDS: High-pressure throttling, pasteurization, blueberry, whey

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DEDICATION

This work is dedicated to the greater glory of God and to the memory of my parents.

For my son.

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I would like to express my gratitude to all of the persons who have guided, inspired and aided me throughout my academic career. Special thanks should go to the faculty, students and staff of the Food Science and Technology Department of the University of Georgia. During my stay here, I have had the good fortune to meet and befriend people for all over the world. Their friendship has enriched my life and, I believe, helped to make me a better person. Certain persons deserve special mention, beginning with to members of my committee who have tolerated my peculiarities of character during my extended tenure in the department.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

One of the author's earliest recollections was the gathering of wild blueberries for breakfast in the Great Smokey Mountains of eastern Tennessee. Until the last fifty years or so, only people that lived near stands or barrens of wild blueberries could enjoy this delicious and healthful fruit. Today, thanks to new cultivars, refrigeration and effective freezing and frozen storage techniques, blueberries are available much of the year. Convenience must be built into a commercial product to make it easier for the public to consume more fruit products. The use of fruit in beverages is one way to meet consumers' demand for convenient products.

The term whey refers to the greenish translucent liquid that separates from the curd during the coagulation of milk during the manufacture of cheese. Approximately 9 kilograms of whey are produced from every 10 kilograms of milk used in cheese manufacture. The watery residue, or whey, is a huge reservoir of high-value milk proteins and minerals. The whey is often literally poured down the drain, wasting valuable nutrients and increasing the biological oxygen demand (BOD) of the waste water from a processing facility. The whey that is recovered is often dried and whey powder is a valuable addition to foods as a source of nutrients because it contains approximately 50 percent of the nutrients in the original milk. The use of whey as an ingredient is especially attractive today as the use of fluid milk as a beverage continues to drop. This drop in consumption is responsible for the emerging problem of calcium deficiency in the American diet today. In the last ten years alone, the per capita consumption of fluid milk and cream has declined nearly 10% from 229 pounds in 1992 to 208 pounds in 2001 (Anonymous, 2003). The

use of whey in various roles in contemporary foods is one possible method of counterbalancing the decline in the consumption of fluid milk in the United States.

The watery residue, or whey, contains high-value milk proteins and minerals, particularly calcium and phosphates. Small processors literally pour whey down the drain, wasting valuable nutrients and increasing the biological oxygen demand (BOD) of the liquid effluent from the processing facility. Large processors may dry the whey directly to produce a powder suitable for animal feed. Some will process the whey to reduce lactose and minerals before drying the product into a protein concentrate. Depending upon the targeted usage, a high-protein beverage with the flavor of milk and fruit may be produced, or delactosed whey may be used as the beverage base which may contain most of the original minerals in whey. A whey-fruit juice beverage may also be produced by a small cheese processor utilizing the whole whey byproduct. Producers of fruit juice-protein beverages are often challenged by the problem of protein aggregation leading to precipitation, and the formation of off-flavors due to heat applied during pasteurization of the product.

The thermal preservation of beverages can be traced to the pioneering work of Pasteur in the nineteenth century. The application of heat to liquid products at a sufficient temperature for an adequate time to inactivate vegetative pathogens is recognized as the most practical method of rendering such products into a shelf-stable form. However, there can be disadvantages to such processes. Flavor components and nutrients can be altered or lost during thermal processing. The result can be the characteristic “cooked” flavor or flavor notes in the finished product and loss of health-functional nutrients. Today’s quality conscious consumers are often inclined to pay a premium for improved flavor and/or nutritional attributes. Food preservation techniques that utilize mild heat treatments have caught the attention of food technologists in recent times.

For the past hundred years, food scientists have been aware of the possibilities of using high-pressure to inactivate pathogens and spoilage microorganisms and thereby preserve food products (Hite, 1899). The primary difficulty then was the lack of metallurgical and mechanical skill to manufacture practical high-pressure processing equipment. The production of reliable high-pressure processing machinery remains a challenge to this day. However, the demand for high-pressure equipment, not only in the food industry but in the chemical industries as well, has stimulated great improvements in the mechanical reliability and capabilities of high-pressure systems. Today we can see high hydrostatic pressure research dealing with food applications and high-pressure processed foods are now commercially available in certain markets. High hydrostatic pressure treatments are by definition a batch process which limits production rates and requires high labor costs. However, a continuous high-pressure processing system, in place of a thermal pasteurization step, is feasible for liquid products. This process requires relatively low pressure compared to hydrostatic pressure systems. The liquid food product is pressurized to 45,000 pounds per square inch and then released instantaneously at a throttling valve. Hence the system is referred to as a high-pressure throttling device or HPT. The objectives of this research is to formulate a beverage consisting of blueberry and whey, test the physical stability of the proteins and pulp in the system, and to determine the effect of high pressure processing relative to heat pasteurization on sensory attributes and physico-chemical properties of the beverage.

1.2 BLUEBERRIES

There are several types of blueberries with various differences, including size, morphology, and chill requirements. The two main divisions are lowbush and highbush blueberries. Low

blueberries or “wild” blueberries are of two species, *Vaccinium angustifolium* and *V. myrtilloides*. As the name implies, lowbush blueberries are a shorter shrub than the more commonly cultivated highbush types. Furthermore, the fruit of lowbush shrubs is often a dull or glossy black and range in size from 2 to 12 mm in diameter. The wild blueberries are grown from New Hampshire to Maine and up into Canada. Commercial production also occurs in areas surrounding Lake Superior, which are the states of Wisconsin and Minnesota, as well as the northern peninsula of Michigan.

The three varieties of highbush blueberries are northern highbush, rabbiteye and southern highbush. Nearly all cultivated blueberries have a mixed genome, from multiple species, giving them those characteristics which provide benefits for the specific area where they are grown. Northern highbush are primarily *Vaccinium corymbosum* that been crossed with various other *Vaccinium* species to improve their desirable properties. Rabbiteye blueberries were originally classed as *V. corymbosum*, then *V. corymbosum* var. *ashei* and now as *V. ashei*. Southern highbush blueberries are recent introductions: they are hybrids designed to have the positive characteristics of both northern highbush and rabbiteye types. The development of southern highbush crosses involved more than simply crossing rabbiteye and northern highbush cultivars, as they are genetically incompatible. *V. ashei* is 6n and *V. corymbosum* is 4n, necessitating the use of intermediate crosses with other species such as *V. angustifolium* (2n).

Rabbiteye blueberry fruit appears nearly identical to the northern highbush types but there are differences in the shrubs, especially in regards to the chill requirement. Blueberries require a period of winter dormancy before they will break dormancy and begin growth. This requirement varies from species to species and between different cultivars of the same species. It is not uncommon for a blueberry patch to receive its quota of chill hours by mid-winter. However,

normal winter temperatures keep the bushes in dormancy until the arrival of the growing season. Rabbiteye plants require only 400 to 500 hours below 7°C to break dormancy, as compared 1,000 hours or more needed by northern highbush plants. Southern highbush are intermediate, with 400 to 660 hours below 7°C required to crop. The rabbiteye blueberry is of primary concern here, and wild *V. ashei* have a range from central Florida to eastern North Carolina and western Arkansas, and westward to eastern Texas (Trehane, 2004).

The cultivation of rabbiteye blueberries began in 1887 in northeastern Florida. In the 1890s, timber man Moses A. Sapp began the first commercial cultivation of rabbiteye blueberries near Crestview, Florida. The plants Sapp selected and set out continued to bear fruit of the next 35 years. The cultivation of wild rabbiteye blueberries increased until in 1930 there were 2000 acres growing in Florida. The cultivation of rabbiteye blueberries in Florida declined in the following years; however this beginning led to the development of the rabbiteye blueberry industry as it is known today.

Scientific research in the breeding of rabbiteye blueberries began in 1926 at the University of Georgia's (UGA) Coastal Plain Experiment Station, Tifton, Georgia. Five plants from each of twelve selections were planted out. Among those contributing selections was Moses A. Sapp's son, W.B. Sapp; J.T. Bush, a railroad engineer; H.H. Hagood, a parson and W.M. Walker. In 1939, a breeding program was begun to select the best characteristics from these wild selections, thus producing superior cultivars. The breeding process continues to this day, with most of the earlier cultivars being superceded by improved ones from more recent breeding.

