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QUALITY OF TART CHERRY NUTRACEUTICAL JUICE: A COMPARISON OF JUICE PACKAGED IN FLEXIBLE POUCHES AND BOTTLES MADE FROM GLASS, PET AND ALUMINUM

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QUALITY OF TART CHERRY NUTRACEUTICAL JUICE: A COMPARISON OF JUICE PACKAGED IN FLEXIBLE POUCHES AND BOTTLES MADE FROM GLASS, PET AND ALUMINUM

By

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ABSTRACT

QUALITY OF TART CHERRY NUTRACEUTICAL JUICE: A COMPARISON OF JUICE PACKAGED IN FLEXIBLE POUCHES AND BOTTLES MADE FROM GLASS, PET AND ALUMINUM

By

Maria-Paz Gonzalez-Mulet

The main objective of this research is to develop a protective, convenient and consumer oriented product for the cherry industry: a pasteurized single-strength tart cherry juice made from concentrate, which was hot filled in four different commercially available packages with nitrogen flushed headspace: composite stand-up pouches with a multilayer structure (Pet/Al/LDPE) and bottles made from glass, aluminum and PET.

A more specific aim is to analyze the stability of anthocyanins and other key properties over 12-months of storage under controlled ambient conditions (23°C) and 50% RH, without light exposure. Gas liquid chromatography-mass spectrometry was used to measure the anthocyanin content over time. Analyses were likewise conducted monthly for color, bacteria, yeast and mold, solids and pH.

The results show that the tart cherry juice can be pasteurized and packed for 12 months in two different packages without suffering a dramatic anthocyanin loss glass as expected and aluminum. Aluminum bottles performed nearly as well as glass. With good gas and moisture barriers and good antioxidant retention, aluminum bottles are becoming more widely available for processors, and aluminum

Bottles are recloseable and easily recycled. One issue that did arise with the trained sensory panelist was an off-flavor that developed in the juice in aluminum bottles after six months. The performance of the aluminum bottles was dependent on the constitution of the inner lining which, if compromised, could cause chelating in the juice with the resulting off-flavor. The plastic bottles in the test performed the least well compared to glass and aluminum. The plastic bottles showed a loss of 50 percentage of the antioxidants in the juice after six months. Plastic is readily available to processors, but again, as generic packages that are easily reclosed and are recyclable.

The performance of the stand up pouches was not evaluated through all the 12 months since the packages failed during the fill stage of the project.

Results were favorable for the tart cherry juice industry. From the consumers' points of view results show a statistically significant preference for the 12 month-stored juice packed in glass and aluminum bottles compared to fresh juice.

DEDICATION

To my parents: Jorge and Mireille
To my husband: Luis
And my daughter Cloe
They have always believed in me.

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I. INTRODUCTION

Historically the United States (U.S.) tart cherry supply was provided primarily by growers located in areas bordering the Great Lakes. The Michigan "fruit-belt" encompasses the state's western coast where Lake Michigan creates a micro-climate suitable for fruit production. Tart cherries are a very important fruit crop to the Michigan economy. Currently, tart cherry production in Michigan represents approximately seventy percent of the total tart cherry production of the United States (Cherry Marketing Institute 2003). Primary producing counties include Leelanau, Oceana, Grand Traverse, Antrim, and Mason, all located along Michigan's west coast (Figure 1). Tart cherry production decreased 30 percent from 2005 to 2008 when the production went from 190 million tons in 2005 to 135 million tons in 2008 (USDA/NASS 2008).

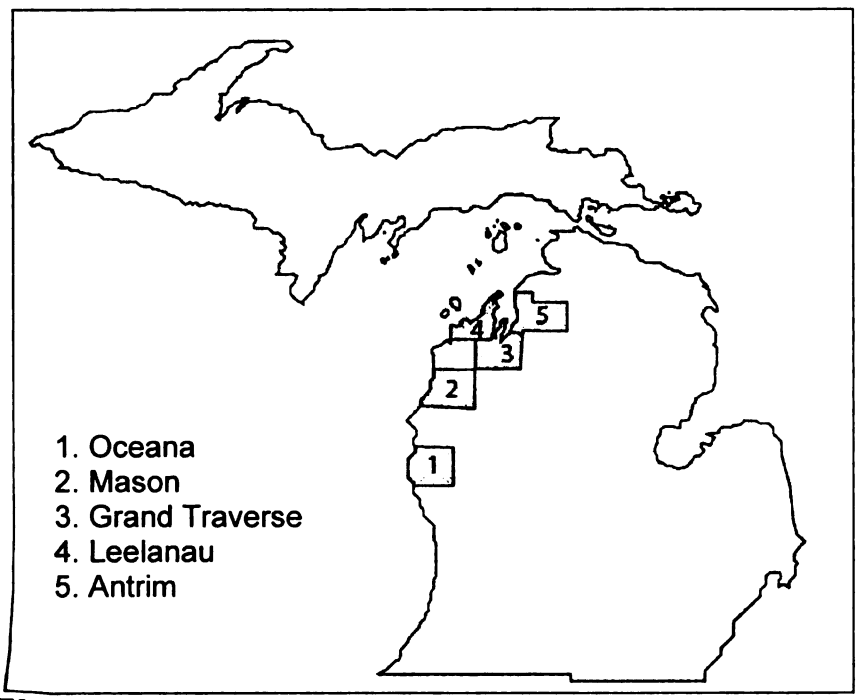

Figure 1. Map of primary producing counties in Michigan, Adapted from Geocities.com.

Among the primary producing states (Michigan, New York, Pennsylvania and Wisconsin), Michigan has been the largest producer, and the price of cherries is greatly determined by the Michigan output during each growing season. For instance, prices go up in years when Michigan has a short crop, while prices can go significantly down in years of bountiful harvests. The economics behind this phenomenon are partially explained by the fact that cherries only account for a small percentage of the overall cost of manufactured food products that includes cherries as one of their many ingredients. Therefore, buyers are willing to pay more per pound of cherries during short crop years as this will not significantly affect the final cost of their products. On the other hand, when output increases, prices fall because there is little demand for cherries other than what is purchased for use in manufactured food products. Furthermore, the knowledge of the economics during short crops following years of large crops have created some reluctance in the manufacturing and retail sector to invest in greater numbers of cherry-based products as shortages of main ingredients can become a problem in satisfying the created demand for new products (USDA 1996).

The industry's economic behavior has led to government-issued Marketing Orders. Marketing Orders can have different objectives in support of the industry. Marketing orders exist in the U.S. for products such as milk, walnuts and certain fruits and vegetables. These orders can be state-specific or federal. In the case of the Tart Cherry Marketing Order, the organized cherry production and

marketing industry is federally authorized (USDA 2001) to harvest only a percentage of the total production during large crops. The percentage harvested is defined as utilized production, and it is calculated by the USDA each year. In years when the estimated total harvest will be larger than the estimated utilized production 20 percent or more of total production may remain unharvested. In years with small crops all the cherries are harvested and utilized. Finally, another factor influencing tart cherry supply and price are the carry-over stocks of frozen or canned cherries from previous seasons.

More recently tart cherry supply has shifted to include greater global participation (Zepp et al 1996). Countries such as Poland, Turkey and Hungary are producing tart cherries as well, (Morello varieties principally) with similar qualities to those produced in Michigan. These countries have increased their world share with more global exports to satisfy the demand (Thornsbury 2005). Increased global competition in tart cherry production has further increased price instability since about twenty percent of the crop is sold to foreign buyers.

Bad weather in Michigan has also contributed to the strength of its competitors by widening their market windows of opportunity. For example, when the 2002 production was lost due to frost during the spring, Michigan processors had to import from other states and countries, giving competitors the opportunity to establish new and stronger market relationships with typical Michigan buyers in state, and around the world (Rowley 2005).

Currently, Michigan tart cherries (Montmorency variety) are marketed primarily in five different forms: (1) frozen, (2) canned, (3) dried, (4) fresh (in a

small window of three to four weeks in July), and (5) concentrated juice (Michigan Cherry Committee 2003). Tart cherries are mostly processed, leaving less than 0.3 % for the fresh market (Table 1). All five products are based on minimum value added characteristics with no significant differentiation among competitors.

Table 1. Processed and fresh tart cherry output.

	Processed tart cherries Million pounds	Fresh tart cherries Million pounds
2005	207.5	0.5
2006	179.8	0.5
2007	192.5	0.5

Source: USDA/NASS, Non citrus and Nuts fruit report, 2007, preliminary summary.

Demand for frozen cherries depends on the form. Individually quick frozen (IQF) fruit is mostly used for pies, pastries and fruit preserves. In processed form the most stable quantity of IQF tart cherries has been produced since 1997. The demand for the second form, "5+1" (fruit packed in sugar)[1], has declined partially due to the lower quality of the fruit and to the large amount of sugar added.

