Effects of Thermal Processing on Antioxidant, Phenolic and Anthocyanin Levels in Blackcurrant Juice

by

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and approved by

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ABSTRACT OF THESIS

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Health and Wellness continues to be a major driver for consumers within the current marketplace. Given this climate, superfruits such as blackcurrant (*Ribes nigrum*) are gaining interest among beverage manufacturers due to their high content of antioxidants and anthocyanins. Blackcurrant juice, while very popular in Europe, is just beginning to gain acceptance in the domestic marketplace.

Various thermal processes are required throughout the production of a shelf stable juice product at both the raw material and finished beverage stages. The goal of this research is to evaluate the effect of these thermal processes on the retention of heat sensitive compounds such as phenolics, anthocyanins and overall antioxidants in the final consumer beverage. To this end, bulk samples of 13 brix flash pasteurized and 65 brix concentrated blackcurrant juice were obtained and further processed at beverage scale. The beverage scale processing entailed the three key thermal processes utilized by retail manufacturers: aseptic, hotfill and tunnel pasteurization. The raw material juices and fully processed samples were then analyzed for anthocyanin content, total phenolic

content and antioxidant capacity to understand retention of these nutrients in the post process beverage.

The findings of this study show marked losses at the raw material level of all measured components with a reduction in Phenolic Content of approximately 35%, a reduction of Antioxidant Capacity by 48% and, most significantly, a reduction of Anthocyanin Content of approximately 80% in the concentrated juice as compared to the flash pasteurized Not From Concentrate (NFC) juice. The anthocyanin content was seen to undergo additional degradation (40-50%) by further processing the juice at beverage level, while little or no further change in either antioxidant capacity or phenolic content was seen. There was little to no difference in the impact of aseptic, hotfill or tunnel pasteurization as compared to each other.

Understanding the relationship between process and retention will allow industry to leverage the proper processes required to deliver the desired health benefits to consumers.

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I. INTRODUCTION

The food market today is strongly driven by a consumer desire for health and wellness. Current issues of obesity, metabolic syndrome, cardiovascular disease, diabetes, inflammatory diseases, cancer and other chronic illness have fueled a desire by consumers for health, youth and energy which has influenced the food industry to put much effort into developing "better for you" and "good for you" food and drink options. A major element of this development has been the desire by formulators to leverage functional foods, specifically those with benefits of which consumers have a pre-existing awareness. One key element of this is the prevalence of "superfruits" within the food, and especially juice industry. While there is currently no scientific definition of the term superfruit, they are typically considered to be fruits valued highly in their native environments for medicinal or health promoting properties. They also have a characteristically high antioxidant content – as defined by their ORAC (Oxygen Radical Absorbance Capacity) scores – often double, triple or more as compared to other fruits (Starling, 2008).

Examples such as blueberries and pomegranates provide antioxidant benefits as well as high levels of vitamins and anthocyanins. These fruits are also very well known to American consumers, and thus are prominent in the beverage market within the United States. Blackcurrants, and blackcurrant juice, are very well known in international markets such as Europe and Asia as members of this superfruit family. As the American market for superfruits grows, blackcurrants are poised to be one of the next popular additions to the category. Due to the antioxidant components in blackcurrant juice, there

is strong evidence for health benefits associated with consumption. Oxidative stresses contribute to cancer, cardiovascular disease and neuronal diseases among others (Ames, et al., 1993).

Commercially available juice products typically undergo one or more of a variety of thermal processes. Initial processes prepare the raw pressed juice for industrial sale, and subsequently beverage manufacturers will process the juice further as it is packaged for retail sale. In developing juice products that will play effectively in the Health & Wellness sector as superfruit products, it is extremely helpful for formulators to understand how these processes are affecting the levels of nutraceuticals that are ultimately delivered to consumers.

The objective of this study is to understand how each thermal process impacts the levels of nutraceutical compounds inherent within blackcurrant juice. The study will include industrial samples prepared as single strength (100%) Not From Concentrate (NFC) juice, as well as juice From Concentrate (FC) that has been diluted back to single strength. The juice will be further processed using three typical methods common in the shelf-stable juice industry: tunnel pasteurization, hotfill pasteurization, and aseptic pasteurization. Our results indicate that the industrial level concentration process, as compared to NFC flash pasteurization had the largest impact on antioxidant capacity, phenolic content and anthocyanin content. Beyond this, our results show that there is little difference in phenolic, anthocyanin or overall antioxidant capacity retention between the three beverage processes.

These findings have a direct industrial impact by allowing shelf-stable juice manufacturers to understand how best to deliver against antioxidant and anthocyanin

claims in their blackcurrant juice beverages. These findings show that utilizing a raw material source of premium NFC blackcurrant juice will deliver the highest antioxidant capacity, including the highest phenolic and anthocyanin contributions. This information could be combined with research on the bioavailability of antioxidants within blackcurrant juice to aid manufacturers in developing products to deliver an optimized level of antioxidant that is both cost effective as a product and, most importantly, providing adequate health benefits to the consumer.

II. LITERATURE REVIEW

II.A. Health & Wellness trends in the Beverage Industry

Health & Wellness trends in food industry have been growing ever more prevalent over the past several years. Consumer desires for disease prevention and general health maintenance, as well as the obesity epidemic, have spurred the growth of this sector within the food industry. The International Food Information Council (IFIC) and the IFIC Foundation have sponsored a number of studies over the past decade to investigate consumer perceptions on food and health. The 2005 study found that "consumer awareness of long-held associations between food and health remains high." Consumers questioned in this survey also identified an awareness of antioxidants as protecting against free radical damage, as well as stating the addition of antioxidant rich foods to their diets. In addition, consumers "overwhelmingly" indicated that food and nutrition play the greatest role in maintaining or improving health (IFIC, 2005). In both the 2007 and 2008 surveys, IFIC found that more than 80 percent of Americans are "currently consuming or would be interested in consuming foods or beverages for benefits." The 2008 survey also showed over two-thirds of Americans indicating an interest in reading or hearing about relationships between food and health. Moreover, the study found that 67 percent of Americans are currently "making an effort to improve the healthfulness of their diet." This figure is a significant 10 percent increase from 2006, showing the amplification of this trend (IFIC Foundation, 2008). From an industrial perspective, in 2006 this awareness translated to $25 billion in functional food and beverage sales in the US market (Coleman & Williams, 2007).

II.B. Prevalence of "Superfruits" in Market

The term "superfruits" has become very common in today's food market. As indicated above there is no agreed upon scientific definition for this term, rather it is a term used by marketers to convey a message to consumers regarding the inherent healthful value of a particular fruit. "Superfruits are the result of bringing together science and marketing in order to create a new, value-added niche in the nutrition market" (Mellentin, 2008). The term superfruits evolved out of the term "superfoods" popularized by Stephen Pratt in 2004 with the publication of his book Superfoods Rx. This book outlined fourteen foods, including some fruits, that Pratt termed "super" due to their high contents of micronutrients such as phytonutrients, carotenoids and antioxidants (Pratt, 2004). As this superfood concept grew in popularity, the term was adopted and customized to "superfruit" by the fruit and juice industries for classifying nutrient dense, often exotic, fruits that were high in antioxidants and other nutraceuticals and communicating the benefits of these fruits to consumers. Although this terminology is new, the concept of marketing juice for its functionality dates back to the 1960's with the launch of a cranberry/apple juice blend by Ocean Spray (Starling, 2008).

Due to the prevalence of Health & Wellness trends, superfruits have gained popularity with both consumers and the industry resulting in a search for fruits linked to natural functionality (Netzel, et al., 2007). Many of the new hit superfruits are sourced from exotic locales such as the Amazon or Africa however, as is the case with cranberry, this is not a requirement for the category. Fruits such as blackcurrant and blueberry fit into this trend very nicely. One key distinguishing factor about superfruit consumption is that it is almost exclusively in a processed format, typically as juice. This is due to the

bitter taste associated with many of the compounds that provide the functional basis for their desirability (Starling, 2008). Consumption as juice makes these products convenient to the consumer and accessible across diverse markets.

II.C. Blackcurrants and Blackcurrant Juice

Blackcurrants (*Ribes nigrum*) are small round berries, up to 10mm in diameter, and have a shiny black appearance. The parent genus, *Ribes*, encompasses over 150 species, a portion of which constitute edible berries (Hummer & Barney, 2002). Blackcurrants have been found to be high in Vitamin C, antioxidants, total phenolics and anthocyanins (Hummer & Barney, 2002; Moyer, et al., 2002). The berry has been cultivated for around 400 years, and the blackcurrant species is of the greatest economic importance within the genus. This economic importance is primarily throughout Europe (Hummer & Barney, 2002). Currants were cultivated in North America in the 1800's, however, they are not a commercially significant crop on this continent today due to widespread *Ribes* eradication in the first half of the 20th century (Benedict, 1981; Maloy, 2001; Hummer & Barney, 2002).

In the early 1900's, a fungal tree disease known as white pine blister rust was brought from Europe to the United States on white pine seedlings. *Ribes* species act as an intermediate host to the disease during its life cycle allowing the spread of the disease to become rampant. White pine was a significant economic crop in North America, and as the disease was foreign there was no inherent immunity to it in the North American crop. These factors, among others, led to the formation of a *Ribes* eradication plan throughout the United States as a way of halting the spread of the disease. *Ribes* eradication was

carried out from 1916 through 1967, and included the banning of *Ribes nigrum* cultivation specifically (Benedict, 1981; Maloy, 2001).

Blackcurrants, while sometimes eaten fresh, are most often processed as juices, jams, jellies or baked goods (Hummer & Barney, 2002). Blackcurrant juice is popular today in many markets, the most prevalent being Europe and Asia. Commercial blackcurrant juice product development was taking place in Europe as early as the 1930's. Blackcurrant syrups were developed for use as cordials intended for use in milk. These cordials were distributed to children in Britain during WWII as part of their war rations, possibly due to the high Vitamin C content as other sources of Vitamin C were scarce (Tressler & Joslyn, 1961). Currently several brands, primarily within Europe and Asia, market blackcurrant juices, juice drinks and cordials as well as jams, jellies, baked goods and other blackcurrant products.

II.D. Nutraceuticals in Blackcurrants

Blackcurrants contain an array of nutrients and nutraceuticals, which allow them to considered superfruits. The fruit is comprised of a wide variety of vitamins, minerals and trace elements, and is a source of dietary fiber (Souci, et al., 2008). Blackcurrants provide a considerable amount of ascorbic acid, but are rich in many phenolic compounds as well. Anthocyanins are a key source of the antioxidant content in blackcurrants, and the specific anthocyanins of interest are discussed in section II.G. In addition, blackcurrants contain polyphenols such as kaempferol, quercetin and myricetin; fruit acids such as quinic, ferulic, caffeic, para-coumaric, protocatechuic and salicylic (Souci, et al., 2008).

II.E. Antioxidants

In order to fully understand the mechanism of action of antioxidants, one must understand the mechanism of chemical oxidation. Oxidation, in the broadest sense, is simply the removal of an electron from an atom or molecule. This is typically paired with a reduction reaction in which the electron or electrons involved are added to another atom or molecule. In biological, and food, systems, oxidation reactions can generate a reactive species and initiate a free radical chain reaction. An example of this is illustrated below:

Initiation

$$R_2N_2 \rightarrow 2R^\bullet + N_2$$

$$R^\bullet + O_2 \rightarrow ROO^\bullet$$

$$ROO^\bullet + LH \rightarrow ROOH + L^\bullet$$

Propagation

$$L^\bullet + O_2 \rightarrow LOO^\bullet$$

$$LOO^\bullet + LH \rightarrow LOOH + L^\bullet$$

Inhibition

$$LOO^\bullet + AH \rightarrow LOOH + A^\bullet$$

Termination

$$A^\bullet + (n - 1)LOO^\bullet \rightarrow \text{nonradical products}$$

$$LOO^\bullet + LOO^\bullet \rightarrow \text{nonradical products}$$

(Huang, et al., 2005)

Historically, antioxidants have been broadly described as "all substances that inhibited oxidation reactions regardless of the mechanism," and narrowly as "those compounds that interrupt the free-radical chain reaction involved in lipid oxidation and those that scavenge singlet oxygen" (Lindsay, 1996). There should, however, be a distinction between the chemical terms of *reductant* and *oxidant* as compared to the biological terms of *antioxidant* and *pro-oxidant* (Prior & Cao, 1999). The biological term *antioxidant* refers to "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell, 1995). Reactive species known as *pro-oxidants* can be defined as "a toxic substance that can cause oxidative damage to lipids, proteins and nucleic acids, resulting in various pathologic events and/or diseases" (Prior & Cao, 1999). It is important to note that "an antioxidant is a reductant, but a reductant is not necessarily and antioxidant" (Prior & Cao, 1999). These definitions emphasize that the value of an antioxidant is in its ability to inhibit the free radical chain reaction. This is essential for stability in food systems and for health promotion *in vivo*.