The cooperative breeding program begun in 1939 had E.B. Morrow, North Carolina Agriculture Experiment Station; O.J. Woodard, UGA Coastal Plain Experiment Station and G.M. Darrow, U.S. Department of Agriculture in Beltsville, Maryland as its principle investigators. In

1944, the state of Georgia, under the direction of Dr. W.T. Brightwell, began a rabbiteye blueberry research farm on 25 acres near Alapaha, Georgia. Thousands upon thousands of seedling have been evaluated by Georgia and the USDA's cooperative breeding program. Notable cultivars developed include 'Tifblue', released in 1955, the most popular rabbiteye blueberry cultivar in the world for the next thirty years. Others types popular in the state of Georgia include 'Woodard' released in 1960 and 'Climax' released in 1974. 'Tifblue' is a mid- to late season cultivar, while 'Woodard' and 'Climax' are early ripening. After 30 years of work improving rabbiteye blueberries, Dr. Brightwell retired from UGA in 1974 and in 1983 the 'Brightwell' cultivar was released, named in his honor. The 'Baldwin' variety of rabbiteye blueberries were released for cultivation during UGA's bicentennial year, 1985, and are named in honor of that institutions founder and first president, Abraham Baldwin. The 'Baldwin' cultivar is currently popular with the "pick-your-own" type of operation, due largely to its lengthy ripening period of more than six weeks (Austin, 1994).

The healthful qualities of blueberries are largely due the chemical compounds that are responsible for their characteristic color. Anthocyanins, a subclass of the flavonoids, are the highly colored compounds responsible for the deep purplish blue color of blueberries as well as the red, blue and violet colors observed in many other fruits and flowers, as well as other tissues. In plants, the role of these pigments is not limited to coloration. Anthocyanins, and other plant pigments, create a contrast to the predominately green colors of plants, which is due to their chlorophyll content (Hutchings, 1999). The contrast is required to attract the animals necessary for pollination and seed dispersal. As food items, colors define the aesthetic value of the product, modulates the appetite and determines the consumers' expectation of flavor and taste (Bayarri et al, 2001; Clydesdale, 1993).

Anthocyanins are thought to increase the antioxidant response of plants which aids in maintaining normal physiological functioning in plant tissues under various stresses. Anthocyanins are known to protect chloroplasts from high light intensities, preventing photoinhibition (Pietrini et al, 2002) At the same time, anthocyanins absorb at the same wavelength as chlorophyll b giving them a role in plant tissue protection and in nutrient retrieval during senescence when chlorophyll is degraded (Field et al, 2001; Hoch et al, 2001). Anthocyanins can also function as transport vehicles for monosaccharides and as osmotic regulators during periods of certain stresses, such as drought or low temperature (Chalker-Scott, 1999). Anthocyanin accumulation due to mechanical wounding and nutrient deficiencies has been reported (Gould et al, 2002; Steyn et al, 2002).

In humans, anthocyanins are thought to have an array of beneficial effects on heath and well-being. Many studies have shown a high positive correlation between fruit or vegetable pigment content and antioxidant capabilities (Cao et al, 2001, 1996; Halvorsen et al, 2002; Moyer et al., 2002; Wang et al., 1996; Wang et al., 2000). Reactive oxygen species including hydroxyl, peroxyl, and superoxide anion radicals as well as reactive nitrogen species including nitric oxide are constantly being produced as a result of normal metabolic reactions in humans and other animals. Free radical synthesis can exceed the natural antioxidant capacity in animal systems, which is provided by antioxidant enzymes such as glutathione peroxidase, catalyase and superoxide dismutase, as well as antioxidant compounds such as ascorbic acid, glutathione and tocopherol. DNA, proteins and lipids thus become the target of free radical attack resulting in dysfunction and damage to genetic material, enzymes and cell membranes (Dröge, 2002; Fang et al., 2002). Therefore, it is hardly surprising that interest in the role of anthocyanins as well as other phytochemicals in supporting human defense mechanisms is increasing.

Free radicals are simply molecules with an unpaired electron in the outer orbital. Free radicals are normally unstable and very reactive. Anthocyanins do not react with free radicals directly; however they provide the reducing capacity in the cycling of antioxidant compounds. In other words, anthocyanins indirectly adsorb the unpaired electron from the outer orbital of free radicals. There are important biological roles that free radicals play in living organisms. Oxygen radicals are known to have critical roles in signal transduction, gene transcription and in the regulation of soluble guanylate cyclase activity in living cells (Lander, 1997). Nitric oxide also functions in a wide variety of roles in living organisms. These roles include the relaxation and proliferation of vascular smooth muscle tissue, leukocyte adhesion, angiogenesis, thrombosis, platelet aggregation, vascular tone and hemodymanics in the circulatory system (Ignarro et al., 1999). In addition, nitric oxide acts as a neurotransmitter and is important in the mediation of the immune response (McCord, 2000).

1.3 WHEY

Whey and especially whey proteins are rapidly becoming acknowledged as possessing many healthful properties as well as having many useful functional properties that can be useful to the food processing industries. Whey proteins and amino acid supplements have gained a strong position in the sports nutrition market based on the purported quality of proteins and amino acids they provide (Ha and Zemel, 2003). Whey and whey products, such as whey protein concentrate (WPC), are widely used as ingredients in the food industry. Products containing whey and whey derivatives include dairy, bakery, meat, beverage and infant formula. Whey proteins are widely used as ingredients in foods because of their unique functional properties, including

emulsification, gelation, thickening, foaming and water-binding capacity. Furthermore, with the decline of milk consumption by the population of the US, many people, especially children, do not receive enough calcium in their diets. The calcium found in milk mineral is of the most bioavailable form. Thus, fortification by and/or use of whey in commonly consumed products can help address this need. Whey has antioxidant properties as well. Lactoferrin and lactoferricin, two minor proteins in whey, function as antioxidants via their iron binding capacity. Lactoferrin is only 8-30% saturated in its native state, a condition that enables chelation of iron and subsequent inhibition of bacterial growth or oxidative reactions. Whey might also enhance antioxidant capacity by contributing cysteine rich proteins which are important in the synthesis of glutathione, a major intracellular antioxidant (Walzem et al, 2002). An improvement in muscular performance (assessed by leg isokinetic cycling) in subjects who consumed a whey-based supplement for 30 days compared to a casein control group has been reported (Lands, 1999). Lymphocyte glutathione, measured as an index of intracellular glutathione, was significantly increased in the whey supplemented group. Noting that oxidative stress contributes to muscular fatigue, the authors propose that increased biosynthesis of intracellular glutathione and its antioxidant activity was the mechanism behind performance improvement. Other developing avenues of research explore health benefits of whey that extend beyond protein and basic nutrition. Many bioactive components derived from whey are under study for their ability to offer specific health benefits. These functions are being investigated predominantly in tissue culture systems and animal models. The capacity of these compounds to modulate adiposity and to enhance immune function and anti-oxidant activity presents new applications potentially suited to the needs of those individuals with active lifestyles.

1.4 HIGH-PRESSURE PROCESSING

High hydrostatic pressure has been known to have food preservation potential for the past one hundred years (Hite, 1899). At the same time, Auguste Gaulin developed the world's first modern homogenizer, patented in 1899 (French Patent number 295,596) and shown to a receptive world at the 1900 World Fair in Paris, France. Since that time, homogenation has become standard practice in many areas of the food industry, most notably in the dairy industry and other areas dealing with oil and water emulsions. In the 1980's, there were introduced new technologies that allowed higher pressures than the 500 bar maximum pressure of the early homogenizers. 3000-5000 bar can be developed by systems such as Microfluid™ technology (Microfluidics, Inc., Newton, Massachusetts), Nanojet™ jet homogenizers (Haskel International, Inc., Burbank, California) and the Emulsiflex™ system (Avestin, Inc., Ottawa, Canada) among others. The new systems are being researched not only for the ability to disrupt microorganisms but also the modification of other food constituents, such as macromolecules and colloids.