Demand for canned tart cherries has also declined. This is due, at least in part, to health concerns regarding the high sugar content, fewer people cooking at home, and the shortage in production during the previous three years. The canned sector is divided into two different packages: Water pack in institutional #10 cans (105 ounces), and supermarket can size (14 to 16 ounces). The large cans are mainly exported to Europe and Asia (Facer 2005). Table two shows the price changes in processed cherries.

[1] "5+1 products conversion factor is 33.33 pounds of fresh tart cherries for 5+1 fruit-to-sugar ratio calculated by multiplying finished weight by 1.11 to determine RPE". (Cherry Marketing Institute Statistical Handbook, 2003). RPE is the acronym for Raw Product Equivalent in the Code of Regulations of the U.S. government.

Table 2. Price of processed tart cherry per pound in U.S. Dollars.

		Price of processed tart cherries $/lb		
	Processed in Million pounds from 2005-2007	2005	2006	2007
Canned	51.0	0.240	0.160	0.260
Frozen	146.0	0.230	0.210	0.260
Other	10.5	0.141	0.153	0.161

Source: USDA/NASS, Non citrus and Nuts fruit report, 2007, preliminary summary.

Dried cherries and juice concentrate are the newest product forms. Growth in sales for both has been limited by inconsistent quality standards, packaging, labeling, and marketing strategies towards end-users. These products represent a great opportunity for growth, given the right marketing mix since they are new concepts that appear to have great potential.

Dried cherry consumption has remained stable because most sales are made in bulk through the USDA school lunch program. Sales through the USDA results in a very low commodity price because large amounts are ordered. The price has declined, from $10.00 to $4.00 per pound during the last five years (Nugent et al 2005).

Tart cherry juice is sold almost exclusively in concentrate form, and then only in limited geographic areas and/or in large industrial-size quantities. This segment has also declined, from 250 million gallons in 2001 to 160 million gallons in 2003, and supply is influenced by low volume crops in 2002 and the Marketing Order that influenced output in the subsequent years. The price of dried cherries and concentrate juice, represented in Table 2 as "others". These

products had a more steady and regular price growth than the canned and frozen categories, due principally to their novelty.

But product awareness is growing among consumers as the tart cherry's antioxidant properties have become better understood. Changes in consumer life styles, the growth of new healthy foods, and alternatives to conventional medicine have increased the pattern of demand for traditional tart cherry products.

As a result, tart cherry processors are looking for new tart cherry value added products to satisfy this growing potential demand. As in many other agricultural industries, marketing and economic changes have increased pressure on the Michigan fruit sector. First, demand for more traditional products or commodities has lagged behind increases in output, which makes the demand for tart cherries elastic; this means that grower prices rise sharply during years of a small crop, and fall sharply in years with a large crop (USDA 1996). Increasing long-run returns are necessary to cover additional risks for Michigan producers.

In light of these growing challenges, new strategies are needed to increase the sustainability and profitability of agriculture in Michigan's rural economy (Nykiel 2003). Industry strategies to capitalize on consumer demand trends must incorporate science-based decision-making tools (Day et al 1989; Karipidis and Galanopoulos 2000). Developing new products for new segments and markets, and creating value added for commodities are essential steps to minimize risks for the industry. Best (2004) defines innovation and value added products to include every new product in the category with different presentation,

size, and concept that will suit the expectations of the consumers and potential buyers.

When developing a new product many steps are required, in order to minimize the launching risks. The industry needs to evaluate every single step involved in developing a new product to make sure they will be able to deliver a constantly high quality product. Adelaja (2004) presented at the Cherry Marketing Institute a summary of steps to commercialization, adapted in Figure 2. It is very important to understand the raw material first, in order to add value to the product and the supply chain. Then manufacturing processes must be considered because they will transform the product into the desired value added product. Without budgets, loans or cash, investment in equipment limits product development. Simultaneously it is necessary to understand the market targets and trends to indentify the correct market niche. These factors are inter-related when developing a new product.

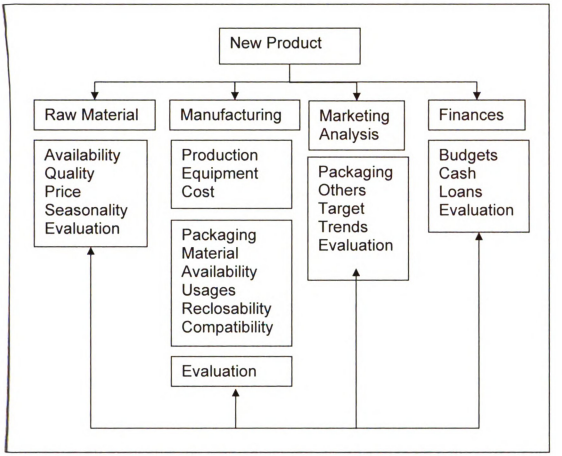

Figure 2. Summary of steps needed when developing a new product
Adapted from Adelaja, 2004

This research focused on the packaging component of the process. It looked for available materials in the area at a reasonable price, evaluating the possible equipment the tart cherry industry has in Michigan without major investments, minimizing the need for loans or expenditure of cash. This research evaluated the compatibility of the package with the tart cherry juice to give information to the tart cherry industry on how to add value to a new product: single strength tart cherry juice. Finally, and the uniqueness of this research was to be a key link between academia and industry focusing on creating a project that could be replicable at the industry level and leaving successfully the lab

benches under the academia settings. This link is important to establish and reinforce both points of view in order to maximize the efforts and the cooperation between both sectors.

Lack of consumer focus in the existing marketing mix of the tart cherry industry has severely limited its long-run sustainability. In an industry that has been primarily oriented towards bulk commodity sales, the packaging, supply chain and logistical requirements will likely change substantially with a focus on end-use purchasers (Hobbs 2002).

Packaging can be used to develop new presentations and sizes, and minimize the risk of failure during the introduction phase in new and different categories by satisfying specific consumer requirements. Therefore, packaging has the potential to provide a valuable contribution to Michigan's agricultural strategies by moving away from commodity trade into more specialized markets and value added products.

The industry is in the process of repositioning tart cherry concentrate from the beverage commodity market to the consumer market for functional foods. This is a strategy that the tart cherry industry has begun to explore based on findings that cherries provide health benefits to human beings (Wang et al 1999). One emerging area of interest is single-strength tart cherry juice and juice from concentrate.

Several studies at Michigan State University and other institutes have found that tart cherries have a high concentration of antioxidants and other components that benefit those suffering from arthritis, chronic pain and cancer

(Chaovanalikit 2004; Raloff 1999). Most beneficial are the anthocyanins, the pigments responsible for the red and blue colors of many fruits and vegetables, which are found in high concentrations in tart cherries.

Repositioning tart cherry concentrate or juice poses a number of challenges to the tart cherry industry. There is a lack of market information and benchmark retail price-points. The supply chain and marketing efforts are insufficient. Most of the packaging is poor, with a lack of quality control, standards, tamper evidence and ease of use. Without a consumer focused product, including the necessary packaging and marketing mix, the antioxidant value that has been identified will not be transformed into sales value-added for the industry.

There is a need to develop a packaged tart cherry juice product that is shelf-stable. To preserve the health benefits, the juice needs to be protected from oxidation which is exacerbated by UV light and from sorption of the pigments and anthocyanins by the packaging material. The packaging material should not interact with the juice, and it should preserve the juice's health benefits, flavor and perceived quality.

The goal of this research is to characterize the change in juice quality over time in four different containers: bottles made from glass, aluminum and PET and stand-up pouches. The factors to be evaluated are: anthocyanin content, color, flavor, microbial activity, pH levels and Brix degrees. The objective is to identify the best packaging material to retain the quality and health benefits of the tart

cherry juice. The most common deteriorative mechanisms are oxidation and flavor scalping.

This research will provide a benchmark for future reference and development of other tart cherry products. Stability of the tart cherry juice, hot filled, in bottles made from glass, PET and aluminum, as well as PET/aluminum/PE pouches will be evaluated. Two working hypotheses will be evaluated:

Null hypothesis: Not all the containers will perform equally.

Hypothesis 1: The four container systems will perform equally regarding the stability of the juice.

Hypothesis 1: Juice packed in glass bottles will have higher quality than PET bottles, stand-up pouches and aluminum bottles.

Two major benefits are expected from the selected package. (1) It will best protect the juice's nutritional value, taste and health benefits. (2) It will protect the product from inside and outside contamination and degradation.

Simultaneously, complementary consumer perception research on different packaging forms (materials, shapes, sizes, labels and graphics) for the juice has been conducted (Whaling 2007). Combined, the results of these research projects will enable us to make recommendations about the best package for tart cherry juice.