As foods are extremely complex systems it is very difficult to determine the impact of each antioxidant compound individually. To this end, there are many assays available to measure the antioxidant capacity of a food or food system as a whole. One of the most commonly used of these is the Oxygen Radical Absorbance Capacity (ORAC) assay. This assay measures the antioxidant capability of a given sample in terms of peroxyl radical ($ROO^{\bullet}$) scavenging ability as compared to standardized solutions of Trolox. Samples are mixed with a fluorescein probe and inoculated with a free radical initiator, as fluorescein is oxidized the fluorescence intensity of the sample decreases. The presence

of anti-oxidant in the sample slows this reaction. As fluorescence is measured over time, a curve of intensity versus time is generated. The area under the curve (AUC) is analyzed as compared to a curve generated by the Trolox standard to yield antioxidant capacity in Trolox equivalents (Huang, et al., 2005). It must be noted, however, that as ORAC measures the ability of the antioxidants present in the sample to reduce peroxyl radicals, antioxidant capacity to scavenge other types of radicals may go undetected.

Several types of compounds can contribute to the antioxidant activity of a food system. Compounds such as tocopherols, ascorbic acid, carotenoids and phenolics are some commonly researched antioxidant compounds (Moyer, et al., 2002; Dubost, et al., 2007). Of interest in blackcurrant juice are polyphenols, such as anthocyanins and anthocyanidins, and ascorbic acid (Moyer, et al., 2002; Miller & Rice-Evans 1997).

II.F. Phenolics

Phenolic compounds are a key source of antioxidant activity in fruits. Flavonoids, the fraction of phenolics comprised of such compounds as flavones, isoflavones, flavonones and anthocyanins are known to be potent antioxidants in vitro (Moyer, et al., 2002). Polyphenols are able to act as reducing agents, hydrogen donating antioxidants, as well as singlet oxygen quenchers (Dubost, et al., 2007). Phenolic compounds are known to terminate oxidation by participating in the reactions through resonance stabilized free radical forms, as well as acting as free radical scavengers (Lindsay, 1996). As suggested by Dubost et al. (2007), hydrogen donation may be a key mechanism of action for the antioxidant activity of phenolic compounds.

II.G. Anthocyanins

According to the findings of Miller and Rice-Evans (1997), much of the antioxidant function of blackcurrant juice is attributable to anthocyanins. Anthocyanins are comprised of an anthocyanidin conjugated with a sugar moiety. Cyanidin and delphinidin have been identified as the key anthocyanidins occurring in blackcurrant juice (Goiffon, et al., 1999; Miller & Rice-Evans, 1997). The structures of each are as follows:

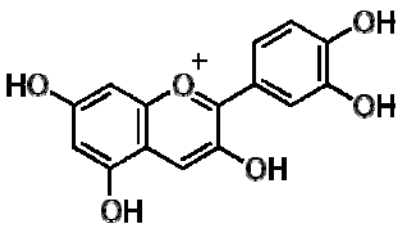


Figure 1: Structure of Cyanidin

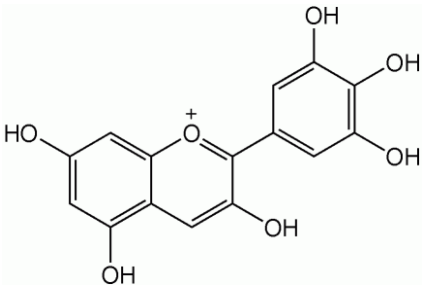


Figure 2: Structure of Delphinidin

In blackcurrant juice these are most commonly conjugated with glucose and rutinose to form cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and delphinidin-3-rutinoside (Goiffon, et al., 1999).

II.H. Health Benefits

Antioxidants, including phenols such as anthocyanins, have been studied extensively for their health benefits. This research has focused on several key areas, most of which relate to oxidative stress. Studies have focused on degenerative aging disorders such as cancer and cardiovascular disease, as well as some work in the field of ophthalmology. Oxidative damage to DNA, proteins and lipids is a key contributing factor to these diseases (Ames, et al., 1993; Ames, et al., 1995). Much data focuses on effects of dietary consumption of fruits and vegetables in general as substantial sources of antioxidants to mitigate this damage. More specifically, research has pointed to the polyphenolic compounds within these foods as important contributors of antioxidant activity (Salah, et al., 1995; Hertog, et al., 1993).

A review of epidemiological evidence regarding cancer prevention in relation to fruit and vegetable intake indicates a statistically significant protective effect in 128 of 156 studies. This meta-study shows a doubled risk of cancer for the lower one fourth of the population in terms of fruit and vegetable intake (Block, et al., 1992). In addition, a meta-study carried out by Steinmetz and Potter (1996) found a protective effect against cancer associated with fruit and vegetable consumption in nineteen of twenty cohort studies. The study also summarized 174 case-control studies which indicated strong evidence for a protective effect against cancers of the lung, stomach and esophagus, as well as a probable effect against cancers of the oral cavity and pharanx, colon, breast, pancreas, and bladder.

Similar meta-studies have been conducted regarding cardiovascular disease. Ness and Powles (1997) found nine of ten ecological studies, two of three case-control studies

and six of sixteen cohort studies indicating a significant protective effect against coronary heart disease through consumption of fruits and vegetables. Additionally, the study found three of five ecological studies, and six of eight cohort studies reporting a significant protective effect against stroke. Within specific studies, research indicates that risk of death in elderly men and post menopausal women due to coronary heart disease may be reduced through regular consumption of foods containing flavonoids (Hertog, et al., 1993; Yochum, et al., 1999). The relationship of red wine consumption to reduced cardiovascular disease has been elucidated as the so called "French Paradox" (Renaud & de Lorgeril, 1992). It has since been suggested that the active components in this relationship are phenolics such as procyanidins, and further that berry juices rich in anthocyanins could provide comparable benefits (Shrikhande, 2000).

Blackcurrant anthocyanosides, in particular, have been shown have an ophthalmic benefit. Nakaishi, et al. (2000) studied the effect of oral intake of the four key blackcurrant anthocyanosides (BCA) on dark adaptation, video display terminal work-induced transient refractive alteration, and visual fatigue. The study resulted in a significant improvement in dark adaptation, prevention of myopic refractory shift after visual tasks on video display terminals (at a dosage of 50mg BCA concentrate), and reduction of visual fatigue in subjects.

II.I. Thermal Processing in the Juice Industry

Thermal processing is very important to the juice industry. At the raw material level, freshly pressed juice must either be flash-pasteurized and stored for use in Not From Concentrate products or concentrated to a high brix (solids content) for use in reconstituted juices and juice drinks. In the manufacture of shelf stable consumer products, juice must undergo further processing when packaged for retail sale.

II.I.1. Not From Concentrate Flash Pasteurization

Premium juice products typically consist of Not From Concentrate juice which has, after being pressed from the fruit, remained at single strength throughout the supply chain. This juice must be processed industrially to prevent microbial growth, and thus a high-temperature-short-time (HTST) flash pasteurization step is applied. The process applied is minimally heat abusive and does not result in a commercially sterile product, however, the adequate control of both pathogenic and spoilage organisms is achieved through this pasteurization (Downes, 1995).

The juice to be pasteurized is pumped through a heat exchanger, typically plate heat exchangers are used in this process, and held for a short time prior to being rapidly cooled (Downes, 1995; Singh & Heldman, 2001). The exact time and temperature parameters used are typically juice dependent and considered proprietary by the juice manufacturers. Once the juice is flash pasteurized, it can be stored in clean, temperature controlled tanks, shipped in specialized tankers, or filled directly into consumer packaging for chilled distribution. Figure 3 below illustrates a schematic diagram of the flash pasteurization process.

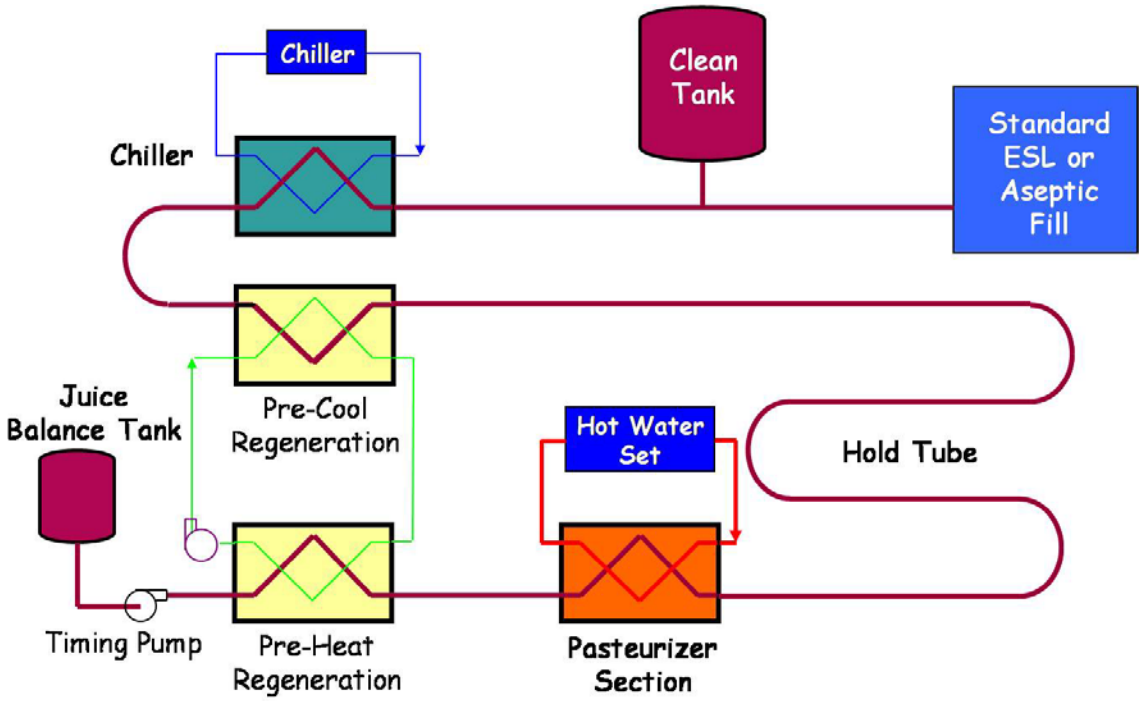


Figure 3: Schematic of Flash Pasteurization

The key benefit of this process, as mentioned above, is that it results in a premium product. The organoleptic quality of the juice is maintained to a high degree through the utilization of this process. On the other hand, the premium nature restricts the use of this process to markets that can bear the associated cost. The juice is shipped and stored as single strength (large quantity), must be chilled throughout the supply chain and handled properly (clean transfers, aseptic storage, etc.). These factors add considerably to the cost of the overall manufacturing system.

II.I.2. Juice Concentrate Manufacture

A more economical way to manage juice distribution is through concentration. By concentrating juice after pressing, quantity (and weight) is reduced allowing juice to be shipped globally at a much lower cost than NFC. Additionally, concentration aids in preservation of the juice through the increase in soluble solids (Downes, 1995). The juice can then be reconstituted in a given market and bottled for sale to consumers. The resulting product is typically of lower quality, from a taste and color perspective, than NFC juice due to the increased thermal abuse via the concentration process. Evaporation technology strives to minimize this loss in quality, and to this end evaporators designed to allow minimal heat contact periods are most commonly employed in the juice industry. The most common designs include rising film, falling film and expanding flow centrifugal evaporators (Downes, 1995).

In rising film evaporators (Figure 4), juice is fed from below, and boiled in 3-15 meter long tubes which are heated externally with steam. As the product boils, the resultant steam forces the juice to form a thin film rising along the inside of the tubes resulting in optimal heat transfer conditions. Product then flows out of the top of the apparatus and is collected. One of the key drawbacks to this system is the low level of evaporation obtained in a single pass. Due to this, product must be passed through multiple times to achieve a desired concentration, thereby increasing thermal abuse (Downes, 1995; Singh & Heldman, 2001).