In this research, a modified high-pressure homogenizer manufactured by Standstead LTD was employed in the high-pressure pasteurization process. The system was capable of generating pressures of up to 3000 bar (45,000 psi). The machine was unique in that, instead of a standard homogenation valve, it was equipped with a throttling valve. Most high-pressure homogenation valves employ a split flow arrangement. In such systems, the two halves of the product flow are directed into each other at 180°. The impact of the two fluid jets has excellent homogenation properties. Such valves generally product a certain amount of back pressure. The throttling valve was a high-pressure needle valve which allowed instantaneous release of the processing pressure. Such a release of pressure creates tremendous shear stresses at the valve orifice, as opposed to

high hydrostatic systems which rely on the effect of high pressure alone. One advantage of the throttling system is its rapid nature due to the fact that it is a continuous process. High hydrostatic pressure systems are necessarily batch systems with the inherent disadvantages of loading and unloading, as well as the necessity of starting and stopping the pressure generating equipment. These limitations slow production and can increase wear on the equipment. Two of the main shortcomings of thermal pasteurization processes are the loss of nutritional quality and changes in flavor. The flavor changes can be loss of flavor or the development of off-flavors, especially the characteristic ‘cooked’ flavor thermally processed foods. High hydrostatic pressure has been shown to little alter the flavor or nutritional qualities of fruit products (Kimura et al, 1994) or dairy products (Drake et al, 1997). One objective of this study was to verify that same high quality was present in beverages treated by high-pressure throttling.

CHAPTER 2

COMPAIRISON BY SENSORY EVALUATION OF HIGH-PRESSURE THROTTLED AND THERMALLY PASTEURIZED BLUEBERRY WHEY BEVERAGE

2.1 INTRODUCTION

Sensory analysis of foods can be one of the most difficult types of research for the food scientist. This fact is due to the subjective nature of sensory tests but also the complexity of the human perception of flavor and the vast array of chemicals that can effect flavor perception. Humans can perceive four different tastes: salty, sweet, sour and bitter. These four tastes, in conjunction with olfaction (smell), create the flavors of various food products. Taste and smell are often referred to, along with thermo-, mechano- and nociception, as ‘lower senses’ as opposed to the ‘higher senses’ of hearing and sight (von Skramlik, 1926). But the ‘chemical senses’ of taste and smell should not be thought of as lesser then the audio and visual senses. This is especially true when one considers the chemical senses’ primary importance in feeding and reproduction (Plattig, 1988).

The perception of the four taste qualities, salty, sweet, sour and bitter, originates with the taste receptor cells on the tongue. These receptor cells are organized in papillae, often referred to as taste buds. There are four types of these papillae: fungiform, foliate, vallate and filiform. This last type does not contain any taste receptor cells, but is thought to be involved in mechanically hold food constituents on or near the other taste buds. It has long been known that certain areas of the tongue are primarily responsible of certain tastes (Boring, 1942). Sweet taste primarily arises from the tip of the tongue, followed in the direction of the throat, by areas that mainly sense salty tastes. Further back, chiefly along the sides of the tongue, are regions that are especially sensitive to sour. The receptor cells for these three tastes are primarily located in fungiform papillae. Approaching the base of the tongue are the areas that sense bitter stimuli.

These receptor cells are mainly found in vallate papillae. Then receptor cells are excited; they transmit through taste nerves to the brain. It is interesting to note that substances can elicit different taste responses at different concentrations. Potassium chloride, for example, tastes sweet at 0.01M, in aqueous solution, but if the concentration is increased to 0.03M a bitter taste results. As the concentration increases to 0.05M, salty taste is added to the bitter. At 0.20M, the taste of a solution of potassium chloride is a combination of salty, bitter and sour, in that order (Plattig, 1988).

The function of smell in the evaluation of food by humans begins before ingestion. Volatiles from the food are drawn through the nose and create an odor sensation. If the smell is acceptable, the food item is placed in the mouth and mastication proceeds. Thus begins the second stage of chemosensory analysis of food flavor, which is the synthesis of all the oral and nasal stimuli by the brain into a single complex sensation. In the nasal cavity resides the olfactory epithelium that contains the cells that are responsible for the detection of the volatiles that cause odors. These cells are of four types: receptor neurons, microvillar cells, supporting cells and basal cells. The physical site of the odor-receptor cell interaction is believed to be the cilia of the receptor cells. On the molecular level, there are receptor sites that are protein or lipid in nature. The nerve impulses proceed to the brain, passing through the olfactory bulbs. In the olfactory bulbs, a single output cell serves about 250 receptor cells. This arrangement is responsible for the low threshold of many odors, as only a few stimulated receptor cells will trigger an output cell. One should also bear in mind that there exist various olfactory modulators that affect olfactory sensitivity. These factors include the sniffing technique, mixing of stimuli, adaptation, hunger and aging, as well as autonomic and hormonal influences (Maruniak, 1988). The objectives of this study were to detect and define any differences in taste and/or flavor, through sensory

difference and preference tests, in a blueberry-whey beverage processed by high-pressure throttling and thermal pasteurization.

2.2 METHODS AND MATERIALS

Beverage

Rabbiteye blueberries, *Vaccinium ashei*, of the Tifblue cultivar were obtained through the good offices of the Bacon County, Georgia extension agent, Mr. Daniel Staniland. The blueberries were stored at 4°C until ready to be used. The juice was extracted from the blueberries by a combination of enzymatic and filtration methods and formulated into a beverage. The high concentration of pectins in blueberries entraps the juice in the mash. However, the use of a pectoyltic enzyme preparation from *Aspergillus aculeatus* (Pectinex Ultra SP-L, Novozymes A/S, Switzerland, distributed by Sigma-Aldrich, St. Louis, MO, Number P2611, Lot 11K1089) provided satisfactory yields of around 70% of fresh weight. A conventional thermal process and high pressure throttling were applied to the beverage. The processed products were then subjected to sensory evaluation and color analysis and a comparison of the characteristics of the two treatments was made.

In processing the juice, the blueberries were first passed through a meat grinder (Model 4612, Hobart Corp., Troy, OH) with a 6.35 mm plate. The resulting mash was heated in a steam kettle (Model TWP-20, Waynesboro Industries, Waynesboro, GA) to 40°C. Then, 45,000 units of pectinase per kilogram were added to the heated product, which was mixed thoroughly and allowed to rest for 1 hr. The treated blueberries were transferred to a micro filtration unit (Model FoodTech LabStar, Bucher-Guyer Ltd., Niederweningen, Switzerland) equipped with 0.2 μm

coated sintered stainless steel filters. The resulting juice was frozen and held at -20°C until ready to use. For freezing, 1L portions of the juice were placed in 4L Zip-Lock Plastic bags. The bags were laid flat on stainless steel trays and placed in a -20°C freezer, thus ensuring rapid rate of freezing.

Spray dried kosher sweet whey powder was purchased from Dairy Farmers of America, New Wilmington, Pennsylvania (lot 02103), and was stored at 20°C until use. The whey powder was of the standard production variety and therefore assumed to be manufactured by a high temperature process. A blueberry-whey beverage was formulated to contain: 20% blueberry juice, 7% spray dried whey powder, 3% sucrose and 70% water. The water was purified with a reverse osmosis system (Model 1200A, Water and Power Technologies, Columbia, SC) and had a pH of 7.2. All pH measurements employed a bench top pH/ISE meter (Model 710A with Model 8102BNU electrode, Orion Research, Inc., Beverly, MA). To avoid any possible complications with regards to solubility, the dry ingredient were dissolved separately. As an example, for each 50L batch of blueberry-whey beverage, 3,000 g of spray dried whey powder were mixed with 12,500 g of RO water and 1,500g of sucrose were added to 12,500g of RO water. When both portions were fully dissolved, they, along with 10,000g of blueberry juice, were mixed in a 120L stainless steel mixing tank. The resulting beverage was adjusted to a pH of 4.4 by meter with anhydrous citric acid powder (J.T. Baker Chemical Co., Phillipsburg, PA, Number 0122, Lot 38956) in the tank during continuous stirring. The pH of 4.4 was selected to ensure that the product was well in the range of an acid food, which is below 4.6. The value of 4.4 before processing was intended to provide a cushion in case either process affected the pH. The concentration of citric acid required varied slightly between the various batches, but the average amount used was 0.08%, w/w, or about 0.8g per L.

The blueberry-whey beverage was processed by two methods. High-pressure throttling was performed with a modified high-pressure homogenizer (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, U.K.). The primary modification was the replacement of the homogenation head with a high-pressure metering valve of the needle type (Series 60VM, Autoclave Engineers, Erie, PA). After the throttling valve, the temperate of the blueberry-whey beverage was adiabatically increased to between 77-79°C from an inlet temperature of between 2-3°C. Next, the product was passed through a tube and ice water heat exchanger and the temperature was reduced to approximately 20°C. The rate of flow through the system was measured at 1300mL/min. The blueberry-whey beverage was bottled in 450mL glass bottles using a custom built aseptic filler employing free-flowing steam. The bottles and caps were pre-sterilized by autoclaving with approximately 50mL of RO water in them to ensure adequate steam was present in the bottles. The water was dumped from the closed bottles immediately prior to filling. The filled bottles were placed on ice and then stored at 2°C until needed.