Industry and academia are not always following the same path and to validate research efforts a link between the academia and the industry is necessary. The uniqueness of this research is that created this necessary link to

benefit all the participants. Bringing science to the specific needs of the industry considering their limitations and risks is a very viable and sustained way of transferring knowledge and technology to the industry.

II. LITERATURE REVIEW

In this section the relevant literature is reviewed to frame and develop the experimental design for the tart cherry juice product development. It begins with a definition of juice in order to create the correct juice proportion from concentrate. This is followed by a discussion about functional foods and the specific antioxidants present in the cherry juice (anthocyanins). The last two sections of this chapter explain how to pack a shelf stable tart cherry juice and describe the four commercially available packages chosen for this experiment.

2.1. Definition of juice

The FAO defines juice as the "extractable fluid content in the fruit cells of tissues" (FAO 2005). Codex Alimentarius, defines juice as "unfermented but fermentable liquid intended for direct consumption, obtained by mechanical process from sound, ripe fruits preserved exclusively by physical means" (Codex Alimentarius 1991). Juice can be clear (clarified) or turbid (puree); it can be "single-strength" (direct consumption) or "from concentrate".

"From concentrate" means a juice obtained by reconstituting concentrate juice with drinkable water and pasteurizing it prior to packaging, as shown in Table 3. The label must clearly show that the juice is made from concentrated fruit juice. Sugars and acids can be added but must follow country-specific regulations (FAO, 1992). The reconstitution levels differ by fruit, country and institution. The Michigan tart cherry juice used for this research is reconstituted and pasteurized juice made from frozen concentrate.

Table 3. Common juice designation.

Term	Contents	Properties
Pure juice 100%	100% juice	No additives, no adjustments and not from concentrate
Fresh squeezed	Not pasteurized	Held refrigerated, short shelf-life, food safety concerns
Chilled, ready to serve	100% juice	Held refrigerated, made from concentrate or pasteurized juice
Not from concentrate	Single-strength	Pasteurized after extraction and clarification
From concentrate	Made with concentrate juice	Reconstituted and pasteurized
Fresh frozen	Unpasteurized	Single-strength, frozen after extraction
Juice blend	100% juice	A mixture of pure juices
Puree	Contains pulp	100% fruit with pulp contents
Nectar	Pulpy or clear	Sugar, water and acid are added can have 25 to 50% juice *
Nectar base	Requires reconstitution	Needs dilution for consumption
Juice drink	Low in juice	10 to 20% juice *
Juice beverage	Low in juice	10 to 20% juice *
Juice Cocktail	Low in juice	10 to 20% juice *
Fruit + aid	Lemonade, orangeade	> 10% juice + sugar and water *
Juice extract	Water extract	Fruit extracted by water then concentrated*
Fruit punch	Token juice	1% juice + natural flavors
Natural flavored	Token juice	More than 1% juice

Source: FAO, 2005
*Differs in different countries.

A Brix degree (B°) is a measurement of the mass ratio of dissolved sucrose to water in a liquid as defined by the U.S. Code of Federal Regulations (21CFR 120.24 subpart B). The FDA requires higher levels of Brix degrees for fruit juice than does Codex Alimentarius (Table 4), (Pollard 1990 and FAO 2005). Tart cherry juice from concentrate must be in a range between 14 to 14.3

degrees Brix. Fourteen degrees Brix means that the juice has fourteen % soluble solids.

Table 4. U.S. and Codex Alimentarius Brix values for juice from concentrate.

Name of the fruit	Codex Alimentarius Brix Value	US. Brix Value
Apple	11.5	13.3
Apricot	11.7	14.3
Blackberry	10.0	10.0
Boysenberry	10.0	10.0
Cherry	**14**	**14.3**
Guava	7.7	7.7
Loganberry	10.5	10.5
Mango	13.0	13.0
Nectarine	11.8	11.8
Papaya	11.5	11.5
Passion fruit	14.0	14.5
Peach	10.5	11.8
Pear	12.0	15.4
Pineapple	12.8	13.0
Plum	14.3	14.3

Source: Pollard 1990 and FAO 2005

2.2. Tart cherry juice from concentrate

Fresh tart cherries have a very short shelf-life, and the fresh window for U.S. supplies is only three to four weeks in July (after harvesting). To ensure a year-long supply the cherries or juice concentrate must be frozen or canned. Concentrated forms of juice have influenced the nature of the global juice industry in product development, stability and transportation.

There are many different methods of concentration including open pan boiling, vacuum evaporation, freeze concentration, reverse osmosis and electro-dialysis. Each method has limitations regarding cost, quality of the output, energy required to process and speed, as shown in Table 5 (FAO 2005). Most Michigan tart cherry juice is concentrated via open pan boiling in the smaller operations

and vacuum evaporation in the larger facilities. Open pan boiling has the lowest processing cost, but it has high energy consumption, a slow speed and the quality of the output is lower. Vacuum vaporization provides a higher quality than the open pan boiling system, and the energy consumption is very low, but the equipment cost is high, and not all the processors have access to this technology.

Table 5. Juice concentration systems.

Method	Cost	Quality	Energy	Speed	Other
Open Pan Boiling	Low	Low	High	Slow	
Vacuum Evaporation	High	Medium	Low	n/a	
Freeze Concentration	High	High	n/a	Slow	Limited solids
Reverse Osmosis	High	High	n/a	Slow	Limited solids and clear only
Electro-dialysis	High	high	n/a	Slow	Limited solids and clear only

Source: FAO, 2005

To maintain stability, concentrate is kept frozen to prevent quality loss and Maillard browning.[2] Normally this reaction occurs with rising temperatures and in products with high viscosity. Results are a brown coloration of the product (Davies and Labuza 2005).

2.3. Functional foods

"Let your food be your medicine, let your medicine to be your food" (Hippocrates 400 BCE).

Functional foods do not have a universally accepted definition. Stephen Defelice, founder and chairman of the Foundation for Innovation in Medicine proposed the following definition: "Functional food is a food (or part of a food)

[2] Maillard browning is a non-enzymatic browning process of simple sugars (carbonyl group) and amino acids.

that provides medical or health benefits including the preventing and or treatment of a disease" (Kalra 2003). The main markets for functional foods include Japan, Western Europe and North America (McDonald 2004). It is difficult to quantify the market potential for functional foods due to the complexity and diversity of the products. Euromonitor (2008) reported that the US share of this market segment has grown from US$ 5,786.1 million in 2002 to US$ 13.533.7 million in 2007, as shown in Table 6.

Table 6. Estimated market size for functional foods for specific regions (US $ Millions).

	2002	2003	2004	2005	2006	2007
North America	5,786.1	6,692.9	8,058.8	10,482.4	12,142.2	13,533.7
Europe	89.1	144.5	200.4	254.8	326.0	422.3
Japan	6,247.5	6,377.0	7,235.4	7,239.5	7,177.1	7,344.8
World	15,364.6	17,262.1	20,269.5	23,996.3	26,616.2	28,833.6

Source: Euromonitor 2008

The Japanese market, for functional foods is the market with more rules and standards for this category, and this market has taken the most steps toward using food as preventive medicine. In 1991, Japan was the first country to develop a regulatory system regarding functional foods. Here functional foods are treated as a special category called: Foods for Specific Health Use (FOSHU).[3] This is a different category from vitamins and minerals.

Foods require a specific approval to carry the FOSHU claims (AFIC 2003). FOSHU must be proven to improve human nutrition and health by researchers, doctors and dietitians. FOSHU must guarantee balanced nourishment, and the

[3] FOSHU are defined as: "foods in case of which specified effects contributing to maintain health can be expected based on the available data concerning the relationship between the foods/food's contents and health, as well as foods with permitted labeling which indicates the consumer can expect certain health effects upon intake of these particular foods" (McDonald 2004, 13).

active components must be scientifically confirmed in the product. The product has to be consumed in a normal way and not in a medicine or pill form, and the active component must come from a natural source (Nill 2001). Japan has a large and diverse range of FOSHU foods. In 2006 they were more than five hundred different products in this category (Functional Foods Japan 2006).

The European market is less structured and refined than the Japanese market. The European market also has a greater focus on alternative medicine and alternative sources of health. Germany has the largest market share followed by Great Britain and France (McDonald 2004). The rest of the European countries represent smaller markets for functional foods.

The U. S. is the largest single country market for functional foods, with a market of $13,533.7 million in 2007 (Euromonitor 2008). But the U.S. market is less demanding than Japan and the EU, and its regulatory system only supports some food claims for preventing heart disease and cancer, as shown in Table 7.

Table 7. Health claims for food packaging labeling approved by the FDA.

Nutrient	Disease
Selenium	Cancer
Antioxidants (Vitamin C and E)	Cancer
Nuts (specially Walnuts)	Heart disease
Omega-3 (fatty acids)	Coronary and Heart diseases
B Vitamins	Vascular disease
Phosphatidyl serine	Cognitive dysfunction and dementia
0.8 mg folic acid	Neural tube birth defects
Lutein	Eye health
Soy protein	Cancer
Green tea	Cancer
Calcium	Bone fractures, kidney/urinary stones, menstrual disorders, various cancers and hypertension
Lycopene from tomatoes	Cancer

Source:U.S. Food and Drug Administration 2003.