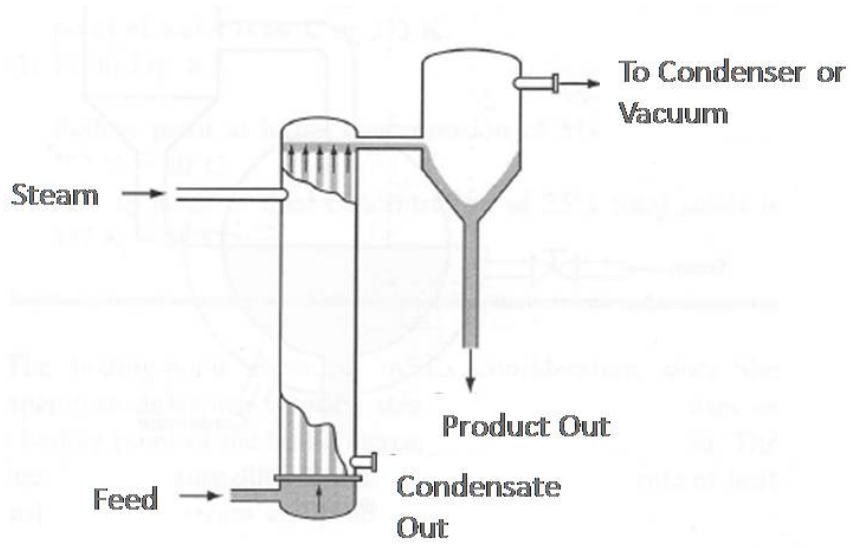


Figure 4: Rising Film Evaporator (Singh & Heldman, 2001)

Falling film evaporators (Figure 5), conversely, use gravity to move the film through the heat transfer tubes or plates. Product is fed into the top of the vertical tubes and is heated as it passes in a film down the heat transfer surface. This design is able to achieve higher evaporation efficiencies and therefore reduce residence time and thermal abuse as compared to the rising film design. The drawback to this system is the difficulty in maintaining uniform flow throughout the system. Care must be taken to ensure that an even distribution of product is maintained to avoid over exposure to the heating elements (Downes, 1995; Singh & Heldman, 2001).

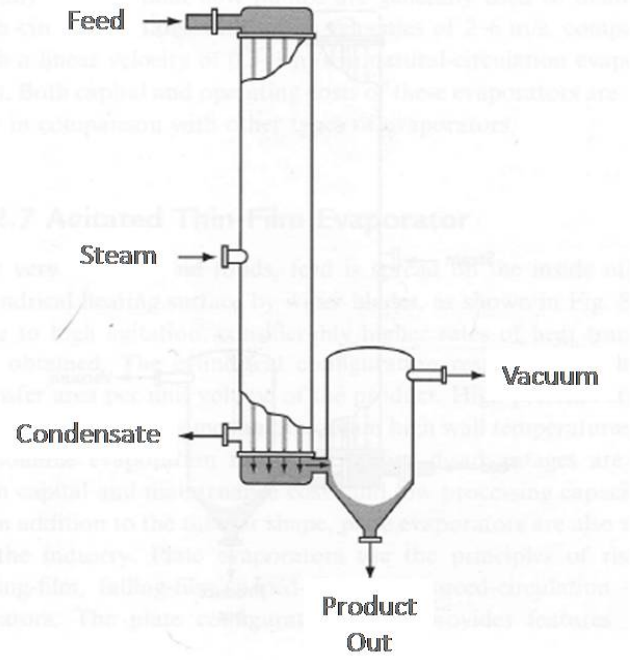
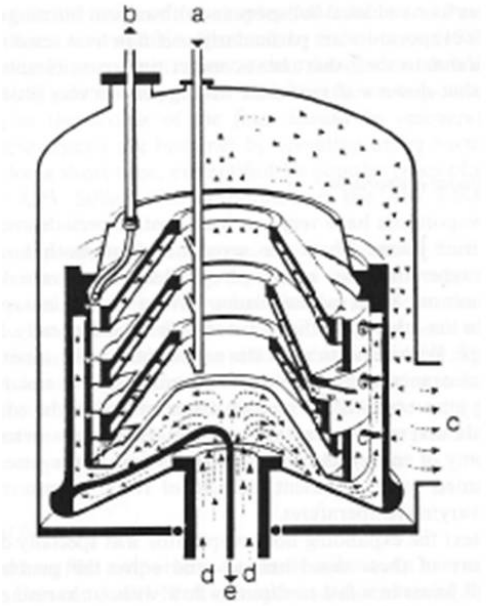


Figure 5: Falling Film Evaporator (Singh & Heldman, 2001)

The third common evaporator design, the expanding flow centrifugal evaporator (Figure 6), utilizes centrifugal force to move the juice and concentrate through the system. In this system, juice is sprayed onto the inner apex of the cones, and as the apparatus spins centrifugal force pushes the juice into a thin film across the heat transfer surface. As the juice is concentrated, the mass is pushed toward the outer edge of the cone where it is collected and removed. A partial vacuum within the apparatus allows for a reduced boiling temperature, and this combined with the rapid and continuous thin film flow of product as well as the high heat transfer coefficients achieved allow for single pass concentration and greatly minimize heat contact times (Downes, 1995).



(a) Process liquid in; (b) concentrate out; (c) vapor to condenser; (d) steam in; (e) steam condensate out

Figure 6: Expanding Flow Centrifugal Evaporator (Downes, 1995)

II.I.3. Beverage Level Processes

The raw materials resulting from the above processes must be further processed prior to filling for consumer use under shelf stable conditions. There are three main processes employed that result in a shelf stable packaged juice product. Shelf stable products must be microbiologically stable at ambient temperatures over a commercially viable shelf life, typically six months to one year. The processes commonly used are aseptic pasteurization, hotfilling and tunnel pasteurization.

Aseptic pasteurization is the least heat abusive of these three processes. The pasteurization step is a high-temperature-short-time (HTST) process whereby the product is heated rapidly (typically to temperatures of 85-95˚C) in a plate or tubular heat exchanger and passed through a holding tube for a period of 4-20 seconds, dependent

upon the specific product parameters and lethality of process required. The product is then passed through a second heat exchanger to be rapidly cooled to ambient temperature prior to filling. In this process, the packaging material is sterilized separately from the product, and filling is carried out in an aseptic chamber (Castberg, et al., 1995; Singh & Heldman, 2001).

Hotfill pasteurization utilizes a similar pasteurization step; however, the two processes vary in the filling step. In this process, the product is filled into the packaging material while still hot enough to ensure sterilization of both bottle and closure. The packaged product is subsequently passed through a cooling tunnel where water is sprayed over the packages to cool the product to ambient temperature. The tunnel is typically staged such that the water becomes progressively cooler as the product moves through in order to avoid thermal shock to the package. This elongated cooling process as compared to the aseptic process above subjects the product to an increased level of heat abuse. In the hofill process, the product is exposed to high temperatures for minutes as opposed to the seconds of high temperature seen in the aseptic process (Castberg, et al., 1995).

The third standard practice is the most heat abusive of the three. Tunnel pasteurized, product is filled with no prior pasteurization step, after which the product and package are then pasteurized together. The packaged product is carried via conveyer at a controlled speed through a tunnel where water mists of varying degrees are sprayed onto the product. The tunnels typically consist of three to five stages with the initial stages slowly increasing the temperature of the product. The middle of the tunnel is akin to the holding tubes seen in the pasteurization steps discussed above, whereas the water sprayed on the product is maintaining the product temperature at a desired level to achieve the

necessary lethality. The final stage or stages then gradually cool the product back to ambient temperatures. This process exposes the product to elevated temperatures for much longer periods of time than either aseptic or hotfill pasteurization methods (Downes, 1995).

III. HYPOTHESIS AND OBJECTIVE

III.A. Hypothesis

Thermal processing has a measurable adverse effect on antioxidant capacity, total phenolic content and anthocyanin levels in blackcurrant (*Ribes nigrum*) juice.

III.B. Objective

To determine the degree of degradation of antioxidant capacity, total phenolic content and anthocyanin levels in blackcurrant juice attributable to:

- Concentration vs. Flash Pasteurization of raw juice for industrial use
- Tunnel Pasteurization vs. Hotfill Pasteurization vs. Aseptic Pasteurization for consumer use

IV. MATERIALS AND METHODS

IV.A. Materials and Equipment

Samples of blackcurrant (*Ribes nigrum*) single strength juice (13 brix) and juice concentrate (65 brix) were obtained from Iprona (Lana, Italy). The juice was processed for hotfill and aseptic experiments using a Microthermics (Microthermics, Inc., Raleigh, NC). For tunnel pasteurization experiments, the juice was processed in a hot water bath. Hydrophilic Oxygen Radical Absorbance Capacity (ORAC), total phenolic content and total anthocyanin content assays were performed by Dr. Boxin Ou and team at Brunswick Laboratories (Norton, MA). Duplicate beverage samples were each analyzed in triplicate. JMP statistical analysis software was used for data analysis.

IV.B. Juice Preparation

All raw juice was stored frozen (-19ºC) and thawed immediately prior to use. The sample of 13 brix single strength (100% juice) Not From Concentrate (NFC) blackcurrant juice was divided into three batches. A 100ml sample was retained and frozen for future baseline analysis.

The sample of 65 brix concentrated (FC) blackcurrant juice was diluted with filtered, deionized water to single strength (13 brix). The single strength (100% juice) FC sample was then divided into three batches. Again, a sample was retained and frozen for future baseline analysis.

IV.C. Benchtop Tunnel Pasteurization

One batch of NFC and one batch of FC blackcurrant juice were hand filled under ambient temperature conditions into 240ml glass bottles. The bottles of juice, as well as an identical bottle containing water and a temperature probe were then placed in a sink bath of warm water. The bath was gradually heated through the constant flow of hot (49ºC) tap water until the temperature probe read 43 ºC. At this point the bottles were transferred to a hot water bath containing water at 77ºC. The temperature of the bottles was monitored until they reached 71ºC. The product was held at 71ºC for 10 minutes. After 10 minutes, the bottles were then transferred back to the sink bath containing hot water. The bath was gradually cooled through the addition of tepid (37ºC) water. When the temperature of the product had cooled to below 38ºC, the incoming water was turned to cold (7ºC) and the flow was continued until the product reached room temperature (21ºC). The lethality of the process (F^{5}_{90}) is 0.23 seconds at 90ºC given a z-value of 5ºC.

Tunnel Pasteurization				
Stage	Initial Time (t_i) min	Final Time (t_f) min	Initial Temp (T_i) ºC	Final Temp (T_f) ºC
1 - Sink (49°C)	0	14	21	43
2 - Bath (77°C)	14	29.5	43	71
3 - Hold (77°C)	29.5	39.5	71	71
4 - Sink (37°C)	39.5	52	71	38
5 - Sink (7°C)	52	55	38	21

Table 1: Tunnel Pasteurization Thermal Process Parameters

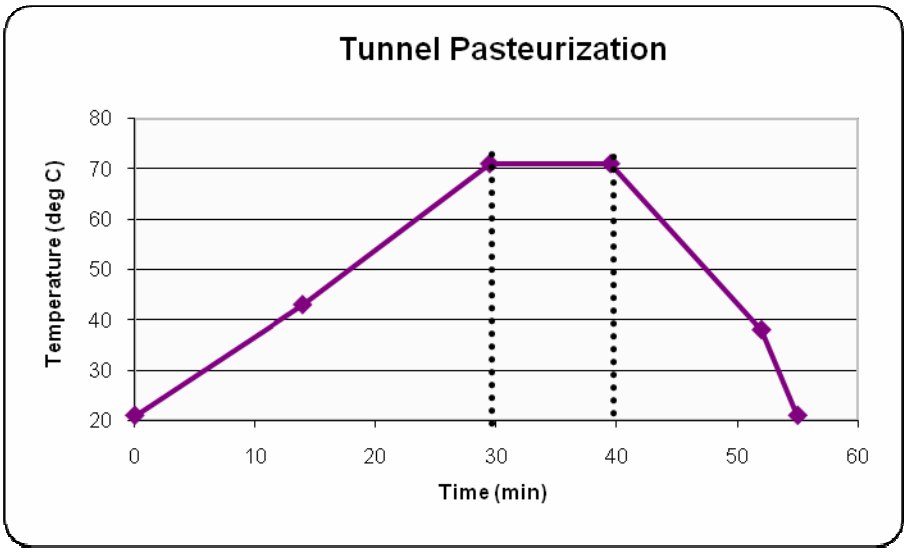


Figure 7: Tunnel Pasteurization Thermal Process Curve

IV.D. Benchtop Hotfill Pasteurization

One batch of NFC and one batch of FC blackcurrant juice were processed through the Microthermics unit using a hotfill protocol. The hotfill process used for these batches heated product rapidly to 88ºC and held the product at 88ºC for 45 seconds. The product was then filled at a temperature of 82ºC into 240ml glass bottles. The bottles were then placed in a warm sink bath and gradually cooled to room temperature (21ºC) through the constant addition of cold (7ºF) water. The lethality of the process (F^{5}_{90}) is 37.50 seconds at 90ºC given a z-value of 5ºC.