The thermal process was achieved by the use of a Cherry-Burrell Unitherm IV NoBac pasteurization system (Waukesha Cherry-Burrell, Delavan, WI). The unit consisted of a high-pressure pump, pre-heated, heater, holding tube, pre-cooler, homogenation head and final cooler. The same aseptic filler as used with the high-pressure unit was utilized with the N0Bac. The target temperatures were 95°C for the pre-heater and 125°C for the heater. The 125°C was measured after the holding tube. The blueberry-whey beverage had a residence time of 6 seconds in the holding tube. From the holding tube the product passed through a pre-cooler that reduced the temperature to 80°C. The product then passed through the homogenation head at a pressure of 1500psi. The final temperature of the product at bottling was approximately 12°C, and similar 450mL bottles were filled using the same aseptic filler as with the high-pressure throttling

system. The blueberry-whey beverage was then placed on ice, and then stored at 2°C until needed.

Sensory evaluation

The sensory evaluation consisted of two types of test. First, a difference test, in this research a triangle test, was carried out to demonstrate the fact that there existed a difference in flavor between blueberry-whey beverage processed via high-pressure and traditional thermal pasteurizations. The panelists consisted of faculty, students and employees of the University of Georgia, primarily from the Department of Food Science and Technology where the research was conducted. In the triangle test, panelists were asked to taste three samples from left to right. The presentation was designed so that every possible combination of the two processes was equally represented (AAB, ABA, BAA, BBA, BAB, and ABB). Two random numbers were assigned to each of the treatments (Amerine, 1965). The panelists were seated in individual booths with sodium lighting to mask any color differences. The 30 mL test samples were presented to the panelists through a rotating double door system that precluded any view of the sample preparation area by the judges. The samples were presented on clean white trays in 1 oz clear plastic soufflé cups (No. P101M, Solo Inc., Urbana, IL). The panelists were asked to identify the odd sample on the provided scoring sheets (see appendix A). Unsalted saltine crackers and deionized water were provided and the judges were instructed to cleanse the palate between samples (Frijters, 1988).

The results showed that a difference did indeed exist between high-pressure and thermal pasteurizations with a $p = 0.01$ at day 4. Therefore, consumer preference tests of the two-tailed difference type were performed at day 5 and day 35 to determine which sample was preferred by the judges and therefore assumed to be of higher quality. As before, panelists were seated in the

booths under similar conditions. The judges were presented two samples, one half with the high-pressure processed on the right side and the other half on the left to eliminate any possible bias. As before, two different random numbers were assigned to each treatment. Again unsalted saltine crackers and deionized water were provided. The panelists were asked to indicate the preferred sample on the score sheets provided (Appendix A). Probabilities were taken from Amerine, Pangborn and Roessler (1965).

2.3 RESULTS AND DISCUSSION

In the preliminary taste preference (triangle) test 20 of 36 panelists were able to correctly identify the odd sample 4 days after processing. According to standard tables (Amerine et al, 1965), this number of correct judgments indicates that there existed a difference in the two types of blueberry-whey beverage. Statistically, the tables indicate that $p = 0.01$ or that there was a 1 in 100 probability that the result was due to pure chance. Having established that a difference existed between the two treatments, it was attempted to determine which product was of higher quality. Therefore, a two-tailed preference test was to be preformed at days 5, 35, 65 after processing. At day 5, 73 out of 126 panelists selected the high-pressure processed product as superior. A clear majority (58%), but not sufficient to be deemed significant statistically ($p > 0.05$). After 35 days of storage, 76 of 112 panelists (68%) preferred the high-pressure processed product to the thermally processed beverage. The numbers translate to a $p = 0.01$ meaning that the number of judges who preferred the high-pressure pasteurized beverage was statistically significant. The preference test planned for 65 days was not carried out due to visible mold growth in many of the bottles of thermally processed blueberry-whey beverage. At that time, the

high-pressure processed product did not have any visible spoilage and was thought to smell and taste satisfactory to the researchers.

The panelist's preference for the high-pressure throttling blueberry whey beverage is in accord with other research indicating higher retention of flavors in such products as opposed to thermally processed items (Drake et al, 1997; Kimura et al, 1994). High-pressure evidently does not degrade flavor volatiles to the same degree as thermal processing. The data obtained indicates the high-pressure pasteurized product was of higher sensory quality than the thermally processed beverage. Furthermore, the self-life of the high-pressure throttled beverage was postulated to be greater than the thermally processed product. The assumption was made due to the superior quality of the high-pressure throttled product at day 35 and the visible spoilage of the thermally pasteurized product at day 65.

CHAPTER 3

COMPAIRISON OF PHYSICAL PROPERTIES OF HIGH-PRESSURE THRODDLED AND THERMALLY PASTEURIZED BLUEBERRY-WHEY BEVERAGE

3.1 INTRODUCTION

In this section, the physical properties of the blueberry-whey beverage and the effects of the two processes, thermal pasteurization and high-pressure throttling were investigated. The viscosity of the two products was compared with the use of a dynamic stress rheometer. Particle size was determined with the aid of a laser light scattering system. Sedimentation was quantified by a method of centrifugation and drying (Iordache and Jelen, 2003). Measurements of pH and color were also carried out.

Rheology

Rheology is the study of the deformation and flow of matter and is in essence the "handling properties of matter" (Goodwin, 2000). In processing complex fluids such as many food products, it is imperative to understand the flow behavior of these products. Both solids and liquids possess rheological characteristic and certain food products display solid and fluid behavior. These materials are termed viscoelastic. Viscometers are instruments that measure the flow behavior of fluids. Rheometers are more complex, and can measure deformation of solids as well as flow of fluids and viscoelastic properties.

Two of the fundamental terms in rheology are stress and strain. In layman's terms, stress is the force and strain is the object's reaction to that force. Stress is defined as force per unit area and is normally measured in pascals (newtons per square meter). Pressure is termed a compressive bulk stress while an example of an extensional stress is the stress on a tow rope. The

type of stress that is of greatest concern in rheology is shear stress. To move sandpaper across a wooden plank requires the application of shear stress.

Objects react by deforming when stress is applied. This deformation is termed strain. To make the rheological calculations tractable, strain is defined as deformation per unit length in this field. The length that is used for the term is the length over which the deformation of the object occurs. In a fluid system, motion is produced until the stress is removed. If we consider two surfaces separated by a small liquid filled space, a constant shear stress must be maintained on one surface for it to move at a constant velocity, u. If there is no fluid slip, a gradient will form between the moving and the stationary surface, being zero at the stationary surface. As in simple rheometeric systems, γ is the shear strain and is composed of two components, γ_{xy} and γ_{yx}. For every second the displacement produced is:

$$\gamma = x/y$$

and as $u = dx/dt$, we have

$$d\gamma/dt = du/dz$$

Which is the rate of strain, also called the shear rate or velocity gradient. When the plot of shear stress versus shear rate is linear, the liquid is said to be Newtonian and its flow characteristics are simple. In systems containing whey and other proteins heat treatment can cause thickening due to changes in the protein structure. These changes can cause sediment or soluble polymer formation especially when fruit and/or pectins are present. It was hoped to observe different changes in viscosity and flow characteristics in blueberry-whey beverages processed by high-pressure throttling and traditional thermally processing.

Particle size

The Mastersizer (Malvern Instruments, Malvern, Worcestershire, U.K.) estimates particle size by measuring the scattering of laser light. The scattering and absorption of light by particle in solution has intrigued scientists for many years. The two theories, of the many available, that the Mastersizer employs are the Fraunhofer model and the Mie theory. The Fraunhofer model is useful in predicting the scattering pattern produced when homogeneous opaque discs of a defined size passes through a laser beam. The Fraunhofer model is limited in that few particles are prefect discs and that many particles are transparent. The Mie theory is able to predict the way light is scattered when light is absorbed by, or passes through spherical particles. Although the Mie theory does assume certain information about the particles in question, such as the refractive index and absorption, the Mie theory generally gives a more accurate estimate of a particle's size. The significance of the theories is that if one knows the size and structure of a particle, one can predict its light scattering characteristics. These characteristics are unique to that size particle. The Mastersizer measures the laser light scattering of a test solution or field of particles and works backwards through the applicable theory to estimate the size of the particle that created said field.