The U.S. does not yet have a legal definition of functional foods, and has not yet approved any health claims regarding this food category. The market growth for functional foods around the world is driven by demographics, economics and social trends. The aging population, especially the baby-boom generation, represents a large workforce that spends more in total on food than any other generation and has the highest level of disposable income in history. There is increasing interest in health and nutrition, and an increase in people who desire to understand the link between them. There is an increase in the number of educated people world-wide. Health costs have ballooned, and there has been an explosion of preventive health and acceptance of "alternative treatments" including more products with labels explaining health and functionality (Scott Wolfe Management Inc. 2002).

Tart cherry juice can be targeted to different market segments due its versatility and health benefits. The target market segments for functional foods are baby-boomers[4] and Generation-X[5] principally, responding to lifestyle changes, attitudes and activities developed over the past decades (Scott Wolfe Inc. 2004). The Generation-X population is the key for the new product development, because this group is beginning to develop product consumption habits for themselves that they will pass on to their children who will then adopt new products for their own consumption (Best 2004).

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[4] Baby boomers are defined as a person who was born during the Post-World War II baby boom between 1946 and the early 1960s (Statistics Canada 2006).
[5] Generation-X is defined as people born from 1965 to around 1982 in many countries around the world (Brown 1997).

But the functional foods category has some challenges that can compromise growth in the ensuing years. Wade (2006) reports ten challenges that the functional foods industry has to overcome to continue its growth: (1) price, (2) lack of consumers awareness or demand, (3) taste/flavor quality, (4) lack of category definition, (5) legal and regulatory environment, (6) lack of marketing data, (7) inadequate distribution, (8) absence of long term opportunity, (9) already too competitive and (10) the inadequate source of ingredients supply.

The lack of consumers' awareness of functional foods can be resolved in part by a correct and positive labeling. Labeling is a way to show the consumer the product benefits. It is part of nutrition education. Thus the labeling must show the correct amount of the nutritional components in the product at the end of the shelf-life to inform the consumer.

Claims regarding anthocyanins, such as those found in tart cherries, have not yet been approved, and so labeling must be done carefully (McDonald 2004). Some labeling concerns for tart cherries were raised by FDA since 2006 due to health claims printed on the packages and websites. Processors had to change their labels and web pages for the tart cherry products because they contained non-approved health claims (Good Fruit Growers 2006).

Since there is no regulation against identifying the amount of nutritional components like anthocyanins, it is important to understand their stability over time so that labels can accurately reflect the amount. This can inform consumers and avoid possible liability and legal issues with FDA. However, no literature has been found on stability of anthocyanin content in functional beverages.

2.4. Anthocyanins

Anthocyanins are antioxidants, meaning that these compounds prevent the degradation of human cells by inhibiting the initiation of free-radicals that cause cell degradation (Cao et al 1993). They are the water-soluble pigments that impart color to flowers, and other plant parts like fruits -- colors ranging from violet and blue to most shades of red (Harborne 1967).

Anthocyanins are a subclass of flavonoids and belong to the group called polyphenols. They can be found in the vacuole of the cell dissolved in the cell sap. They have in the main molecular chain a $C_6C_3C_6$ skeleton that is typical of flavonoids. Their main part is an aglycone that contains double bonds responsible for the absorption of light around 500 nm making the pigment visible to the human eye.

There are 22 different anthocyanins but only six are common in food. These are pelargonidin, cyanidin, peonidin, delphinidin, malvidin and petunidin and are represented in Figure 3 (Francis 1989). The most common are pelargonidin, cyanidin and delphinidin. The main differences between these aglycone are the number of hydroxyl groups at carbon 3, 4 and 7 in the A ring, and methoxyl groups. In the B ring they change; pelargonidin has one hydroxyl group at 4', cyanidin has one hydroxyl group at 4' and a second one at carbon 3' and finally delphinidin has the hydroxyl groups at carbons 3', 4', and 5' (Paul and Palmer 1972). Products rich in cyanidin, pelargonidin or delphinidin are less color stable than products containing petunidin or delphinidin because the hydroxyl group is blocked, and sugar moiety influences stability, therefore this mechanism still is not well understood and more research is being done (Von

Elbe and Schwartz 1996). Anthocyanins are greatly impacted by pH, temperature and oxygen and in less proportion by degradative enzymes, ascorbic acid, sulfur dioxide, metal ions and sugars (Von Elbe and Schwartz 1996)

Cyanidin is the most recurrent anthocyanin in woody plants. Cyanidin can shift from the red to the blue of delphinidin with additional hydroxyl groups. Methoxyl groups can replace the hydroxyl groups causing the color change. Another factor that affects color stability is the sugar residue attached by glycosil linkage to one or more hydroxyl groups (Paul and Palmer 1972). This means that when the sugar starts to dissolve a color change occurs in the product.

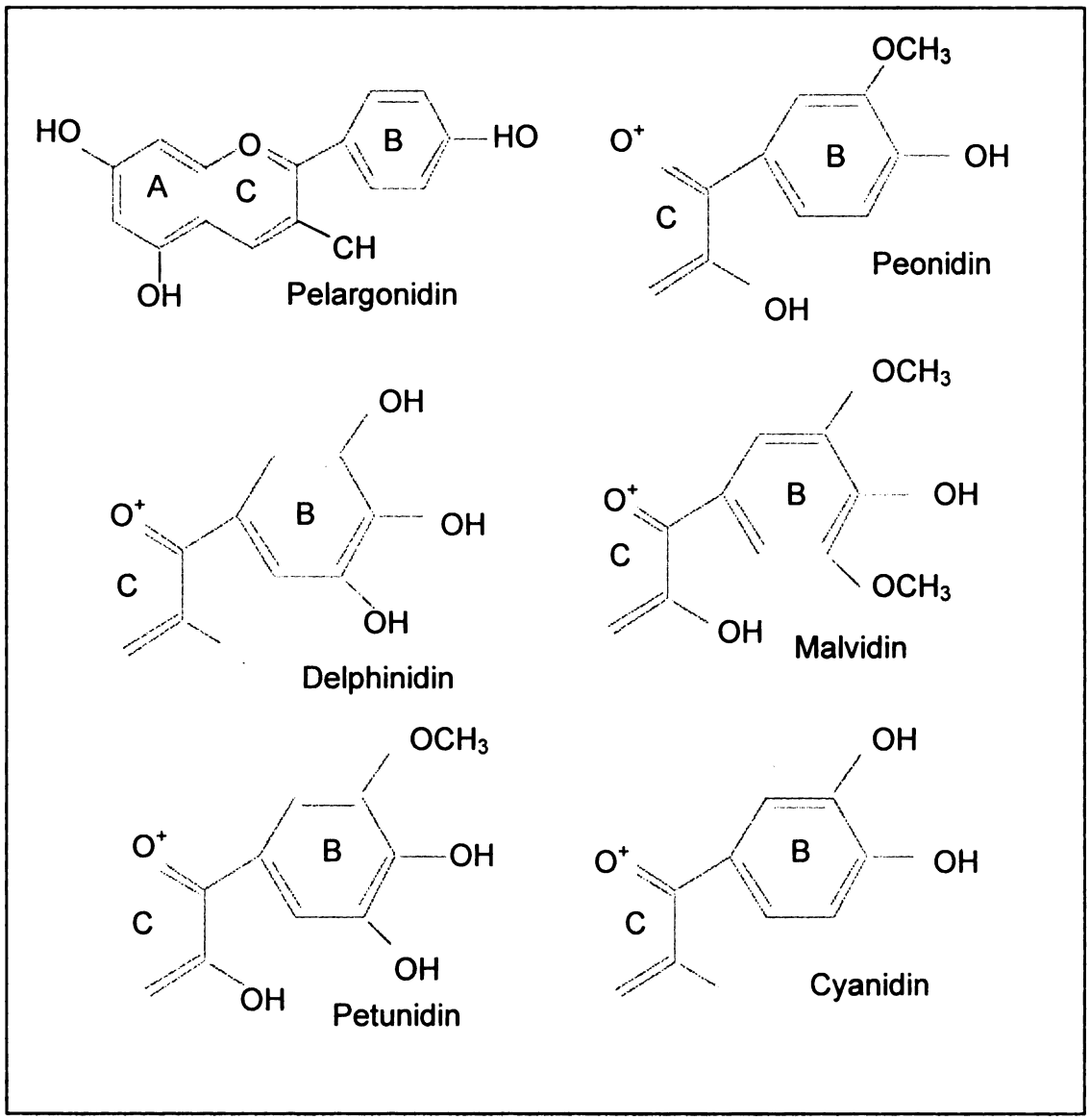

Figure 3. Six common anthocyanins formed in plants,
Adapted from Francis 1989.