Hotfill Pasteurization				
Stage	Initial Time (t_i) sec	Final Time (t_f) sec	Initial Temp (T_i) ºC	Final Temp (T_f) ºC
1 – CUT	0	85	21	88
2 – Hold	85	125	88	88
3 – Exit	125	135	88	82
4 – Closure	135	165	82	80
5 – Sink (7°C)	165	567	80	21

Table 2: Hotfill Pasteurization Thermal Process Parameters

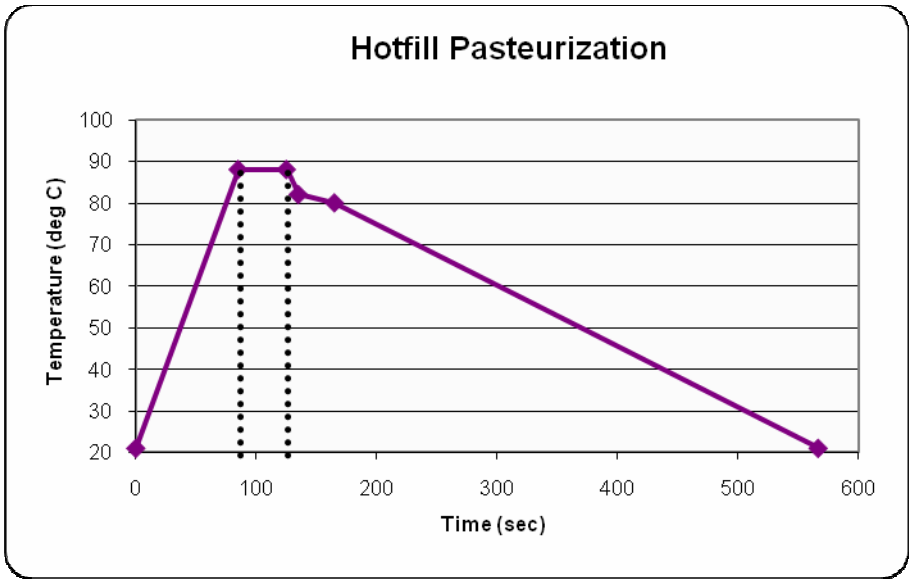


Figure 8: Hotfill Pasteurization Thermal Process Curve

IV.E. Benchtop Aseptic Pasteurization

One batch of NFC and one batch of FC blackcurrant juice were processed through the Microthermics unit using an aseptic protocol. The aseptic process used here heated the product rapidly to 94ºC and held the product for 30 seconds. The product was then rapidly cooled to 21ºC within the unit. Finally, the product was filled in a positive pressure clean-fill hood into 240ml glass bottles which had been pre-sterilized in a peracetic acid bath. The lethality of the process (F^{5}_{90}) is 514.23 seconds at 90ºC given a z-value of 5ºC.

Aseptic Pasteurization				
Stage	Initial Time (t_i) sec	Final Time (t_f) sec	Initial Temp (T_i) ºC	Final Temp (T_f) ºC
1 – CUT	0	85	21	94
2 – Hold	85	115	94	94
3 – CDT	115	133	94	21

Table 3: Aseptic Pasteurization Thermal Process Parameters

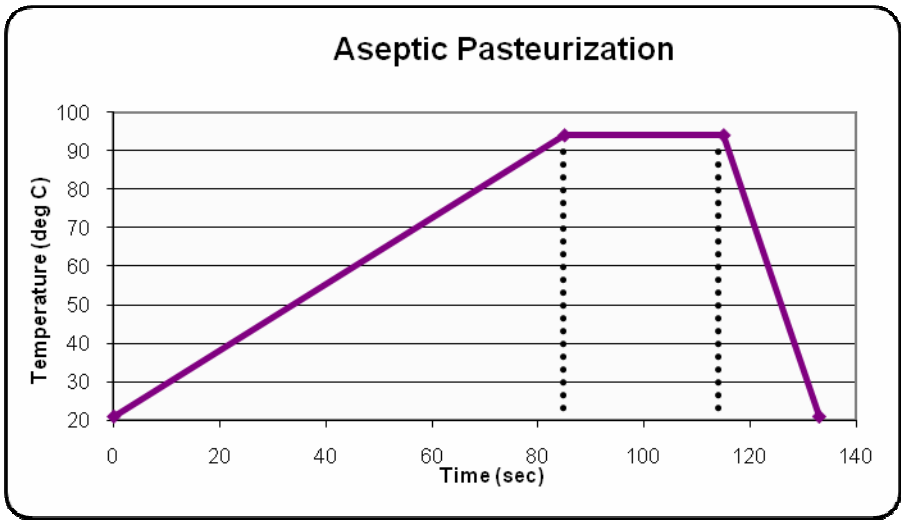


Figure 9: Aseptic Pasteurization Thermal Process Curve

IV.F. Antioxidant Capacity Analysis

Hydrophilic Oxygen Radical Absorbance Capacity (ORAC) analysis as carried out by Brunswick Laboratories (Norton, MA) was as follows: (Ou, et al., 2001; Ou, et al., 2006) The liquid beverage sample was centrifuged and the supernatent was diluted with buffer in preparation for analysis with the COBRA FARAS II analyzer. Buffered sample and reagents are added to the COBRA FARAS II tube and incubated for 30 seconds while the rotor is spinning prior to initial baseline analysis of flouresence. 2,2'-Azobis(2-aminidopropane) dihydrochloride (AAPH) reagent was then added to the sample well to initiate oxidative reaction. Fluorescence readings were then taken at 0.5 seconds, then every minute thereafter for 30 minutes. Samples of Trolox at varying concentrations were used as a control for comparison, and a well containing only buffer used as a blank. Area under the curve (AUC) for the sample and standards was calculated using a regression equation:

$$AUC = 1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + f_4/f_0 \ldots + f_{29}/f_0 + f_{30}/f_0$$

(Ou, et al., 2001; Ou, et al., 2006)

Where f_0 is the fluorescence reading at time zero and f_i is the fluorescence reading at time *i*. The AUC for the sample was compared with that for the Trolox standards and the blank thus determining the relative ORAC value of the sample as Trolox equivalents per gram as follows:

$$\text{Relative ORAC Value} = [(AUC_{Sample} - AUC_{Blank})/(AUC_{Trolox} - AUC_{Blank})] \times (\text{molarity of Trolox/ molarity of Sample})$$

(Ou, et al., 2001; Ou, et al., 2006)

As indicated above, duplicate samples were analyzed in triplicate to allow for statistical analysis. The data was analyzed using a One-way ANOVA (Analysis of Variance) test and results compared using a Tukey-Kramer HSD comparison.

IV.G. Total Phenolic Content Analysis

Analysis of Total Phenolic content was carried out by Brunswick Laboratories (Norton, MA). The Folin-Ciocalteu procedure was used to measure total phenolic content with gallic acid as a standard (Wada & Ou, 2002; Sanchez-Moreno, et al., 2003). Duplicate samples were analyzed in triplicate to allow for statistical analysis. The data was analyzed using a One-way ANOVA test and results compared using a Tukey-Kramer HSD comparison.

IV.H. Anthocyanin Content Analysis

Analysis of Total Anthocyanin content was carried out by Brunswick Laboratories (Norton, MA). A pH differential method was used to estimate total anthocyanin content. Results are expressed as mg of cyanidine-3-glucoside per liter (Wada & Ou, 2002;

Sanchez-Moreno, et al., 2003). Duplicate samples were analyzed in triplicate to allow for statistical analysis. The data was analyzed using a One-way ANOVA test and results compared using a Tukey-Kramer HSD comparison.

V. RESULTS

V.A. Antioxidant Capacity

The results of the study indicate that the largest influence on overall antioxidant capacity can be attributed to the initial industrial processes. There is some further variation upon further processing of the juice into shelf-stable beverage format, however, this degradation is minor, if at all evident, when compared to the initial drop in ORAC value. Interestingly, the results show that the further degradation of antioxidant capacity via beverage processes is evident only in NFC juice, whereas FC juice does not exhibit any further significant degradation.

ORAC (µmoleTE/L)		
Sample	**NFC**	**FC**
"Raw" Juice	67,172	34,758
Tunnel	62,150	35,559
Hotfill	61,608	34,436
Aseptic	60,692	32,465

Table 4: Mean ORAC Values at Each Level of Process

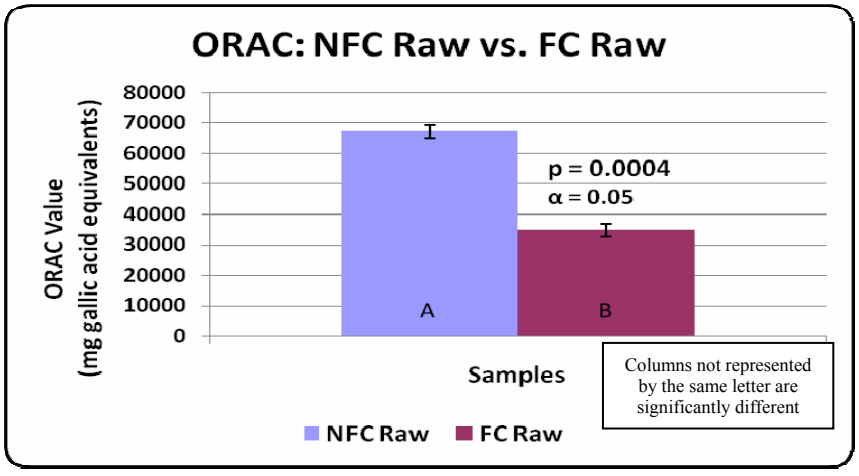


Figure 10: ORAC: NFC Raw vs. FC Raw

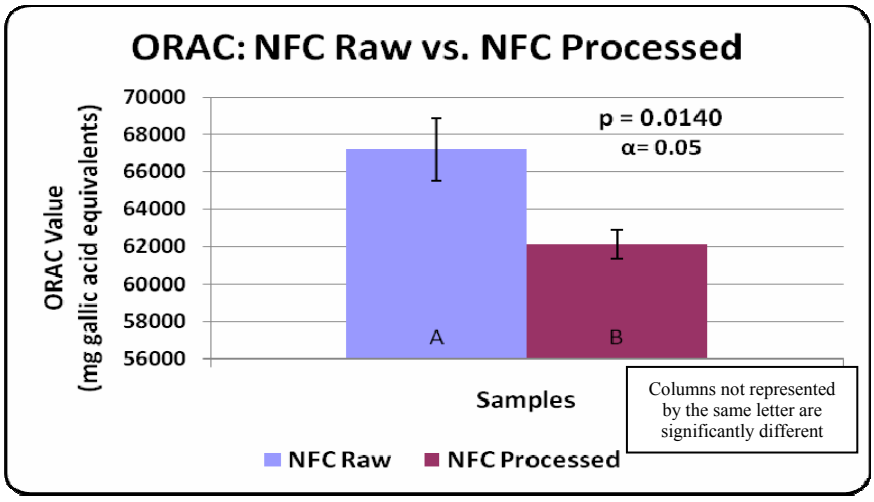


Figure 11: ORAC: NFC Raw vs. NFC Processed

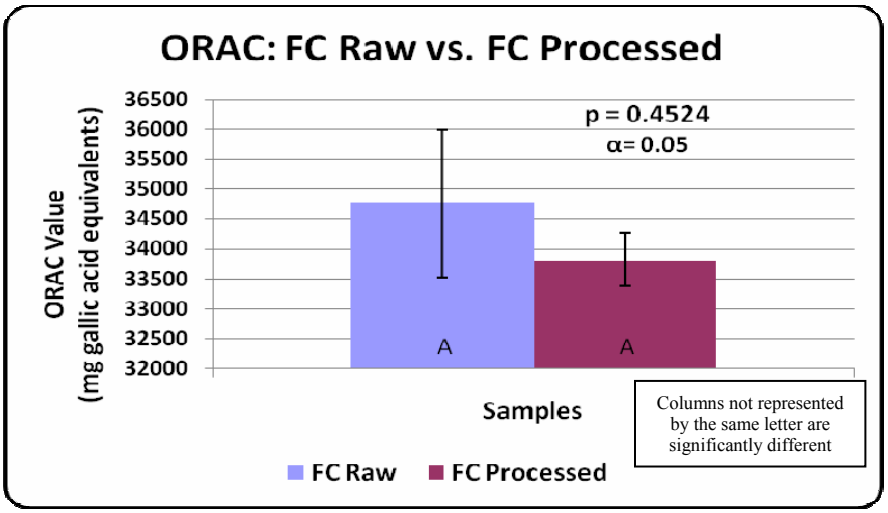


Figure 12: ORAC: FC Raw vs. FC Processed

Comparison of NFC raw juice to FC raw juice reveals a 48% decrease in overall antioxidant capacity (Figure 10). When a comparison is made between the ORAC value of NFC raw juice, and that of the NFC processed juice (a mean of aseptic, hotfill and tunnel results), a further 8% decrease in antioxidant capacity is observed (Figure 11). ANOVA analysis of the data indicates that this is a statistically significant decrease.