The Mastersizer consists of an optical unit, a sample preparation accessory and a computer system. The optical unit consists of a transmitter, which creates the laser beam and the receiver that captures the pattern created. The sample preparation accessory places the field of particles between the transmitter and receiver. In this case, the sample was dispersed in a liquid product that was diluted and circulated through a transparent sample cell. The system employed was a flow cell, there the sample was pumped through the measuring cell and kept in solution by an external accessory. The other types of sample cells available are the stirred cell and the air cell.

With the stirred cell, the sample is dispersed in a liquid as with the flow cell. However, the sample is magnetically stirred inside the optical unit to keep the particles in suspension. The air cell is used for dry powders and the sample is blown or passed free fall through the laser beam by an external accessory.

The receiver collects and stores information gathered by the detector which is the primary component of the receiver. The detector consists of a radial arrangement of photo-diodes. The receiver then sends the data to the computer for analysis. Using especially created software, the computer estimates the size of the particles that created that particular scattering pattern. The software works backward through the Fraunhofer model or the Mie theory depending on the parameters set by the operator. Thus an estimate of the size of the particle is generated and reported. The data can be presented in tabular or graphical forms. The particle size is shown in mean size and by the range in which 80% of the particles fall (Anonymous, 1997).

Sedimentation

The sedimentation of the product was determined in a method similar to solubility index test for dry milk powder (Anonymous, 1971). Irodache and Jelen (2003) employed this method for the determination of sediment from heat denatured whey proteins. However, the procedure was modified in that a dry weight of sediment was reported rather than a wet volume. The modification was felt to be necessary due to the differences in the nature of the sediment between blueberry-whey beverages of the two treatments. There appeared to be significant differences in the densities of the respective sediments (discussed below), therefore it was felt that dry weights would be more accurate for this application.

pH

The pH of the product is an expression that relates how acidic or basic a substance is. The term stands for the log of the concentration of free hydrogen ions available. In neutral water, there are 10^7 hydrogen ions per mole of water, giving a pH of 7.

Color

Color, as perceived by the human eye, is normally the most important visual aspect of any food products. The eye detects 'visible' light or light with a wavelength of between 390 to 750 nm. Light passes into the eyeball through the pupil, the size of which is controlled by the iris diaphragm. The image is focused on the fovea, a depression on the retina, or back of the eye. The retina is a complex, multiplayer structure. There are two main types of light sensing cells in the retina, rods and cones. Rods are receptors of low intensity colorless vision and encircle the fovea. Cones, of which contain three types of pigment, sense color. The cones are concentrated in the fovea, which is a depression on the retina. The lens of the eyeball focuses light on the fovea, which is directly behind the lens. The pigments have absorption peaks at 450, 530 and 560 nm. Thus cone vision is trichromatic and in fact any color of light can be matched by mixing red, green and blue primary lights. The signals from the retina are transferred to the brain via the optic nerve. It is in the brain that the image seen in the mind's eye is created (Francis and Clydesdale, 1975). The origins of qualitative color determination originated in the realm of art, rather than industry. A.H. Munsell devised the first truly successful system of color classification around 1900. His system was based on a three-dimensional color solid, which is a characteristic borrowed by nearly all of the later color classification systems. Furthermore, Munsell assigned a value to each of the three attributes of a color. Hue was the actual color, for instance, red.

Chroma was the intensity or saturation of a color and value denoted the amount of lightness (white) or darkness (black) present in the color. The Munsell book of color, containing removable color chips, is still widely used in art and industry, including food processing. Today, many types of colorimeters are available, based on the trichromatic nature of visible light. In this work, the system of the CIE (Commission Internationale de L'eclairage) and an automatic chroma meter were employed. The 1976 CIE system contains three values, L∗, a∗, b∗ (MacDougall, 1988). Differences in color were also to be detected and defined in terms of the CIE-L*a*b* color system.

3.2 METHODS AND MATERIALS

Rheology

The viscosity of the two types of blueberry-whey beverage, thermal and high pressure processed, were prepared and treated as in Chapter 2, was measured with a dynamic stress rheometer (Model SR-5000, Rheometerics Scientific, Inc., Piscataway, NJ). For these determinations, a couette tool was employed having a cup diameter of 32 mm and a bob diameter of 29 mm. The bob length was 44 mm and the arrangement required approximately 16 ml of sample to fill. To simulate the intended serving temperature of the blueberry-whey beverage, the cup was maintained at 4°C with the circulating water-bath which was an integral part of the instrument. Furthermore, the samples were held on ice until ready to be used and the cup and bob were rinsed with deionized water at 4°C between the samples. A steady state ramp test was used with shear rates of 0 and 50 1/s. The test time zone was set at 300s. Data was plotted as shear stress versus shear rate. The power law model was use to fit the data, giving the equation:

$$\sigma = k \cdot \gamma^n$$

Where σ = viscosity, γ = shear rate, n = flow behavior and k = consistency index.

Particle size determination

Particle size was estimated using Mastersizer S laser light scattering system (Model MAM 5004, Malvern Instruments, Malvern, Worcestershire, U.K.) described above. As the particles were in a liquid product, dispersed in water, a flow type sample cell was employed. The sample cell was filled with approximately 100 ml of RO water, to which sufficient sample was added to attain a transmittance of 20 % (+/- 2 %). This dilution required approximately 10 mL of the thermal processed blueberry-whey beverage and 20mL of the high-pressure treated product to achieve. The impeller was adjusted to 2000 rpm, keeping the products thoroughly mixed and flowing at a constant rate through the internal portion of the sample cell during the measurements.

Sedimentation study

A method similar to American Dry Milk Institute solubility index test for dry milk powder (Anonymous, 1971) was used to determine the sedimentation characteristics of the high-pressure and heat pasteurized blueberry whey beverage. 5g of the beverage was measured into pre-weighed 13 mm x 100mm borosilicate test tubes and centrifuged in a tabletop centrifuge (Centrific Model 228, Fisher Scientific, Pittsburgh, PA) at 3000x*g* for 30min. The supernatant was decanted, and the tubes with the wet pellets were placed in a freeze dryer (Model 25SL Freezemobile with 600L Unitop, Virtis Co., Gardiner, NY) and vacuum dried at 40°for 24 hr. The tubes were then reweighed and the sediment reported as % w/w.

pH

The pH of the samples was standardized to 4.4 before processing. All pH measurements were made using a bench top pH/ISE meter (Model 710A with Model 8102BNU electrode, Orion Research, Inc., Beverly, MA). pH was measured after processing at day 6 and day 36. The pH determinations were done at room temperature, approximately 20°C.

Color measurement

Color was determined with a Minolta Chroma Meter (Model CR-300, Minolta Co. Ltd., Osaka, Japan) as per operating instructions. The blueberry-whey beverage was thoroughly agitated with special care being exercised to ensure that any sediment was dispersed fully. 25mm x 200mm test tubes were filled with 50 ml of sample and placed in the appropriate attachment provided by the manufacturer. Measurements were reported as CIEL*a*b*. Color differences, ΔE^*, were calculated employing CIE-L*a*b* color difference formula:

$$\Delta E^* = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$$

ΔE^* values of above 2 are considered significant in the paint and textile industries. However, in the food industry it is often necessary to examine the individual components and their differences (Hutchings, 1999). Accordingly, the values of lightness difference, ΔL^*, was determined by:

$$\Delta L^* = L^*_1 - L^*_2$$

Metric chroma, C, is defined by the formula:

$$C^* = [a^{*2} + b^{*2}]^{1/2}$$

This term is also known as the saturation index. Differences in saturation, ΔC^*, are defined as:

$$\Delta C^* = C^*_1 - C^*_2$$

The hue difference, ΔH^*, was calculated by the formula:

$$\Delta H^* = [\Delta E^{*2} - \Delta L^{*2} - \Delta C^{*2}]^{1/2}$$

This difference in hue, ΔH^*, is normally the most critical and sensitive parameter in the food processing industry (Anonymous, 1996).

3.3 RESULTS AND DISCUSSION

Rheology

The values for the flow behavior, *n*, are shown in table 3.1.1 and the values for the consistency index, *k*, are shown in Table 3.1.2. The power law model was used to fit the stress versus shear rate data. The formula:

$$\sigma = k \cdot \gamma^n$$

Where σ = viscosity, γ = shear rate, n = flow behavior and k = consistency index. The value of n indicates the type of shear dependency. Shear thinning materials have n values of $0 < n < 1$ and shear thickening materials display values of $n > 1$. Newtonian fluid behavior is indicated by $n = 1$. Here, the n values bracket 1, with no statistical difference between the n values. Therefore it is concluded that, for this blueberry-whey beverage, the flow behaviors were strictly Newtonian in nature, regardless of the method of pasteurization or length of storage. With γ and n being both approximately equal to 1, the values of k become equal to σ. As the viscosity of water is about 0.001 pascal seconds, all of the samples had a very low viscosity. Before and after storage k values were statistically different, it was felt that for practical purposes the viscosities

were so slight as to be insignificant. The failure to observe any real changes in rheological properties was most likely due to the low concentration of proteins, pectins and soluble solids.