Anthocyanins can also suffer dramatic color changes in mediums when the ion concentration or pH changes. In dilute acids, the oxygen in the rings carries a positive charge, resulting in a structure called oxonimum or flavylium ion. At pH less than 3.0 anthocyanin color shifts to more of a red color. If the medium shifts to be less acidic, than the red color changes to purple (quinine form), and in an alkaline solution it turns blue. At pH close to neutral (pH=7.0)

the violet base color is in equilibrium with a form of a pseudo base that many times is colorless. Anthocyanins are more stable in the flavylium ion stage than in the pseudo base where oxidation can start (Paul and Palmer 1972).

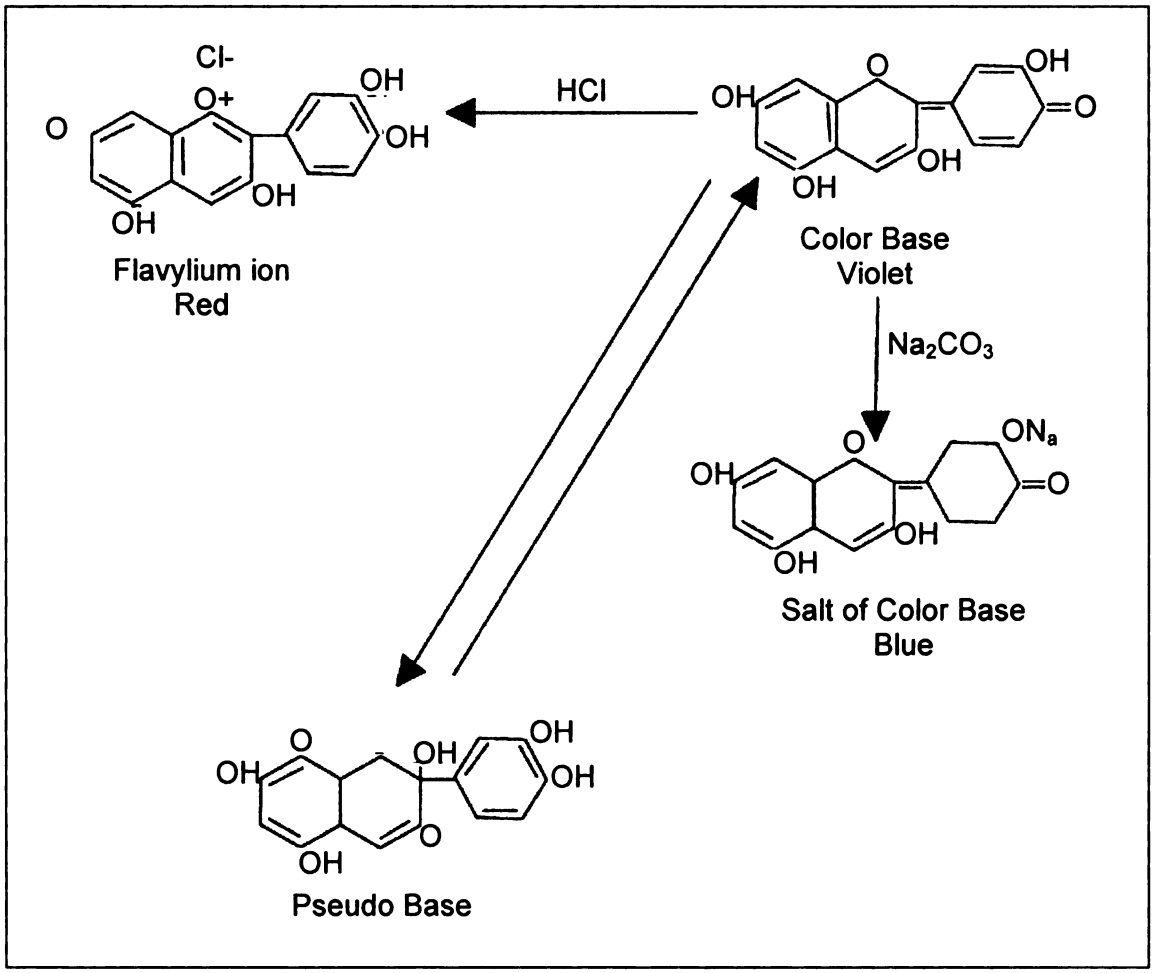

Figure 4. How anthocyanins can change from any stage to any stage with pH change (cyanidin example),
Adapted from Paul and Palmer 1972.

Figure 4 shows the changes suffered by anthocyanins when the pH of the solution changes. Not all anthocyanins will have such a dramatic change. Those with 4 hydroxyl groups in the molecule and one unsubstituted hydroxyl at the 4' position are more prone to change. Cyanidin has these four hydroxyls, and is

one of the most susceptible anthocyanins to color change (Paul and Palmer 1972).

If products containing anthocyanins are packaged in metal packages like cans or bottles, they should have a polymer coating because the anthocyanins and acidity can cause pitting and perforation of the aluminum or steel. Seams or imperfections in the coating plus the acidity of the product (such as juices) can lead to corrosion. Corrosion occurs due to metal ions that have been dissolved by the acid or by a depolarization mechanism where hydrogen is removed (Paul and Palmer 1972).

Furthermore anthocyanins can also react with metals such as aluminum and tin. Anthocyanins with more than two unsubstituted hydroxyl groups are more prone to this reaction; red colors turn more purple or purple changes to blue with a more stale hue making food unappealing. This reaction is called chelating and can disassociate in a more acidic medium. Chelate is a chemical compound in the form of a heterocyclic ring, containing a metal ion attached by coordinate bonds to at least two nonmetal ions. This reaction is non-reversible.

Anthocyanins can react with temperature as well. This chemical reaction is irreversible compared to the acidity related reaction previously described and will create a browning of the pigments. The problem is not the loss of the anthocyanins but a change in the molecule due to degradation. Degradation can be caused by processing temperature, storage temperature, oxygen and UV light. Degradation is promoted by high pH, oxygen in the head space, presence of sugars and the presence of ascorbic acid (Paul and Palmer 1972).

When a molecule has at least one reactive oxygen specie (OH•), UV light or oxygen can start a chain reaction creating free radicals that will lead to product degradation (Burke and Nair 1986).[6] When molecules have reactive oxygen species such as hydroxyl (OH•), then peroxyl radicals (ROO•) and the superoxide anion (O_2•) are produced as a result of metabolic reactions (Wang et al 1999). These reactions can be caused by a source of energy like heat or UV light where the OH and double bonds in the anthocyanin molecule structure are initiated. Once the reaction is initiated; it goes through a chain reaction that will continue creating more free radicals. This chain reaction can be stopped by the bonding of two radical intermediates resulting in a stable molecule or when the molecules are consumed (Figure 5).

[6] Similar reaction occurs with human cells that results in changes to DNA due to free radical formation. These changes can lead cancer cells formation or mutations due to free radical reactions (Burke and Nair 1986)

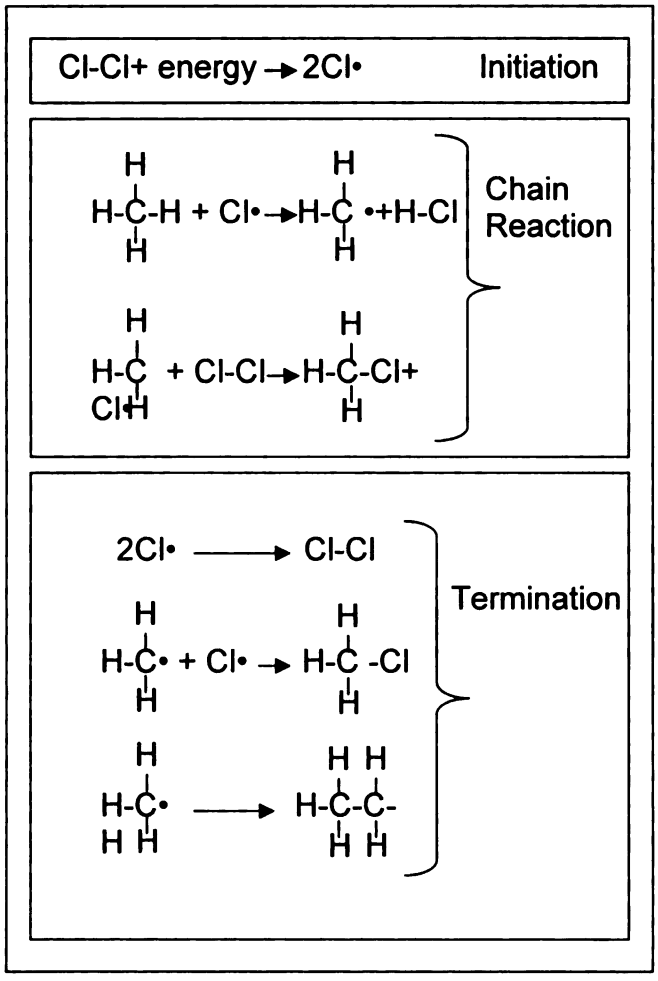

Figure 5. Free radical formation process.
Adapted from Reuch 1999.