Conversely, no statistically significant further decrease was observed when carrying out the same comparison on FC juice data (Figure 12). The ANOVA analysis of the ORAC data is shown in Appendix 1.

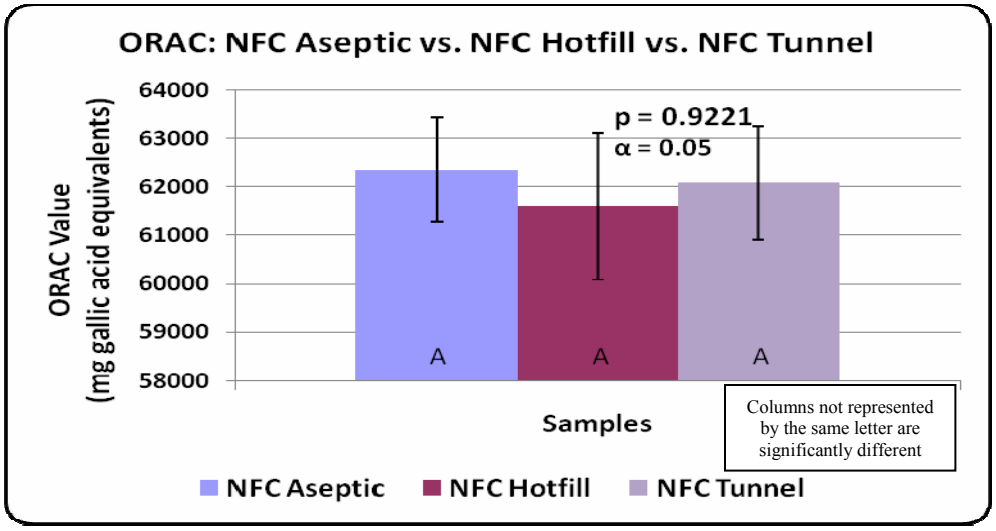


Figure 13: ORAC: NFC Aseptic vs. NFC Hotfill vs. NFC Tunnel

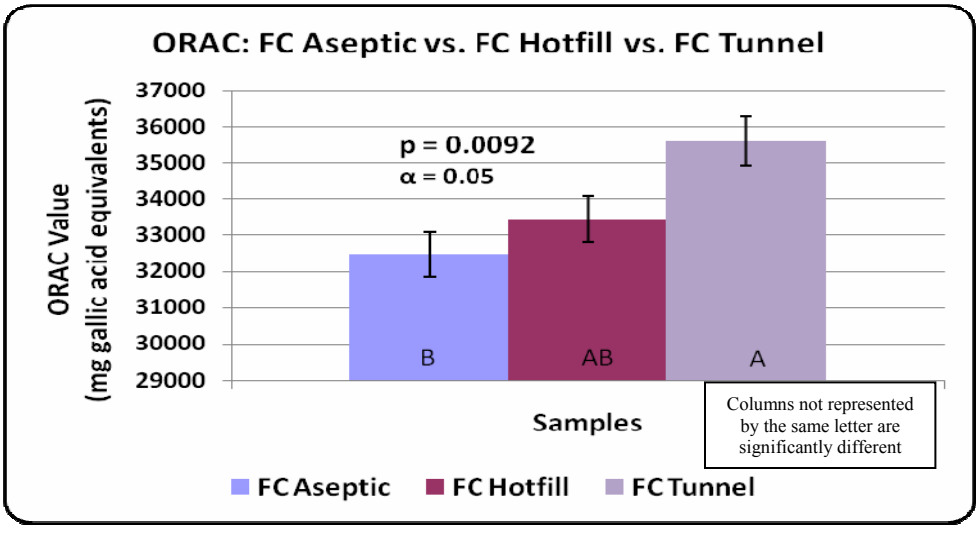


Figure 14: ORAC: FC Aseptic vs. FC Hotfill vs. FC Tunnel

Comparative analysis of the beverage level processes shows a different picture for NFC juice versus FC juice. The data show no significant difference in ORAC value for NFC juice regardless of how the juice was processed at bottling (Figure 13). From Concentrate samples, however, do exhibit some differences. From a statistical perspective, hotfill product contains a similar antioxidant capacity as both aseptic and tunnel pasteurized juice although aseptically produced samples exhibit a significantly lower ORAC value than tunnel pasteurized samples (Figure 14).

In terms of delivery in a commercial product, a standard 8 ounce serving size of juice would deliver ORAC values as described in Table 5 for the beverages produced in this study.

ORAC (µmoleTE)	
per 8oz serving	
NFC Tunnel	14,704
NFC Hotfill	14,576
NFC Aseptic	14,359
FC Tunnel	8,413
FC Hotfill	7,681
FC Aseptic	5,600

Table 5: ORAC Values per 8oz Serving

V.B. Total Phenolic Content

As with the overall antioxidant capacity, the most severe degradation of total phenolic content was seen through the concentration process as compared to flash pasteurization and further significant degradation was again only seen in the NFC juice samples.

Phenolics (mg gallic acid/L)		
Sample	**NFC**	**FC**
"Raw" Juice	5,281.08	3,457.65
Tunnel	5,143.06	3,490.41
Hotfill	4,969.46	3,518.78
Aseptic	4,849.82	3,392.15

Table 6: Mean Phenolic Content at Each Level of Process

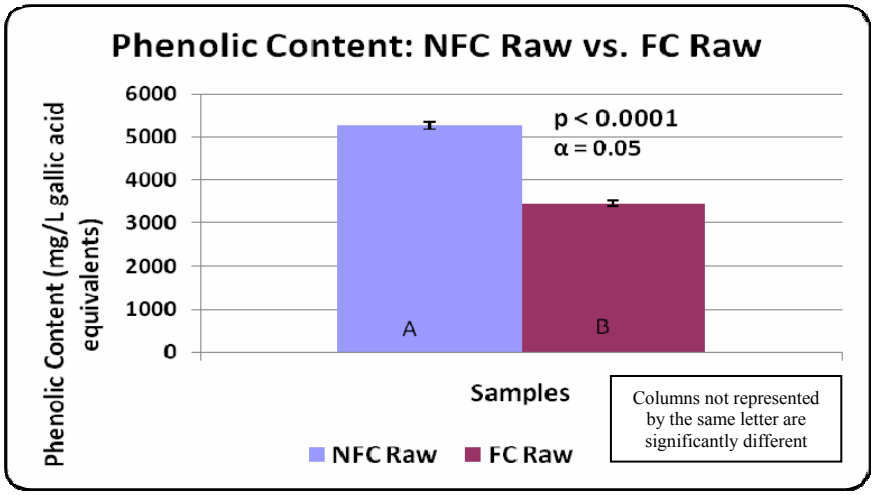


Figure 15: Phenolic Content: NFC Raw vs. FC Raw

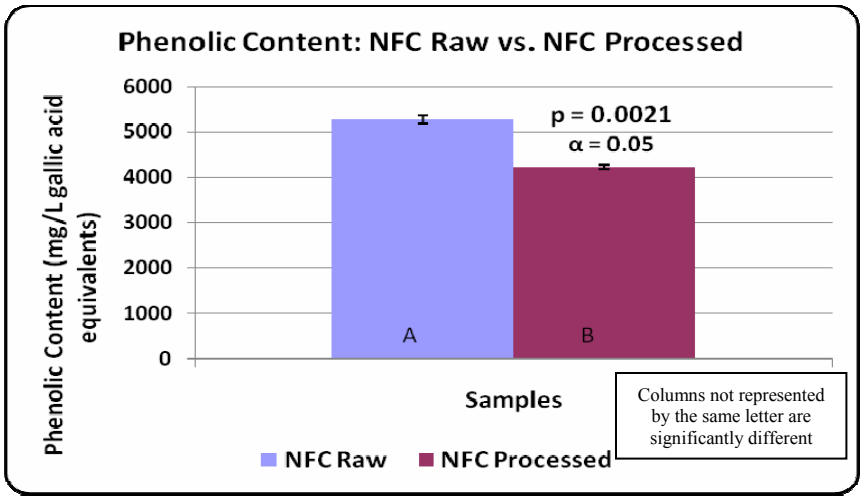


Figure 16: Phenolic Content: NFC Raw vs. NFC Processed

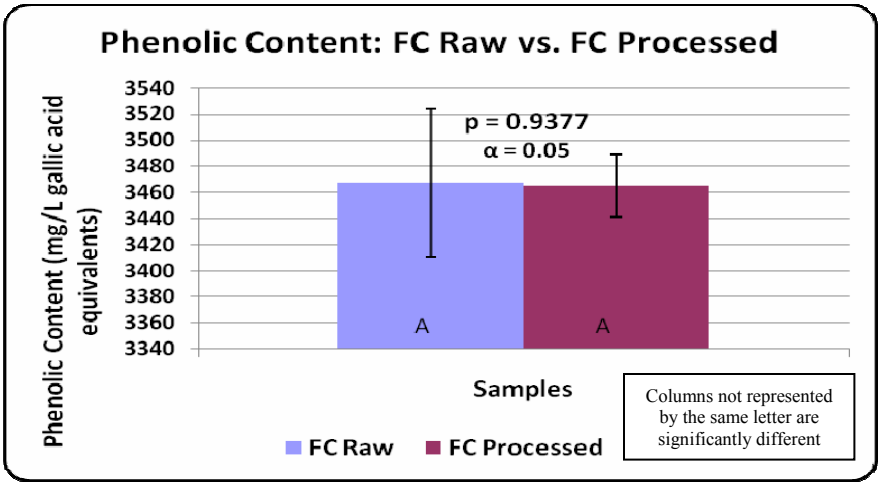


Figure 17: Phenolic Content: FC Raw vs. FC Processed

The data revealed a statistically significant 35% difference in total phenolic content between the raw NFC and FC juice samples (Figure 15). When evaluating the pre- and post-beverage process data, a significant decrease of 6% was seen in the NFC juice (Figure 16). The FC samples showed no significant further decrease in phenolic content as a result of the beverage processes (Figure 17).