Particle size

Particle sizes are summarized in table 3.2. As one might expect, the particle size estimates generated were significantly lower in the high-pressure processed product. An advantage of high-pressure throttling is that excellent homogenation is achieved at the throttling valve, without a separate mechanism. For example, the Cherry-Burrell Unitherm IV system used in this study mounted a remote homogenation head between the pre-cooler and the cooler. Such arrangements can at times cause mechanical difficulties or problems with sanitation. Particle size and particle shape can also affect sedimentation.

The particle size data showed significant differences between treatments and between storage times. Surprisingly, the particle size and range marker values became significantly smaller during storage. One would expect aggregation to continue during storage but this was not the case. Perhaps some polymers were formed during processing which dissociated during storage. From the literature and the sediment observations it would seem that this phenomenon would be more likely in the high-pressure throttled product. However, the reduction in particle size was observed in the thermally processed blueberry-whey beverage as well.

Sedimentation

The amounts of sediment obtain are shown in table 3.3. The heat-treated sample had significantly more sediment recovered than the high-pressure samples. It was noted that during storage the thermal and high-pressure processed blueberry-whey beverage had different sediment characteristics. The thermally processed product developed dense sediment, similar to what one might expect from a packed powder. Even with extremely vigorous agitation, the sediment was difficult to disperse. Conversely, the high-pressure treated product had soft feathery sediment

that was dispersed with the slightest disturbance. One might think the product with the smaller particle size would have the sediment packed more tightly. The difference here however, can be attributed to particle structure rather than size.

The most common cause of precipitation in fluid products containing whey is heat induced insolubilization of whey proteins. When the proteins are denatured by heat, they are prone to form sediment in the presence of fruit components such as pectins, especially when calcium ions are present (de la Fuente, 2002). High hydrostatic pressure is known to cause major structural changes to proteins (Rademacher, 2002) and other substances. The additional stresses of dynamic high-pressure processing may cause additional modifications to the ternary or quaternary structures of the proteins. Thus a protein structure which has not lost all of its solubility or functionality may be formed. A similar effect has been note on mildly heat whey protein solutions at low ionic strengths, termed 'soluble polymers' (Britten, 2002). As there is interest in modifying heat denatured whey proteins with high-pressure homogenation (Iordache, 2003), the changes from the two stresses are assumed to be somewhat interrelated. Therefore, it is difficult to strictly separate the effects from the mild adiabatic heating and the stress from decompression at the throttling valve. But the fact remains that the easily dispersed sediment in high-pressure throttled blueberry-whey beverage is more acceptable, both in appearance and function, than the concrete-like sediment noted in the thermally processed product.

pH

The pH values obtained are summarized in table 3.4. There were no statistical differences observed in pH due to processing method or storage time. The pH stability is no doubt due to the inherent buffering characteristic of complex biological systems such as the product.

Color measurement

The color measurements were taken at the approximately the same times as the taste panels. The L* a* b* values obtained are shown in table 2.1. A value of Δ*E** equal to 1 or less is generally recognized as an industrial match. Values of Δ*E** equal to 2 or greater is a significant difference in color (Anonymous, 2000; Francis and Clydesdale, 1975). All of the samples, with the exception of the 3rd replicate at day 6 displayed Δ*E** values in excess of 2. The 3rd replicate, day 6 had a Δ*E** value which was 1.82. Therefore, one must examine the individual values for Δ*L**, Δ*a**, and Δ*b**. When the three Δ values are approximately equal, then one should assume that the colors are not significantly different. However, if one or two of the Δ values are markedly different, especially if one or more Δ values are greater than 1, the assumption should be made that the colors are noticeably different. The 3rd replicate, day 6 had Δ*L**= 0.22, Δ*a**= 1.23 and Δ*b**= 1.32 which indicates that a difference existed between the high-pressure and thermally processed blueberry whey beverage. Furthermore, the Δ*H** values all except one exceeded 2 CIE units. The other Δ*H** value was 1.95. These numbers indicate a fundamental difference in hue, or color in the two blueberry-whey beverages. Unfortunately, no color data was available for the unprocessed product.

TABLE 3.1.1: RHEOLOGY; FLOW BEHAVIOR, *n*, VALUES

	A1	A2	A3	AveA	B1	B2	B3	AveB
Th1	0.8881	1.0125	0.9664	0.9557	0.8829	0.9556	0.9721	0.9369
HP1	1.0740	1.0212	0.7630	0.9527	0.8748	0.9556	0.9987	0.9430
Th2	0.9849	1.0120	0.9689	0.9886	1.0721	0.8856	0.9096	0.9558
HP2	0.9504	0.9456	0.8938	0.9299	1.1414	1.0456	1.0030	1.0633
Th3	0.8829	0.9556	0.9721	0.9369	0.8889	1.0057	0.9642	0.9529
HP3	1.0340	0.8218	0.6886	0.9519	1.0391	0.9543	0.9921	0.9951

TABLE 3.1.2: RHEOLOGY; CONSISTANCY INDEX, *k*, VALUES (PA·S)

	A1	A2	A3	AveA	B1	B2	B3	AveB
Th1	0.0044	0.0033	0.0039	0.0039	0.0049	0.0039	0.0051	0.0046
HP1	0.0071	0.0047	0.0059	0.0059	0.0068	0.0056	0.0078	0.0067
Th2	0.0062	0.0046	0.0054	0.0054	0.0072	0.0056	0.0069	0.0066
HP2	0.0024	0.0030	0.0027	0.0027	0.0042	0.0056	0.0037	0.0045
Th3	0.0046	0.0029	0.0075	0.0050	0.0052	0.0059	0.0061	0.0057
HP3	0.0042	0.0048	0.0036	0.0042	0.0046	0.0032	0.0058	0.0045

HP = High-Pressure throttled, Th = Thermal processed, Ave = Average value for that duplicate, Number after process designation = replicate, A = day 6, B = day 36, Number after A or B = duplicate

TABLE 3.2: PARTICLE SIZE ESTIMATION (µm)

	HP1A1	HP1A2	HP1A3	AveHP1A	HP1B1	HP1B2	HP1B3	AveHP1B
10% less than	3.59	3.48	3.33	3.47	3.54	1.58	3.35	2.82
Median	6.00	6.31	6.48	6.26	6.59	4.07	5.98	5.55
90% less than	9.15	10.21	8.27	9.21	11.29	8.12	9.94	9.78
	HP2A1	HP2A2	HP2A3	AveHP2A	HP2B1	HP2B2	HP2B3	AveHP2B
10% less than	3.23	3.38	3.34	3.32	1.12	1.12	1.24	1.16
Median	5.96	6.29	5.91	6.05	3.78	4.76	4.04	4.19
90% less than	11.21	14.77	9.84	11.94	8.93	9.54	9.3	9.26
	HP3A1	HP3A2	HP3A3	AveHP3A	HP3B1	HP3B2	HP3B3	AveHP3B
10% less than	1.51	1.53	1.78	1.61	1.80	1.70	1.74	1.75
Median	3.97	4.15	4.42	4.18	4.33	4.22	4.15	4.23
90% less than	7.90	8.05	15.95	10.64	9.05	8.57	7.83	8.48
	Th1A1	Th1A2	Th1A3	AveTh1A	Th1B1	Th1B2	Th1B3	AveTh1B
10% less than	1.2	1.24	1.43	1.29	1.26	1.06	1.05	1.12
Median	8.89	9.21	9.85	9.32	8.61	7.36	7.36	7.78
90% less than	21.14	22.49	27.43	14.63	27.49	17.69	17.61	20.93
	Th2A1	Th2A2	Th2A3	AveTh2A	Th2B1	Th2B2	Th2B3	AveTh2B
10% less than	1.46	1.47	1.78	1.57	1.03	1.00	0.98	1.00
Median	12.46	12.72	13.02	12.73	5.85	5.54	5.33	5.57
90% less than	26.99	27.26	28.25	27.50	16.76	13.37	13.13	14.42
	Th3A1	Th3A2	Th3A3	AveTh3A	Th3B1	Th3B2	Th3B3	AveTh3B
10% less than	0.98	1.03	1.03	1.01	1.73	1.98	1.74	1.82
Median	9.21	9.54	9.54	9.43	7.58	7.70	7.62	7.63
90% less than	25.32	26.31	26.81	26.15	17.61	18.09	17.99	17.9

HP = High-Pressure throttled, Th = Thermal processed, Ave = Average value for that duplicate, 1st number = replicate, A = day 6, B = day 36, 2nd number = duplicate, 10% (of particles) less than (the value given), 90% (of particles) less than (the value given).