Anthocyanins are also affected by anthocyanase, a browning enzyme, in fresh fruits, that is activated when they are bruised. Enzymatic problems can be eliminated by heating the fruit up to 80°C (Paul and Palmer 1972).

Many anthocyanins are found in cherries, and the most important are: cyanidin, delphinidin, peonidin, pelargonidin and petunidin (Chandra et al 1992) Tart cherries have a high Oxygen Radical Absorbance Capacity (ORAC) due to larger quantities of Cyanidin 3-glucoside, Cyanidin 3-rutinoside and Peonidin 3-

rutinoside. Concentration of these anthocyanins can be determined by high pressure liquid chromatography (HPLC), mass spectrometry (MS) and gas chromatography (GS) methods (Esti et al 2001; Kim et al 2005; Hong et al 1990; Wang et al 1999 and Chandra et al 1992).

2.5. Shelf-life stability

The shelf-life of a product is determined by the amount of time the product remains acceptable to consumers, including taste, chemical, physical and microbiological characteristics. Food Processing Technology (2008) defines shelf-life as: "the period of time which a product can be stored, under specified conditions, and remain in optimum condition and suitable for consumption". For a functional food, the product needs to retain its functional components over time, especially when conformance to a labeled health claim is required.

Shelf-life is affected by the structure and composition of the product, the environment outside of the package, and the interaction of the package and product leading to chemical and physical reactions (Vercelino et al. 200; Ayhan et al 2001). These reactions will lead to color and flavor changes, and could even make a product unsafe. There are different methods to lengthen the shelf life of fruit juice including heat, pressure, aseptic technology and electrical conductivity. Each of these methods will have different degrees of advantages and disadvantages. One of the most common methods is pasteurization and hot filling. Hot fill packaging and a low product pH help to control the initial and end of shelf-life microbial burden.

2.5.1. Hot fill and product acidity

Hot filling is a heating process in which the beverage is pasteurized at temperature above 85° C (185° F) for a determined period of time and then packaged while it is still hot. The main purpose of this process is to kill harmful organisms such as bacteria, enzymes, viruses, protozoa, molds, and yeasts, and create a shelf stable product that can be stored at ambient temperatures for several months (Solberg et al 1990). This method was developed by the French scientist Louis Pasteur in the nineteenth century and is used to achieve long shelf-life for juices at low cost.

It is sufficient to inoculate viable vegetative forms of microorganisms but not heat-resistant spores. If the manufacturing plant has good hygiene, hot fill is suitable for non-carbonated beverages and juices.

The hot fill method is simpler than aseptic technology.[7] The package chosen must ensure integrity when exposed to high filling temperatures and prevent post process contamination. It is necessary to choose packages with a high barrier against gas penetration and aroma and sorption. Microorganisms can be destroyed by heat, and each organism will have different resistance and tolerance. Most significant are the *Clostridium. botulinum* (*C. botulinum*) spores, which can be killed at 82° C (180° F). C. *botulinum* is the most poisonous and resistant naturally occurring substance in the world, and if its death is ensured, other organisms are also destroyed (Brin et al 1999; Hersom and Hulland 1980).

[7] Aseptic packaging is the technology and process where food and beverages are first sterilized for a short period of time and then filled and sealed under sterile conditions that include the packaging containers, packaging devices and products to be packaged.

Hot fill temperatures must be high enough to reduce the microbial charge to safe levels and to inactivate fruit enzymes that can produce browning and fermentation. At the same time, temperatures have to be low enough to avoid destroying the bonds in the anthocyanin molecules which would produce free radicals, causing oxidation and changes in color and taste (Marquez et al 2003).

It is especially necessary for non-acidic foods to be heat processed. The Danish biochemist Søren Peter Lauritz defined pH in 1909, as an inverse logarithmic measure of hydrogen ion concentration (H).

$$pH = -\log_{10} a_{H}^{+} \quad ; \text{where } a_{H}^{+} \text{ is hydrogen ion activity.}$$

The amount of acid present in food is measured on the pH scale extending from 0 to 14. It is rare for foods to be in the alkaline range (a pH of 7 or above). Foods with a pH from 0 to 7 are acidic and can be divided into low acid and high acid (Star 2004).

High acidity (with a pH below 4.5) is very important in food processing, because *C. botulinum* cannot grow in this environment. Tart cherry juice is considered to be high acid, with a pH of 3.92. It is essential to the stability of the product to maintain the pH below 4.5. The high acidity will prevent the growth of some bacteria, while heat will destroy most yeast and molds.

2.5.2. Color and anthocyanin stability

Anthocyanins are a very important part of the juice and a very important attribute for product marketing. USDA and FDA regulations state that the nutritional values on the label must be the minimum nutritional values in the

product through the duration of the shelf-life. Therefore, anthocyanin contents in the juice must be evaluated over time.

The stability of color and anthocyanins depends on the pH, temperature, process, anthocyanin concentration, physical-chemical properties, enzymes and storage (Rein 2005 and de Freitas and Mateus 2006).

Anthocyanins and color are degraded by oxidation and elevated temperatures. The combination of oxygen and elevated temperatures produces the greatest color destruction and loss of health benefits (Nebeskey et al 1949; Von Elbe and Schwartz 1996). A brown hue appears as the pigments degrade and polymerize (Kearsley and Rodriguez 1981, Constela and Lozano 2005, Marquez et al 2003, Turker and Erdoğdu 2005). The heat process used for tart cherry juice should be limited in time and temperature to prevent the browning effect.

Enzymatic browning is another concern relative to the color and anthocyanin stability. Enzymes react with other phenolic compounds present in the food (corresponding quinones), which will then react with anthocyanins. This results in degradation, giving a brown coloration to the product (Rein 2005).

Anthocyanins are more stable at low pH values, and will fade as pH is increased. The majority of anthocyanins from plants are non-acylated and are unstable when pH changes, especially cyanidin and its derivates (Stintzing et al 2002).

Turker and Erdoğdu (2005) proposed that anthocyanins are more stable at a pH level of 2 and concluded that the effect of pH change is more dramatic than

the temperature effect. Color loss is reversible (Scaman 2005; Jiang et al 2004; Turker and Erdoğdu 2005) and pigments can return to their original colors if pH values fall. Jiang et al (2004) described how they manipulated the red color of litchi fruit by modifying the pH. If the acidity level is the only change in the juice, color can be manipulated by adding acid. Unfortunately for tart cherry juice producers, pH is not the only change that the juice will undergo.

They are three major physical-chemical reactions that anthocyanins can go through: (1) reaction of anthocyanins with small compounds, (2) condensation between anthocyanins and flavanols mediated by aldehyde and (3) direct condensation between anthocyanins (Freitas and Mateus 2006, Gomez-Cordoves 2004). Pyruvic and phenolic acids and acetaldehyde, are some the small compounds that can react with anthocyanins. Condensation of anthocyanins and flavanols (tannins[8] included) can be done directly or mediated by acetaldehyde present in tart cherry juice. Tannins are mostly colorless; therefore they can also range from yellow to light brown. When copolymerized with anthocyanins, they can result in a bluer tint to the juice (Gomez-Cordovés 2004, Eglinton et al 2004)

The packaging material plays a very import role in protecting the anthocyanins from oxidation. Many packaging materials have good gas and good moisture barriers, especially colored glass and aluminum. Other packaging processing techniques such as the controlling temperature and the eliminating

[8] Tannins: An exact definition of tannins does not exist but, they are a very special phenolic compounds that have the ability of combining with proteins and other polymers like polysaccharides (Von Elbe and Schwartz 1996)

oxygen in the head-space also help to maintain the anthocyanins and color stability of the product.

2.5.3. Flavor stability and food packaging interactions

Products can be monitored analytically to ensure safety for human consumption. However, it is the final consumer who will decide whether or not to purchase a product. If a product does not fulfill customer expectations it will not be repurchased. Sensory evaluation is a science that more and more companies are implementing to minimize the risks of developing new products and reformulating mature products to better satisfy consumer needs and tastes (Stone and Sidel 2004).

Flavor and taste are two very different concepts and they are many different definitions. Therefore in food science two definitions of flavor are accepted. The first definition is by Hall (1968 p. 352): "the sum of those characteristics of any material taken in the mouth, perceived principally by the senses of taste and smell and also by the general tactile and pain receptors in the mouth as received and interpreted by the brain". The second definition of flavor has been accepted by the Society of Flavor Chemists: "the sensation caused by those properties of any substance taken into the mouth which stimulates one or both of the senses of taste and smell and/or also the general pain, tactical and temperature receptors in the mouth (Food Flavor 2007).