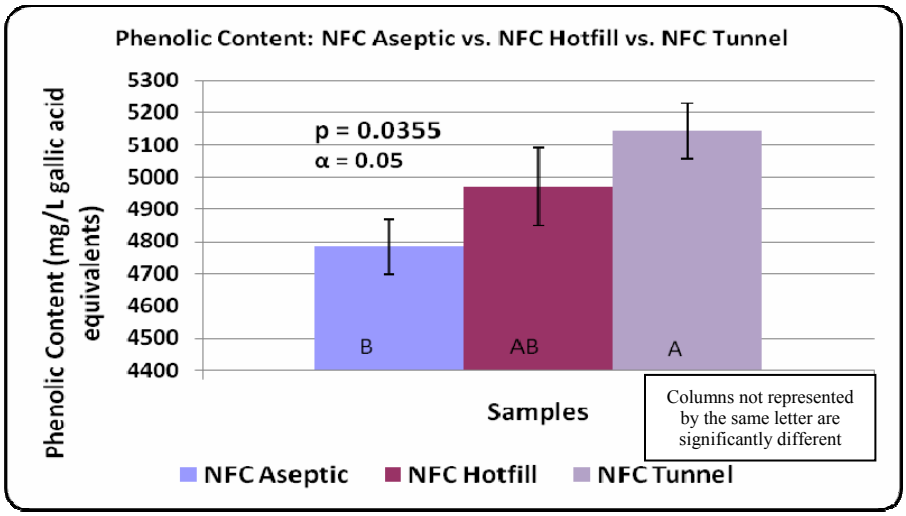


Figure 18: Phenolic Content: NFC Aseptic vs. NFC Hotfill vs. NFC Tunnel

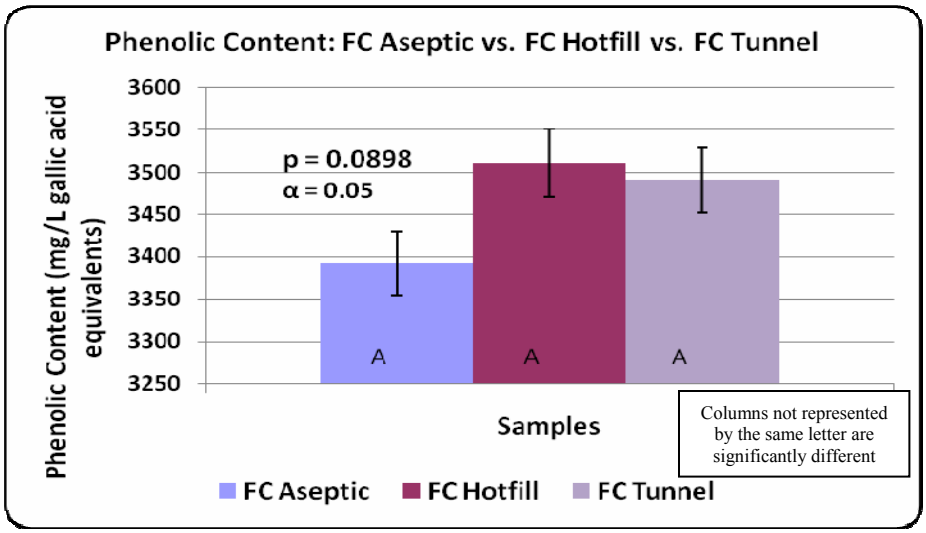


Figure 19: Phenolic Content: FC Aseptic vs. FC Hotfill vs. FC Tunnel

Evaluation of differences due to variation of beverage level process showed a significant difference in phenolic content between NFC aseptic product and NFC tunnel product such that the aseptic product registered the lowest phenolic content, with the hotfill product not significantly different from either (Figure 18). There was no

significant difference across beverage processes for the FC products (Figure 19). ANOVA analysis of the phenolic content data is shown in Appendix 2.

The standard 8oz quantity representative of a commercial product serving size would deliver a phenolic content as described in Table 7 below:

Phenolics (mg gallic acid)	
per 8oz serving	
NFC Tunnel	1,217
NFC Hotfill	1,176
NFC Aseptic	1,147
FC Tunnel	826
FC Hotfill	833
FC Aseptic	803

Table 7: Phenolic Content Value per 8oz Serving

V.C. Anthocyanin Content

The results showed that the anthocyanin degradation due to thermal process was the most extreme of the classes of compound tested. Even so, the trend of degradation was the same as that for overall anthocyanins and total phenolics with one exception. Concentration provided the harshest initial degradation, with bottling processes significantly degrading the anthocyanins in both the NFC and FC juice, as opposed to inducing further significant degradation in NFC only, as was seen with the previous classes of compound.

Anthocyanins (mg cyanidine-3-glucoside/L)		
Sample	**NFC**	**FC**
"Raw" Juice	2,460.76	494.21
Tunnel	1,768.71	246.61
Hotfill	1,817.42	241.535
Aseptic	1,789.01	235.45

Table 8: Mean Anthocyanin Content at Each Level of Process.

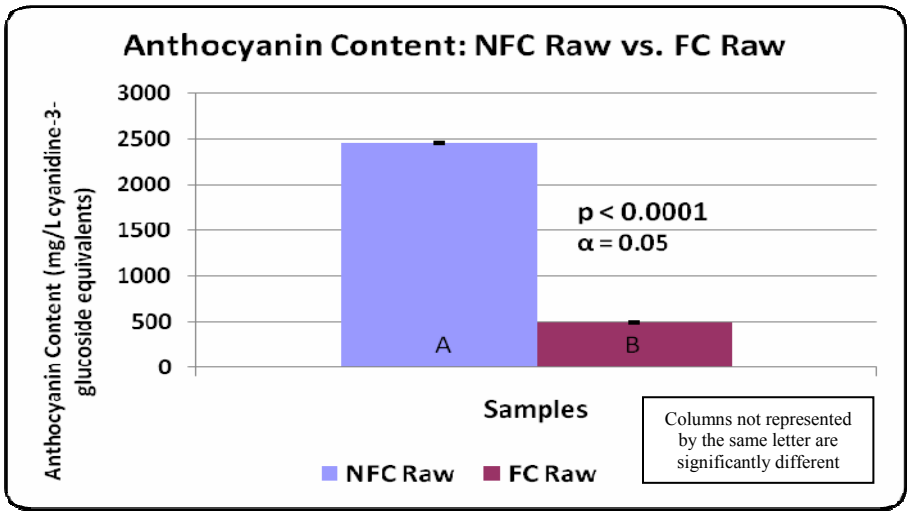


Figure 20: Anthocyanin Content: NFC Raw vs. FC Raw

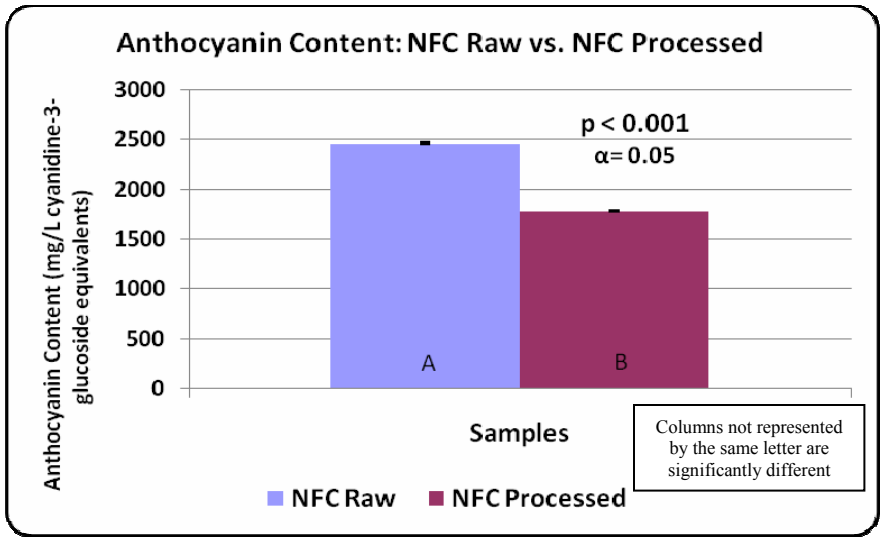


Figure 21: Anthocyanin Content: NFC Raw vs. NFC Processed

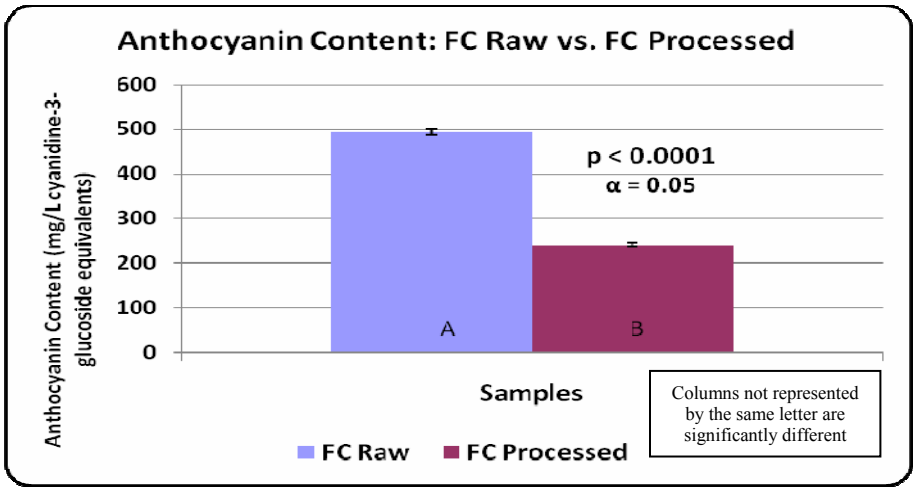


Figure 22: Anthocyanin Content: FC Raw vs. FC Processed

Juice that underwent the concentration process exhibited a massive 80% reduction in anthocyanin levels as compared to juice that was flash pasteurized (Figure 20). The NFC juice exhibited a 27% loss in anthocyanin content when exposed to the beverage level processes (Figure 21) while the FC juice showed a further 67% degradation through this step (Figure 22).

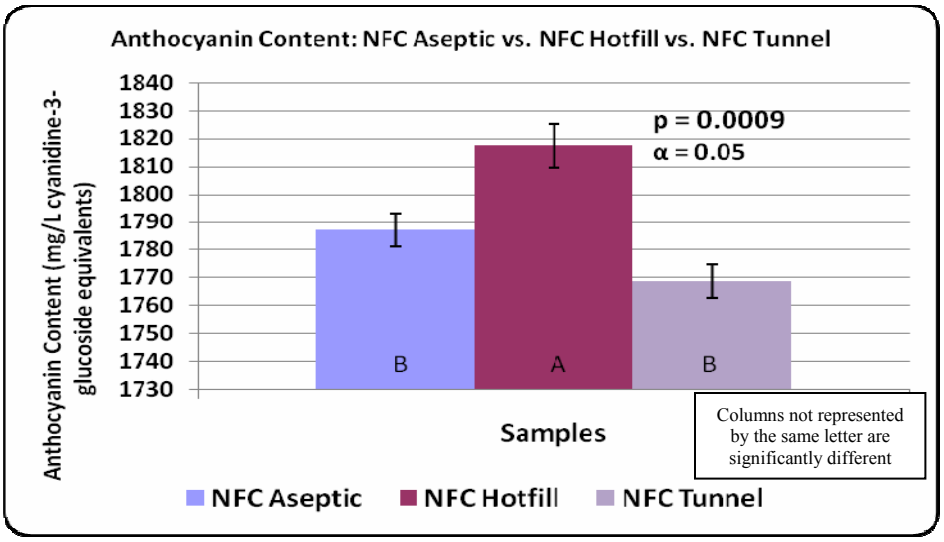


Figure 23: Anthocyanin Content: NFC Aseptic vs. NFC Hotfill vs. NFC Tunnel

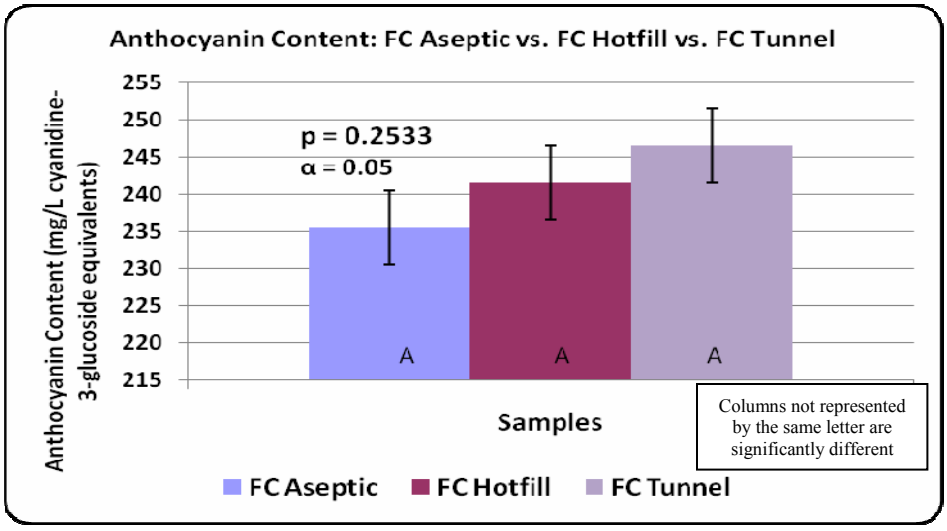


Figure 24: Anthocyanin Content: FC Aseptic vs. FC Hotfill vs. FC Tunnel

In comparing the three beverage processes, statistical analysis showed no difference in anthocyanin retention across these processes in the FC juice samples (Figure 23). A statistically significant difference was found for the hotfilled NFC juice, which exhibited higher retention of anthocyanins than the aseptic and tunnel pasteurized samples (Figure 24). ANOVA Analysis of the anthocyanin content data is shown in Appendix 3.

Once again taking into account the standard commercial beverage serving size, the anthocyanin contents delivered per 8 ounces of juice is expressed in Table 9.

Anthocyanins (mg cyanidine-3-glucoside)	
per 8oz serving	
NFC Tunnel	418
NFC Hotfill	423
NFC Aseptic	320
FC Tunnel	58
FC Hotfill	57
FC Aseptic	56

Table 9: Anthocyanin Content Values per 8oz Serving

VI. DISCUSSION

The results of this study confirm a clear negative impact of thermal process on the antioxidant components in blackcurrant juice. This is to be expected overall due to the heat sensitive nature of the compounds. Two aspects of the analytical results, however, provide interesting insight into how these desirable compounds behave throughout the manufacturing process.