TABLE 3.3: SEDIMENT DETERMINATION (µg/ml)

	B1	B2	B3		B1	B2	B3
Th1	8.2	8.3	8.5	HP1	4.6	6.8	7.2
Th2	8.1	8.4	6.5	HP2	6.7	6.1	6.4
Th3	7.4	8.8	9.2	HP3	6.7	7.3	7.6

HP = High-Pressure throttled, Th = Thermal processed, Number after process designation = replicate, B = day 36, Number after = duplicate

TABLE 3.4: pH VALUES

Th1A1	Th2A1	Th3A1	HP1A1	HP2A1	HP3A1
4.4	4.4	4.5	4.4	4.4	4.5
Th1A2	Th2A2	Th3A2	HP1A2	HP2A2	HP3A2
4.4	4.4	4.5	4.4	4.4	4.5
Th1A3	Th2A3	Th3A3	HP1A3	HPA3	HP3A3
4.4	4.4	4.5	4.4	4.4	4.5
Th1B1	Th2B1	Th3B1	HP1B1	HP2B1	HP3B1
4.4	4.4	4.5	4.4	4.4	4.5
Th1B2	Th2B2	Th3B2	HP1B2	HP2B2	HP3B2
4.4	4.4	4.5	4.4	4.4	4.5
Th1B3	Th2B3	Th3B3	HP13B	HP2B3	HP3B3
4.4	4.4	4.5	4.4	4.4	4.5

HP = High-Pressure throttled, Th = Thermal processed, Number after process designation = replicate, A = day 6, B = day 36, Number after A or B = duplicate

TABLE 3.5: CIE-L*a*b* CHROMA METER MEASUREMENTS

	Day 6				Day 36			
Sample	L*	a*	b*		L*	a*	b*	
				ΔC*=1.26				ΔC*=1.09
Th1A	35.12	3.75	-2.01		35.07	3.76	-2.03	
Th1B	35.14	3.70	-2.07	ΔH*=3.53	35.26	3.76	-1.92	ΔH*=2.62
HP1A	37.03	5.20	-1.67		36.61	5.07	-1.59	
HP1B	37.20	5.38	-1.86	ΔE*=4.24	36.69	5.15	-1.50	ΔE*=2.05
Δ	1.99	1.56	0.24		2.19	1.35	0.43	
				ΔC*=1.31				ΔC*=1.44
Th2A	34.93	3.54	-2.29		35.01	3.49	-2.35	
Th2B	35.01	3.56	-2.18	ΔH*=2.77	34.81	3.46	-2.31	ΔH*=3.11
HP2A	36.57	5.57	-1.57		36.45	5.66	-1.40	
HP2B	35.66	5.01	-1.55	ΔE*=2.19	36.06	5.17	-1.67	ΔE*=2.50
Δ	1.15	1.74	0.68		1.36	1.94	0.79	
				ΔC*=0.53				ΔC*=0.93
Th3A	35.39	3.45	-2.35		35.28	3.33	-2.59	
Th3B	35.22	3.48	-2.49	ΔH*=1.95	35.47	3.36	-2.43	ΔH*=2.65
HP3A	35.65	4.69	-1.03		36.31	5.06	-0.91	
HP3B	35.40	4.70	-1.16	ΔE*=1.82	35.84	4.97	-1.14	ΔE*=2.34
Δ	0.22	1.23	1.32		0.7	1.67	1.48	

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APPENDIX A: SENSORY EVALUATION DOCUMENTS

A.1: CONSENT FORM

I, _____ agree to participate in the research entitled "Comparison of the flavors of a blueberry whey beverage processed by heat and high pressure" which is being conducted in the Food Process Research and Development Laboratory; University of Georgia; Athens, Georgia by Dr. Romeo T. Toledo (706-542-1079) and David C. Peck (706-583-0637); both of the Department of Food Science. I understand that my participation is entirely voluntary. I can withdraw my consent at any time without fear of penalty or retribution, and have the records of my participation, to the extent that they can be identified, returned, removed from the research records, and/or destroyed.

The following have been explained to me:

1. The reason for the research is to determine if a difference in flavor exists between blueberry-whey beverages processed by heat and high-pressure pasteurization.
2. The procedures are as follows: A blueberry-whey beverage was prepared and pasteurized by heat and high-pressure. Panelists will be asked to perform a triangle test to determine if a difference exists between the two. Panelists will be presented with three samples and asked to identify the different one. The experiment should not last more than ten minutes.
3. Participation entails the following risks: **ALLERGIC REACTION TO MILK, WHEY OR BLUEBERRIES.** These risks are considered minimal but in the event that it happens, the investigators will obtain 911 services to obtain medical services as rapidly as possible.
4. In the event of a medical problem no treatment or payment will be provided the investigators or institution.
5. I must make known all known allergies to the investigators. I am allergic to: _____
6. The results of this investigation are anonymous and will not be released without my prior consent, unless required by law.
7. I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in the study. I have been given a copy of this form. The investigators will answer any further question now or during the course of the study.

_____ _____

Investigator Date Participant Date

Please sign two copies, retain one and return to other to the investigators.

For questions or problems about your rights please call or write: Chris A. Joseph, Ph.D., Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu.

Taster No._____ Date_____

A.2: TRIANGLE TEST: BLUEBERRY-WHEY BEVERAGE

INSTRUCTIONS: Taste the samples on the tray from left to right. Two samples are identical; one is different. Select the odd/different sample and indicate it by placing on X next to the code of the odd sample.

Samples on Tray	Odd Sample	Remarks
_____	_____	_____
_____	_____	_____
_____	_____	_____

If you wish to comment on the reasons for your choice or comment on the product characteristics, you may do so under remarks or below.

A.3: PREFERENCE TEST: BLUEBERRY-WHEY BEVERAGE

Taster No._____ Date_____

INSTRUCTIONS: Taste the two samples on the tray from left to right. Select the sample that you preferred and indicate it by placing on X next to the code of the preferred sample.

Sample code	Preferred Sample	Remarks
_____	_____	_____
_____	_____	_____

If you wish to comment on the reasons for your choice or comment on the product characteristics, you may do so under remarks or below.

APPENDIX B: ANOVA TABLES FOR STATISTICAL ANAYLSIS

B.1 RHEOLOGY

The SAS System
General Linear Models Procedure
Class Level Information

Class	Levels	Values
DUP	3	1 2 3
REP	3	1 2 3
TTT	2	1 2
DAYS	2	7 42

Number of observations in data set = 36

The SAS System
General Linear Models Procedure

Dependent Variable: X

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.10176261	0.01453752	1.25	0.3103
Error	28	0.32577554	0.01163484		
Corrected Total	35	0.42753815			

R-Square	C.V.	Root MSE	X Mean
0.238020	11.16206	0.10786492	0.96635278

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DUP	2	0.03747318	0.01873659	1.61	0.2178
REP	2	0.00838956	0.00419478	0.36	0.7005
TTT	1	0.00432745	0.00432745	0.37	0.5469
DAYS	1	0.00239937	0.00239937	0.21	0.6532

TTT*DAYS	1	0.04917306	0.04917306	
		4.23	0.0492	

Source	DF	Type III SS	Mean Square	
		F Value	Pr > F	
DUP	2	0.03747318	0.01873659	
		1.61	0.2178	
REP	2	0.00838956	0.00419478	
		0.36	0.7005	
TTT	1	0.00432745	0.00432745	
		0.37	0.5469	
DAYS	1	0.00239937	0.00239937	
		0.21	0.6532	
TTT*DAYS	1	0.04917306	0.04917306	
		4.23	0.0492	

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: X

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.011635

Number of Means	2	3
Critical Range	.09020	.09478

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DUP
A	0.99842	12	2
A			
A	0.97843	12	1
A			
A	0.92221	12	3

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: X

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.011635

Number of Means	2	3

Critical Range .09020 .09478

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	REP
A	0.98441	12	2
A	0.96758	12	3
A	0.94708	12	1