Taste is perceived when a chemical solution diluted in saliva or with any other liquid is absorbed by the receptor in the taste buds. The tongue is the main taste organ, but taste buds are also found in the hard and soft palate, in the

throat, cheeks and in the floor of the mouth. They all contribute to the taste sensation. Four main tastes exist: sweet, salty, bitter and sour. The existence of other tastes such as umami, astringency, alkaline and metallic have been also described (Carden and Baird 2000).

Cherries have a number of volatile compounds that influence flavor and aroma over time, including alcohols, aldehydes, esters and ketones (Matteis et al 1997). Bauer-Christoph et al (2005), Mattheis et al (1997), and Girard and Kopp (1998) agree that the most important volatile compounds in cherries are: methanol, hexanol, benzaldehyde and acetaldehyde.

Benzaldehyde is responsible for much of the flavor strength, and acetaldehyde gives the cherries their freshness and aroma. (Esti et al 2002, Mattheis et al 1992). Benzaldehyde is also present in almonds and can be recognized as the flavor of almond extract. Benzaldehyde has a benzene ring with an aldehyde substituent. It is the simplest representative of the aromatic aldehydes and one of the most used industrially in its family (European Chemical Bureau 2006). Like all volatile compounds, benzaldehyde decreases over time, but it is not as volatile as acetaldehyde. Amounts remaining in the product are influenced by the packaging material, heat and time (Meheriuk et al 1995).

These compounds can be identified through gas chromatography analysis (GC) as a quantitative method or with a trained panel for qualitative testing.

Packaging plays a very important role in protecting product flavor. Packaging provides a barrier against oxygen, UV light and from the environment in general (permeation). It prevents packaging compounds from migrating to the

products and prevents the absorption of flavor compounds from the product to preserve flavor.

Interaction within the system refers to the different mass and energy exchanges between the food, the packaging material and the external environment that the package system to which it is exposed to (Hotchkiss 1997). Permeation includes to two different mechanisms: diffusion of molecules through the packaging material and absorption or adsorption from/into the internal or external atmospheres. Migration refers to the release of compounds from the material to the food product, which can be harmful for human health. Finally, absorption or scalping refers to the transfer of molecules to the packaging material which causes a loss in flavor, aroma and/or color. Figure 6 shows these three mechanisms that can occur in a packaging-food system.

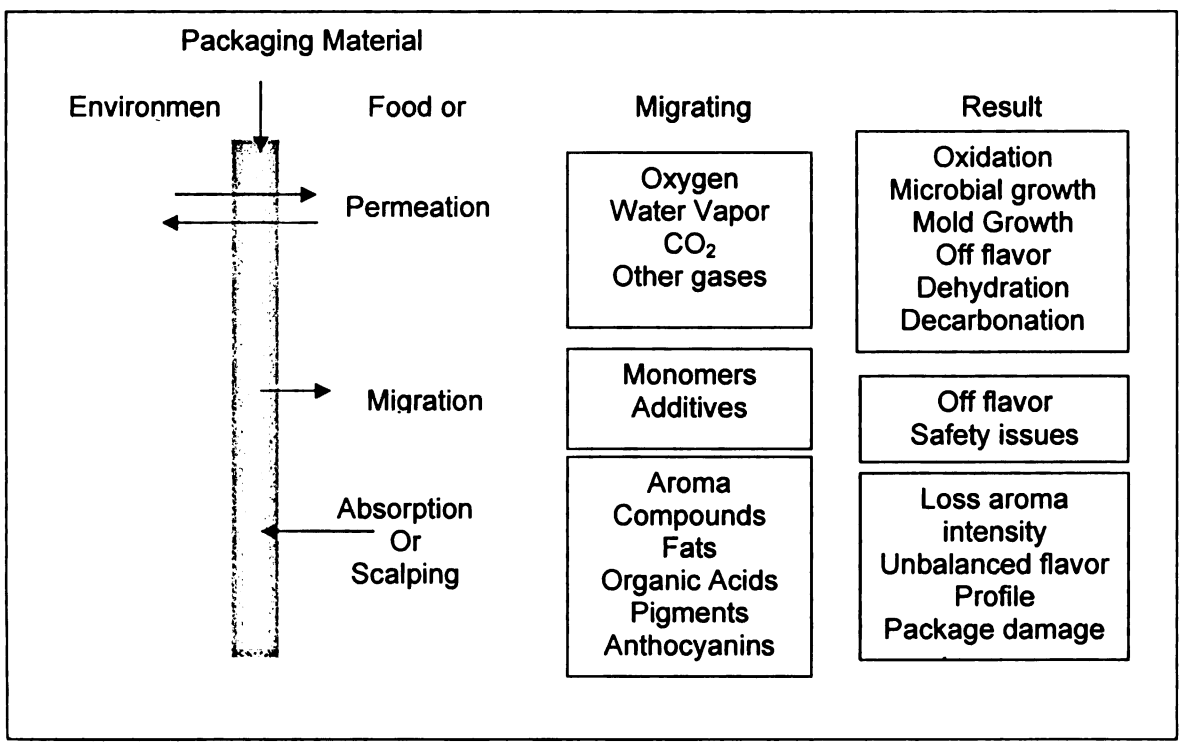

Figure 6. Permeation, migration and absorption.
Adapted from Nielsen and Jägerstad 1994.

The compatibility of the packaging material with a food or beverage is one of the most important problems faced by industry when developing new products. This mechanism can be extremely complex and depends on both the packaging material and food molecular structure. Two of the most important parameters to analyze when choosing a package are: (1) glass transition temperatures (T_g) and (2) polarity of the molecules in the food product and the packaging (van Williege 2002).

Glass transition temperature refers to the temperature where the materials pass from a glassy or brittle stage to a rubbery stage (Wesselingh and Krishna 2000). Of the polymers used for bottles, polyethylene terephthalate (PET) and polyethylene naphthalate (PEN) have a T_g above room temperature. They have very stiff chains that reduce the diffusion coefficient for flavor molecules at low temperatures since these molecules are harder to disrupt. On the other hand, polyethylene (PE) and polypropylene (PP) have chains that are less stiff and have a higher diffusion coefficient, and so volatile compounds can more easily be absorbed by the polymer structure.

The electrical charge of the polymer molecules affects flavor and aroma scalping. It is necessary to find packaging food systems that will prevent scalping. Polyesters (PET and PEN for example) are polar and so they are less attracted to non-polar volatiles or flavor compounds, therefore, they are not prone to flavor or aroma scalping. On other hand, polyolefins like PE and PP are non-

polar and so they will promote the scalping mechanism that can shorten the shelf length of products or minimize the overall quality (Sullivan 2005).

2.6. Materials

Euromonitor (2005) reports that, in the U.S., packaged food grew by 16% from 2000 to 2005. The soft drink category, over the same period of time, grew about 20%. Soft drink, or non-alcoholic beverages, is the category that includes carbonated and non-carbonated beverages. The non-carbonated beverage category includes water, juices, artificial and natural flavored fruit beverages and energy drinks (Hicks 1990 Nermark 2002).

Packaging for this category includes an array of possibilities including glass, aluminum, steel, coated paperboard, different kinds of plastics and flexible packages. Most common are glass, PET, aluminum, Tetra Pack® and flexible packages that in the last ten years have been growing in market share (Euromonitor 2005).

The most important beverage packaging trends for new product development are convenience, recyclability and innovation. Convenience includes portability, reclosability and individual portions. There is a demand for recyclability or minimal waste production due to environmental issues and consumer knowledge. Innovation and quality are part of the product image and communication with the consumer (Getachew and Peterson 2005).

Euromonitor (2005) reports that the most common packages used for functional foods in the European Union (E.U.) are first metal (51 %) and then plastic. Plastic is first in the U.S. (73.61%) and Japan (52.55%), and metal is the

second choice. Glass is the third choice and flexible packaging is the fourth for all three geographic regions. The package used least in the three regions for functional foods is cartons. Cost, availability and recyclability are the big drivers in the processors selection of packaging for functional foods.

For this project, packages were chosen to meet the consumer demand for convenience, recyclability and innovation. Packages were also chosen to be compatible with the tart cherry juice, with the manufacturing requirements, market availability and commercial ability. Four packages were included: glass, aluminum and PET bottles and stand-up pouches.

In general, glass is one of the most inert materials; it provides excellent moisture and oxygen barrier, and scalping is not an issue. PET is a good gas barrier, especially with the help of an oxygen scavenger layer that can trap the incoming oxygen and soluble oxygen present in the juice. Aluminum bottles are a new package for beverages and are also provide an excellent gas and moisture barrier. These three packages can be recyclable and a recycling stream is available in the U.S. as it is in other countries of the E.U. The stand-up pouch is not recyclable due to the different material layers; however, they do produce less waste volume. Following is a review of the properties of each of these materials.