The first aspect of particular interest is the range of degradation rates across the classes of compound. As the classes of compounds studied are related to one another, the rates of degradation are also related. Anthocyanins are phenolic compounds, and therefore were part of the total phenolic content measured. As such, the 35% difference in total phenolics observed in comparing NFC and FC raw juice includes the 80% reduction in anthocyanins observed by that assay. Similarly, the 50% change in overall antioxidant capacity includes the antioxidant capacity being contributed by the phenolics in the juice. The differing rates of degradation clearly indicate a non uniform response of antioxidant compounds to thermal abuse. The data indicates that of the phenolic compounds present in blackcurrant juice, the non anthocyanin compounds are more robust as the decrease in phenolics of 35% when looking at the total leaps to 80% when evaluating anthocyanins alone. This is demonstrated conversely when evaluating the change in overall antioxidant capacity to that of total phenolics. The 35% decrease in total phenolics comprises a portion of the 50% reduction in overall antioxidant capacity reflected in the ORAC scores. This would indicate the presence of non-phenolic antioxidants that are less robust than the phenolics in the juice. Previous research has

shown that total phenolic content does not bear a linear relationship with antioxidant capacity (Wu, et al., 2004; Moyer, et al., 2002) and it has been hypothesized that the high content of ascorbic acid in blackcurrant juice could be a contributing factor in that matrix (Moyer, et al., 2002). Ascorbic acid would be a good fit for this profile as a contributor to the higher rate of loss in overall antioxidant capacity as it is extremely heat labile and is known to be a prominent antioxidant in blackcurrant juice (Miller & Rice-Evans, 1997). Additionally, Miller and Rice-Evans (1997) suggest a major unidentified antioxidant in blackcurrant juice which, if phenolic could be contributing to the higher retention of phenolic content as compared to anthocyanin content.

Also of particular interest is the increased vulnerability of NFC juice to further degradation by beverage level processes. The retention of all classes of compounds in the NFC juice as compared to FC juice was significant. When the juice was further processed, however, it was only the NFC juice that exhibited a significant reduction in overall antioxidant capacity and total phenolic content with significant reductions in anthocyanin content being observed for both types of raw juice. This could suggest that there is a threshold of degradation that is vulnerable to the thermal processes to which the juice is subjected. It is possible that, in the FC juice samples, the most vulnerable compounds had already been degraded through the relatively harsh concentration process, therefore leaving only more robust compounds to undergo further thermal abuse during bottling. On the other hand, these more delicate compounds may have been retained in the less abused NFC juice. When the NFC juice was exposed to the thermal abuse of bottling, some of these more vulnerable compounds were degraded resulting in

the statistically significant reductions in phenolic and anthocyanin contents, and overall antioxidant capacity.

Interesting further study would be an investigation of the profile of the juice at each step in the process, including fresh pressed juice obtained at the source. A detailed chemical profile at each level of process could indicate which specific compounds were most able to withstand the thermal processes during production. Research has been done to investigate anthocyanin or antioxidant capacity of specific varietals of blackcurrant juice (Moyer, et al., 2002; Siksnianas, et al., 2006), although overall content was investigated. A thorough understanding of key, robust compounds as well as what varietals contain these compounds in the greatest concentrations could allow growers to select for berries that would provide the highest antioxidant benefit to consumers post production.

The beverage scale processes used in this study were chosen as these are the most commonly used pasteurization methods in the juice industry. The bench scale adaptations were designed to mimic full scale production as closely as possible. All three processes tend toward being slightly more heat abusive than actual production scale, however, the aseptic process differs most from bench to commercial scale. The results indicated significant differences between bench scale processes, in some cases indicating a lower level of retention in the aseptically processed samples. This result is intriguing given the lower degree of heat abuse in the aseptic process. One possible explanation for this is scalability issues between bench and commercial production, for instance residual sterilant in bottle on bench scale, which could be verified by analyzing samples produced on commercial scale. Overall, the differences between benchtop productions, though

statistically significant, pale in comparison to the difference caused by the industrial process. In terms of product development, the major factor for delivery of an efficacious antioxidant level will be decided by the industrial processes of flash pasteurization versus concentration.

An understanding of the antioxidant capacity of a beverage must be coupled with an understanding of the bioavailability of the antioxidant compounds within that beverage in order to deliver a beneficial product to consumers. Some work has been done to date indicating that antioxidants, including anthocyanins and other phenols, in juices are both bioavailable and functional in humans. Anthocyanins from blackcurrant juice were detected in urine from human subjects unchanged (0.020-0.050%) over a period of 5 hours (Netzel, et al., 2001). This study is unclear as to the fate of the remainder of ingested anthocyanins, hypothesizing a range of possibilities including decomposition in the lumen or elimination in the feces. A study investigating flavonoids in human plasma, however, was able to demonstrate absorption as glycosides (Paganga & Rice-Evans, 1996). A subsequent plasma study has shown both absorption and functionality of berry juice anthocyanins. A 30% increase in plasma antioxidant capacity was seen coupled with an 18% decrease in plasma MDA (an indicator of lipid peroxidation) after juice intake (Netzel, et al., 2002).

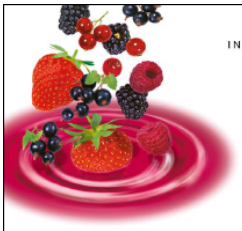
The juice administered in the Netzel plasma study delivered a TEAC value of 16.4 mmol/L Trolox equivalents, a phenolic content of 2,470 mg/L gallic acid equivalents and an anthocyanin content of 415 mg/L (Netzel, et al., 2002). As compared to the findings here, the overall antioxidant capacity delivered in the Netzel study is about half of that seen in the FC blackcurrant juice and one quarter of that seen in the NFC. The phenolic

content in the Netzel study is slightly lower than what is seen for FC juice here, and roughly half the content seen in NFC juice. The anthocyanin content Netzel used was roughly twice as high as the FC, and one quarter of the level found in the NFC blackcurrant juice studied here. Based on this data FC blackcurrant juice, although providing much lower levels of all quantities of compound as compared to NFC, would provide a sufficient level of antioxidant capacity and phenolic content for uptake in plasma as well as a measure of efficacy.

Further study into the bioavailability and functionality of antioxidant compounds delivered orally through juice sources would be helpful in generating a comprehensive picture of ideal "dose" amounts. In addition, this data would need to be coupled with clinical data on efficacious levels required to deliver specific health benefits. Some key questions to be answered would be: "Is there a maximum useful level of these compounds?" "What is the minimum level required to achieve effects *in vivo*?" and "Are there synergies that effect absorption and efficacy?" An understanding these thresholds coupled with a thorough knowledge of juice composition will allow industry to deliver an optimized product that is beneficial to the consumer while remaining commercially viable to the manufacturer.

VII. APPENDICES

VII.A. Appendix 1: Specification for Iprona NFC Blackcurrant Juice



INDUSTRIELLE PRODUKTION NATÜRLICHER LEBENSMITTEL INDUSTRIA PRODOTTI NATURALI PER ALIMENTI

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Ges. Reg. Landesgericht / Reg. Soc, Tribunale BZ 3488

MWST/IVA/AT IT 00207010216

PRODUCT SPECIFICATION

70140000

BLACK CURRANT

CLOUDY JUICE

BX 13

SENSORY PROPERTIES - PRODUCT PROPERTIES

COLOUR:	red
APPEARANCE:	uniform liquid, cloudy
TASTE:	characteristic for black currant

CHEMICAL VALUES

Soluble solids refract (20°C) Brix		11 -	15
pH - value		2,5 -	3,3
Acidity exp. as citric acid (anhydr.) (pH 8,1)	g / kg	21 -	35

MICROBIOLOGICAL VALUES

Yeast	cfu / g	0
Mould	cfu / g	0
Total viable count	cfu / g	< 100

PACKAGING
Aseptically packed in: cartons of 20 l / buckets of 20 l / drums of 200 l

STORAGE
Microbiological shelf life for unopened package: 12 months
Recommended storage temperature: 0/+4°C.
Colour may change at the end of shelf life.
Storage in frozen conditions (-18°C) avoids colour changes.
If package is opened use immediately or freeze at -18°C.
A short storage of the unopened package at ambient temperatures for transport is permissible.

Date : 11.12.2006

Laboratory of Quality Control

QM-System Iprona ISO 9001:2000

VII.B. Appendix 2: Specification for Iprona Blackcurrant Juice Concentrate

IPRONA AG/SPA Update: 29.09.00

PRODUCT SPECIFICATION

BLACKCURRANT

CLEAR FRUIT CONCENTRATE
Bx 65
Code 70140002

A) PRODUCT PROPERTIES - SENSORY PROPERTIES

COLOUR:	intensive red
APPEARANCE:	viscous uniform liquid, clear
TASTE:	characteristic for blackcurrant

B) CHEMICAL VALUES OF CONCENTRATE

Soluble solids refract (20°C) Brix		64 -	66
Density		1,34	
Concentration factor		5,9	
pH - value		2,3 -	2,9
Total acids as citric acid (anhydr.) pH=8,10	g/kg	155 -	185

C) MICROBIOLOGICAL VALUES

Yeast		0 /g
Mould		0 /g
Total viable count	<	100 /g

D) PACKAGING

Aseptically packed in drums of 200 l or in cartons of 20 l.

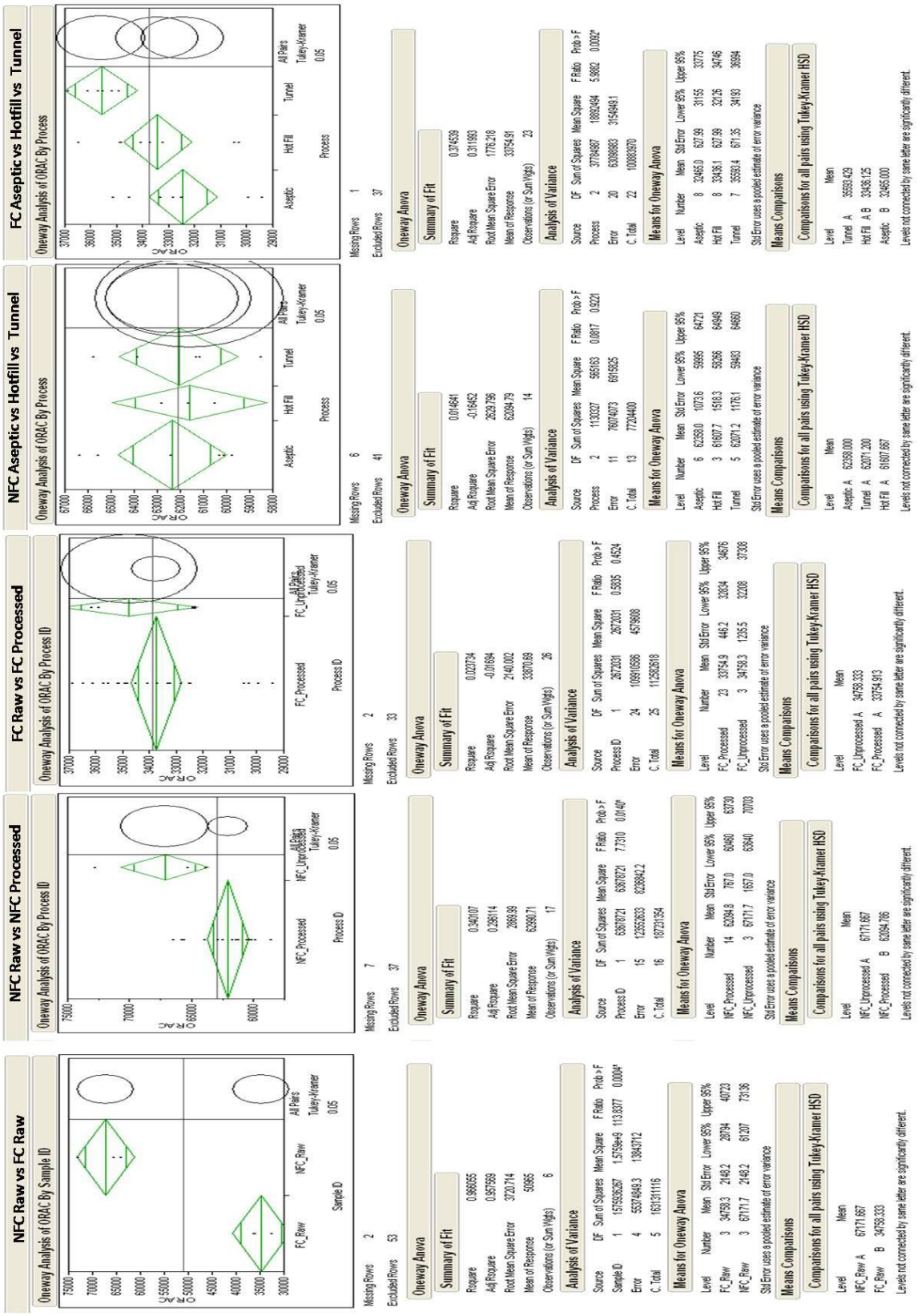
E) STORAGE

Store the product under cool conditions (below 5 degrees Celsius). A shelf-life of up to 12 months is then possible. At the end of maximum durability colouring may change. If a package is opened, use immediately or store at -18/25°C.