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: X

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.011635

Number of Means 2
Critical Range .07365

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TTT
A	0.97732	18	1
A	0.95539	18	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: X

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.011635

Number of Means 2
Critical Range .07365

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DAYS
A	0.97452	18	42
A	0.95819	18	7

Level of TTT	Level of DAYS	N	Mean	SD
1	7	9	1.00611111	0.14111066
1	42	9	0.94852222	0.06396073
2	7	9	0.91026667	0.13048226
2	42	9	1.00051111	0.07366119

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DUP	3	1 2 3
REP	3	1 2 3
TTT	2	1 2
DAYS	2	6 36

Number of observations in data set = 36

The SAS System
General Linear Models Procedure

Dependent Variable: K

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00001719	0.00000286	1.54	0.2020
Error	29	0.00005411	0.00000187		
Corrected Total	35	0.00007130			

R-Square	C.V.	Root MSE	K Mean
0.241083	27.42548	0.00136594	0.00498056

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DUP	2	0.00000584	0.00000292	1.56	0.2264
REP	2	0.00000168	0.00000084	0.45	0.6412
TTT	1	0.00000173	0.00000173	0.93	0.3431
DAYS	1	0.00000793	0.00000793	4.25	0.0483

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DUP	2	0.00000584	0.00000292	1.56	0.2264
REP	2	0.00000168	0.00000084	0.45	0.6412
TTT	1	0.00000173	0.00000173	0.93	0.3431
DAYS	1	0.00000793	0.00000793	4.25	0.0483

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: K

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 29 MSE= 1.866E-6

Number of Means	2	3
Critical Range	.001141	.001198

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DUP
A	0.0053667	12	3
A	0.0051500	12	1
A	0.0044250	12	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: K

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 29 MSE= 1.866E-6

Number of Means	2	3
Critical Range	.001141	.001198

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	REP
A	0.0052833	12	1
A	0.0048667	12	3
A	0.0047917	12	2

General Linear Models Procedure

Duncan's Multiple Range Test for variable: K

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 29 MSE= 1.866E-6

Number of Means	2
Critical Range	.0009312

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TTT
A	0.0052000	18	1
A	0.0047611	18	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: K

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 29 MSE= 1.866E-6

Number of Means 2
Critical Range .0009312

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DAYS
A	0.0054500	18	36
B	0.0045111	18	6

B.2 PARTICLE SIZE

The SAS System
General Linear Models Procedure
Class Level Information

Class	Levels	Values
DUP	3	1 2 3
REP	3	1 2 3
TTT	2	1 2
DAYS	2	7 42

Number of observations in data set = 36

The SAS System
General Linear Models Procedure

Dependent Variable: PSIZE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	217.15624444	31.02232063	13.78	0.0001
Error	28	63.04484444	2.25160159		
Corrected Total	35	280.20108889			

R-Square	C.V.	Root MSE	PSIZE Mean
0.775001	19.96571	1.50053377	7.51555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DUP	2	0.21167222	0.10583611	0.05	0.9542
REP	2	30.38517222	15.19258611	6.75	0.0041
TTT	1	184.50694444	184.50694444	81.94	0.0001
DAYS	1	0.04551111	0.04551111	0.02	0.8880

TTT*DAYS	1	2.00694444	2.00694444
	0.89	0.3532	

Source	DF	Type III SS	Mean Square
	F Value	Pr > F	
DUP	2	0.21167222	0.10583611
	0.05	0.9542	
REP	2	30.38517222	15.19258611
	6.75	0.0041	
TTT	1	184.50694444	184.50694444
	81.94	0.0001	
DAYS	1	0.04551111	0.04551111
	0.02	0.8880	
TTT*DAYS	1	2.00694444	2.00694444
	0.89	0.3532	

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PSIZE

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 2.251602

Number of Means 2 3
Critical Range 1.255 1.318

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DUP
A	7.6125	12	3
A	7.5092	12	2
A	7.4250	12	1

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PSIZE

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 2.251602

Number of Means 2 3

Critical Range 1.255 1.318

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	REP
A	8.5825	12	2
A	7.6242	12	1
B	6.3400	12	3

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PSIZE

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 2.251602

Number of Means 2
Critical Range 1.025

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TTT
A	9.7794	18	1
B	5.2517	18	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PSIZE

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 2.251602

Number of Means 2
Critical Range 1.025

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DAYS
A	7.5511	18	7
A	7.4800	18	42

Level of TTT	Level of DAYS	N	PSIZE Mean	PSIZE SD
1	7	9	9.57888889	1.82624919
1	42	9	9.98000000	2.22163791
2	7	9	5.52333333	1.01017325
2	42	9	4.98000000	1.55367307

B.3 SEDIMENT DETERMINATION

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: SEDIMT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 0.628056

Number of Means 2 3 The SAS System
General Linear Models Procedure
Class Level Information

Class	Levels	Values
DUP	3	1 2 3
REP	3	1 2 3
TTT	2	1 2

Number of observations in data set = 18

The SAS System
General Linear Models Procedure

Dependent Variable: SEDIMT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	14.57444444	2.91488889	4.64	0.0137
Error	12	7.53666667	0.62805556		
Corrected Total	17	22.11111111			

R-Square	C.V.	Root MSE	SEDIMT Mean
0.659146	10.74171	0.79249956	7.37777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DUP	2	1.65444444	0.82722222	1.32	0.3040
REP	2	2.03111111	1.01555556	1.62	0.2389
TTT	1	10.88888889	10.88888889	17.34	0.0013

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DUP	2	1.65444444	0.82722222	1.32	0.3040
REP	2	2.03111111	1.01555556	1.62	0.2389
TTT	1	10.88888889	10.88888889	17.34	0.0013

Critical Range 0.997 1.043

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DUP
A	7.6167	6	2
A	7.5667	6	3
A	6.9500	6	1

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: SEDIMT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 0.628056

Number of Means	2	3
Critical Range	0.997	1.043

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	REP
A	7.8333	6	3
A	7.2667	6	1
A	7.0333	6	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: SEDIMT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 0.628056

Number of Means 2
Critical Range .8140

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TTT
A	8.1556	9	1
B	6.6000	9	2

B.4 pH

The SAS System
General Linear Models Procedure
Class Level Information

Class	Levels	Values
DUP	3	1 2 3
REP	3	1 2 3
TTT	2	1 2
DAYS	2	7 42

Number of observations in data set = 36

The SAS System
General Linear Models Procedure

Dependent Variable: PH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.07764300	0.01109186	205.69	0.0001
Error	28	0.00150989	0.00005392		
Corrected Total	35	0.07915289			

R-Square	C.V.	Root MSE	PH Mean
0.980924	0.165523	0.00734334	4.43644444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DUP	2	0.00004839	0.00002419	0.45	0.6430
REP	2	0.07735706	0.03867853	717.27	0.0001
TTT	1	0.00020544	0.00020544	3.81	0.0610

DAYS	1	0.00000711	0.00000711	
	0.13	0.7192		
TTT*DAYS	1	0.00002500	0.00002500	
	0.46	0.5015		

Source	DF	Type III SS	Mean Square	
	F Value	Pr > F		
DUP	2	0.00004839	0.00002419	
	0.45	0.6430		
REP	2	0.07735706	0.03867853	
	717.27	0.0001		
TTT	1	0.00020544	0.00020544	
	3.81	0.0610		
DAYS	1	0.00000711	0.00000711	
	0.13	0.7192		
TTT*DAYS	1	0.00002500	0.00002500	
	0.46	0.5015		

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PH

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.000054

Number of Means	2	3
Critical Range	.006141	.006452

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DUP
A	4.438083	12	2
A	4.435667	12	3
A	4.435583	12	1

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PH

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.000054

Number of Means 2 3
Critical Range .006141 .006452

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	REP
A	4.502000	12	3
B	4.403917	12	1
B	4.403417	12	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PH

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.000054

Number of Means 2
Critical Range .005014

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TTT
A	4.438833	18	1
A	4.434056	18	2

The SAS System
General Linear Models Procedure

Duncan's Multiple Range Test for variable: PH

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 28 MSE= 0.000054

Number of Means 2
Critical Range .005014

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DAYS
A	4.436889	18	7
A	4.436000	18	42

Level of TTT	Level of DAYS	PH N	PH Mean	PH SD
1	7	9	4.44011111	0.04932151
1	42	9	4.43755556	0.04998778
2	7	9	4.43366667	0.04929757
2	42	9	4.43444444	0.05002777