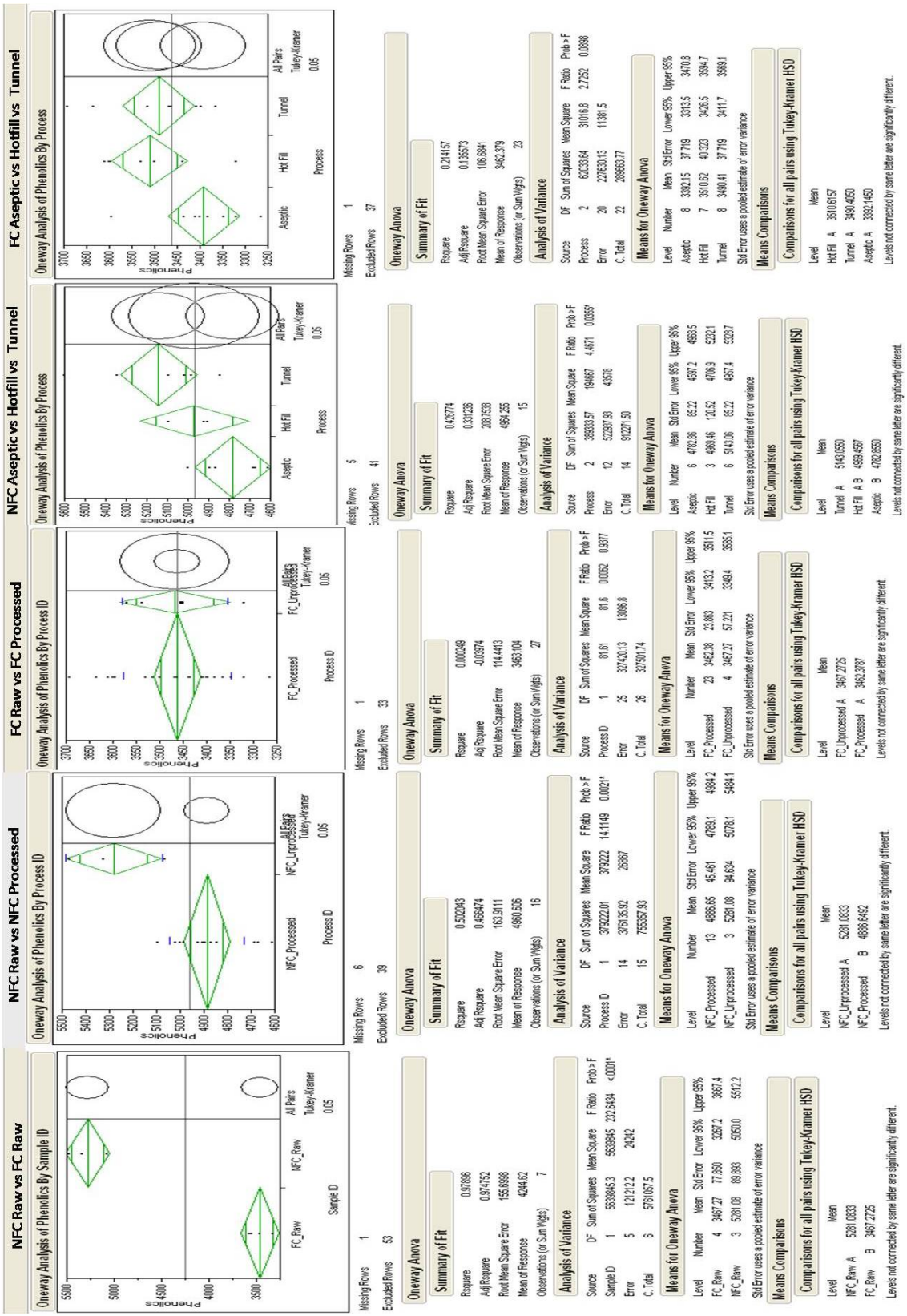
Laboratory of Quality Control
(Dr.Köllemann)

Date : 17.01.05

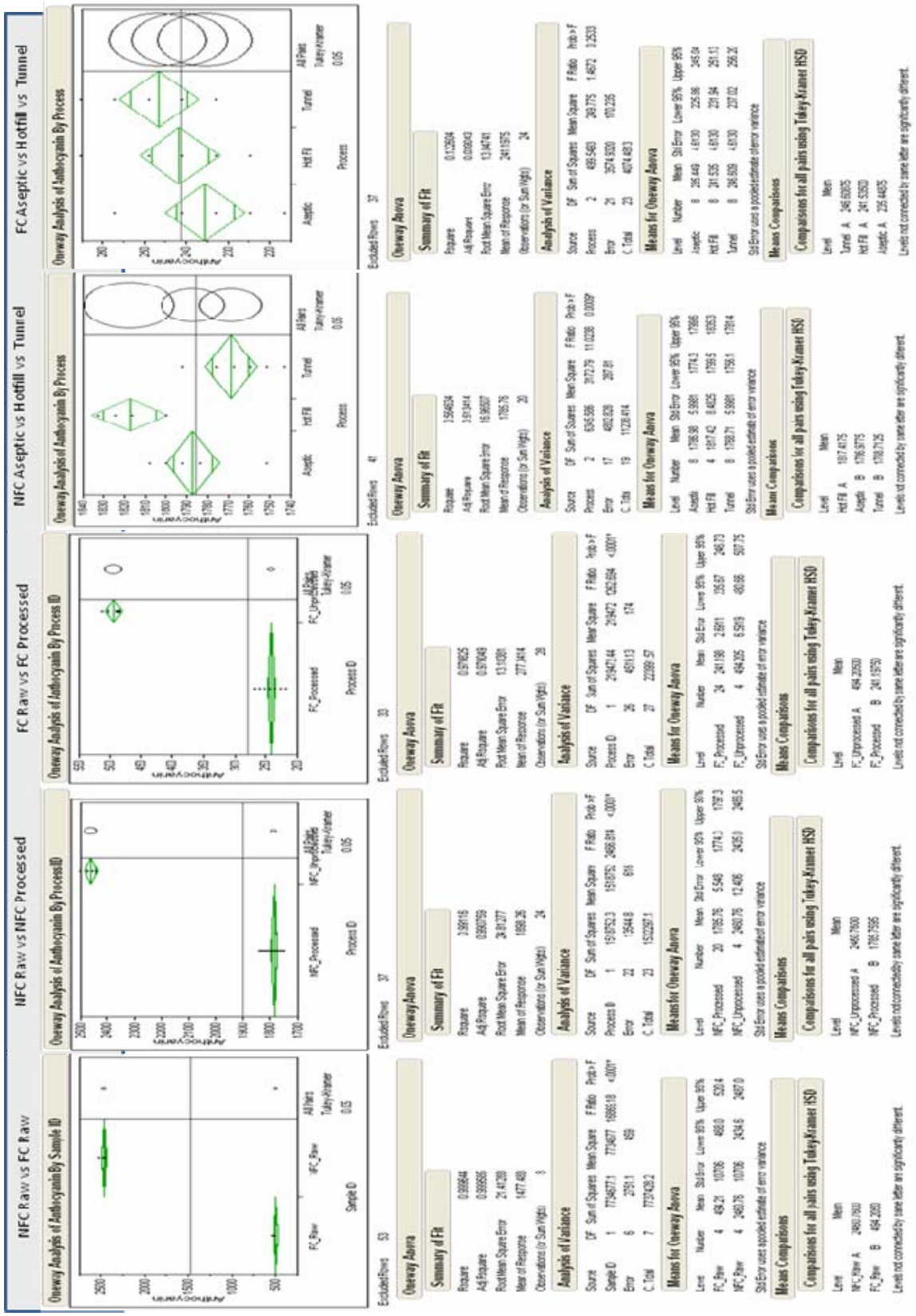
VII.C. Appendix 3: ANOVA Analysis of ORAC Data



VII.D. Appendix 4: ANOVA Analysis of Phenolic Content Data



VII.E. Appendix 5: ANOVA Analysis of Anthocyanin Content Data



VII.F. Appendix 6: Source Data for ANOVA Analyses

Type	Process	Sample ID	Process ID	Lab ID	ORAC	Phenolics	Anthocyanin
FC	Unprocessed	FC_Raw	FC_Unprocessed	Jul-53	35850	3316.84	500.29
FC	Unprocessed	FC_Raw	FC_Unprocessed	Jul-53	36115	3535.7	484.06
FC	Unprocessed	FC_Raw	FC_Unprocessed	Jul-53	32310	3448.77	484.06
FC	Unprocessed	FC_Raw	FC_Unprocessed	Jul-53	.	3567.78	508.41
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824A	35121	3404.13	248.64
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824A	35679	3632.34	256.76
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824A	35147	3511.86	232.4
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824A	.	3395.73	240.52
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824B	36804	3688.91	248.64
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824B	36069	3363.17	248.64
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824B	34850	3461.15	232.4
FC	Tunnel	FC_Tunnel	FC_Processed	07-3824B	35484	3465.95	264.87
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825A	30410	3606.98	264.87
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825A	33177	3527.01	240.52
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825A	34767	3593.78	248.64
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825A	33646	.	248.64
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825B	34005	3497.76	240.52
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825B	34831	3597.23	232.4
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825B	36404	3433.84	232.4
FC	Hot Fill	FC_Hot Fill	FC_Processed	07-3825B	30249	3317.71	224.29
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826A	33280	3433.39	256.76
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826A	32454	3591.38	240.52
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826A	29340	3398.73	224.29
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826A	29874	3317.71	232.4
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826B	34478	3380.72	216.17
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826B	35082	3408.03	256.76
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826B	32442	3344.11	224.29
FC	Aseptic	FC_Aseptic	FC_Processed	07-3826B	32770	3263.09	232.4
NFC	Unprocessed	NFC_Raw	NFC_Unprocessed	Jul-53	65167	5060.09	2432.35
NFC	Unprocessed	NFC_Raw	NFC_Unprocessed	Jul-53	72664	5320.17	2464.82
NFC	Unprocessed	NFC_Raw	NFC_Unprocessed	Jul-53	63684	5462.99	2448.58
NFC	Unprocessed	NFC_Raw	NFC_Unprocessed	Jul-53	.	.	2497.29
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827A	63817	5341.46	1774.8
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827A	61269	5595.93	1750.45
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827A	.	5090.59	1791.04
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827A	.	.	1766.68
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827B	58464	4838.22	1742.33
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827B	61104	4979.56	1774.8
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827B	65702	5012.57	1791.04
NFC	Tunnel	NFC_Tunnel	NFC_Processed	07-3827B	.	.	1758.56
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828A	60431	5087.89	1799.15
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828A	61624	4901.54	1823.51
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828A	62768	4918.94	1831.62
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828A	.	.	1815.39
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B*	32990	2879.3	938.66
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B*	34033	2845.95	946.78
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B*	31382	2587.41	954.89
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B*	29550	2742.9	938.66
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B	.	2607.71	.
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B	.	2879.3	.
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B	.	2775.37	.
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B	.	2703.89	.
NFC	Hot Fill	NFC_Hot Fill	NFC_Processed	07-3828B	.	2602.91	.
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829A	59690	4666.57	1782.92
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829A	60058	4995.16	1782.92
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829A	62327	4887.73	1791.04
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829A	.	.	1799.15
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829B	60243	4678.28	1750.45
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829B	65506	4862.53	1799.15
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829B	66324	4606.86	1782.92
NFC	Aseptic	NFC_Aseptic	NFC_Processed	07-3829B	.	.	1807.27

*Note: Sample 07-3828B was excluded from the data set. Sample was diluted during processing, and data is unreliable.

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IX. CURRICULUM VITA

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1997 – 2001	Iona College Bachelor of Science, Biology	New Rochelle, NY
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