2.6.1. Glass

Glass is one of the oldest materials used for containers although it was not until the early 20th century that the glass container process was automated. Glass bottles can be shaped by a blowing method or a pressing and blowing

method. Glass is a molten mixture of inorganic oxides: 73 % silica sand (SoO_2), 15 % sodium oxide (Na_2O), 10 % calcium oxide (CaO) and 2 % of other materials like aluminum oxide (Al_2O_3) and iron oxide (Fe_2O_3). ASTM C 162-03 defines glass as a non-crystalline material obtained by a melting process. Glass has a very irregular atomic arrangement as shown in Figure 7 (Masayuki and Asahara 2005).

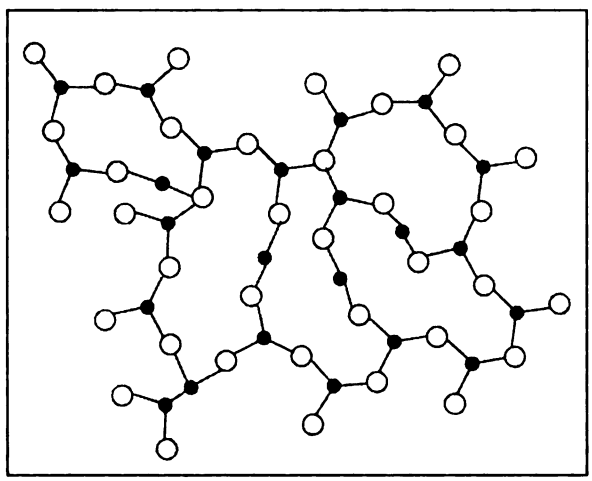

Figure 7. Molecular arrangement of glass.
Adapted from Masayuki and Asahara 2005.

Commercial glass has a density of 2.5 g cm^{-3}, one of the highest among the different types of glasses, a coefficient of thermal expansion between 0 to 300° C of 92 x 10^7 $°C^{-1}$, and a thermal conductivity that ranges between 10 to 11.2 $Wcm^{-1}°C^{-1}$ when temperature changes from 10 to 100° C. It also has one of the lowest thermal shock resistances among the different glass types (Bansal et Doremus 1986).

Glass has been identified as the most chemically inert material available for food packaging and has a very low coefficient of solubility of gases in its solid

state (Bansal and Doremus 1986). It is a great barrier to oxygen and moisture due to its impermeability, absence of pores and homogeneity.

In its solid state, the molecules are bonded tightly together giving glass its excellent barrier characteristics (Halloway 1973). Therefore the food product stored inside the glass can be in direct contact with the internal wall without any coating.

Glass provides many options for labeling and marketing. Labeling options include paper and plastic labels, with applied adhesive or self adhesive, as well as plastic shrink labels. Special molds can be developed for unique and innovative identification, although it is much more expensive to have to use a custom mold compared to a stock bottle (Glass Packaging Institute 2006).

Glass bottles have five very practical operational properties: (1) availability, (2) returnability, (3) ease of packaging, (4) ease of distribution and (5) recyclability. They have four favorable market properties: (1) product recognition, (2) container appeal, (3) flexibility and (4) environmental friendly (Kurylowsky 1996). Glass also offers better gas and moisture barrier properties, better absorption barrier properties, and better UV protection than plastics, and thus gives a longer stability for food products (Coutelieris and Kanavouras 2005).

There are also disadvantages of glass, most notably the high risk of breakage during production, filling, storage, transportation, display and usage. Glass is generally a high cost package due to the energy cost used to blow bottles and transportation energy costs due to the heavy weight.

For over 100 years, glass was the most common package for beverage containers (Nankivel 1997), although much of the beverage industry has now switched to PET and metal cans. U.S. glass industry shipments peaked in 1980 at 47 billion units, declining to 34.5 billion in 2004 due largely to substitution. Glass containers now account for 4.5 % of all shipments of the packaging industry (Impact Marketing Consultants Inc 2006). Glass has been reserved for high-end products especially with the competition of other packaging materials since the seventies (Hook and Heimlich 2006).

EPA reported that in 2003 Americans, generated 12.5 million tons of glass that ended in the municipal waste stream with only 22% of that ending up in the recycling stream.

2.6.2. Polyethylene terephthalate

Plastics are a newer packaging option and 29% of the 2005 production of plastics was used for packaging (American Plastic Council 2006). Polyethylene terephthalate (PET) became available in the 1970s, and its use has increased steadily. The plastic bottle industry volume was over 118 billion units in 2004, of which PET soft drink bottles comprised the largest proportion at about 28% (33 billion units). PET bottles for water was the fastest growing segment: 23 billion units in 2004, up from only 3 billion in 1998, a growth rate of almost 35%/year (Impact Marketing Consultants 2008). The majority of PET used for packaging is for beverages (carbonated and non-carbonated), dressings, and preserves.

PET belongs to the polyester family and is produced by a condensation reaction of ethylene glycol and terephthalic acid or dimethyl terephthalate. Condensation polymers are created using a two step mechanism. The first step produces the amides or the esters as in PET. The second step is a much slower mechanism and it is called polymerization when the polymer is created. When condensation occurs in polymers a small amount of water or methanol is lost, and the most common byproduct is water (Selke et al 2004). PET is generally blow molded to produce bottles.

PET has many beneficial properties for packaging such as clarity, inertness, light weight, strength (especially resistance to pressure), moisture resistance, gas retention for carbonated beverages and good gas barrier. Another advantage of PET is that the polarity of its molecules helps to repel aroma and flavor compounds, although it is never as good as glass.

PET has a glass transition temperature (T_g) of 80°C, and 22 to 25% crystallinity (Reim 2005). Figure 8 shows the molecular structure of PET. It has a benzene ring in the backbone which reduces the crystalline path and two unsaturated bonds.

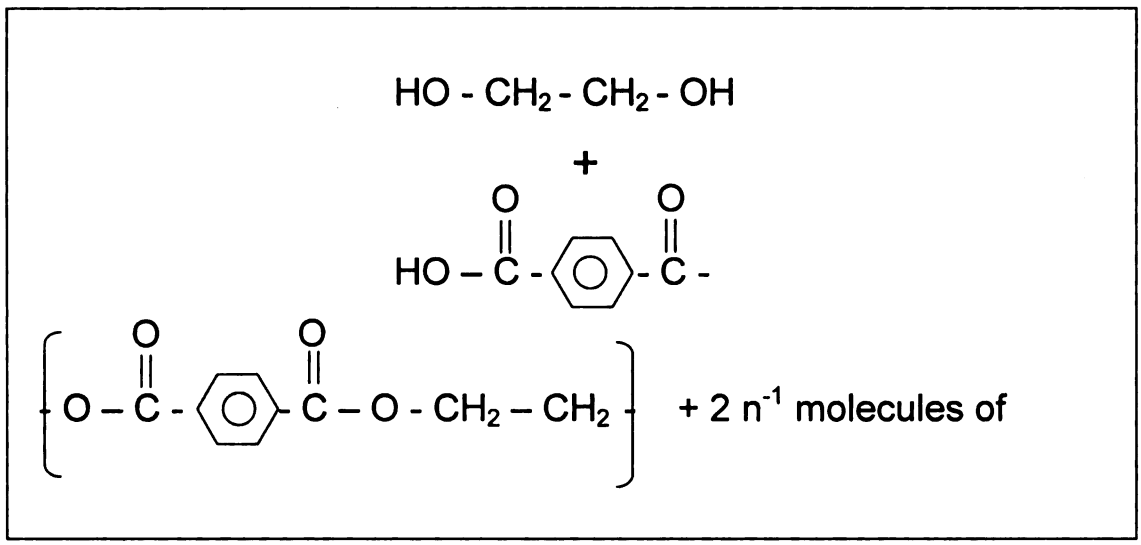

Figure 8. Molecular structure of PET and condensation reaction.
Adapted form Selke et al 2004.

PET has good oxygen and UV resistance. It is resistant to high temperatures and can be coated or coextruded with different additives or oxygen scavengers to improve the barrier to oxygen or moisture although this increases the cost (Risch 1999; Ros-Chumillas et al 2006).

The disadvantages of PET, compared to other resins, is its proportionally higher price, the two-step bottle production process, lack of rigidity, and bottle deformation when the walls are too thin (Packaging Council of New Zealand 2004). PET bottles have different weights depending on the product specifications: for 20 oz bottles, a bottle for water generally weighs between 15 and 19 g; for carbonated beverages the weight should fall between 23 and 27 g; and for hot fill the range should be 33- 38 g to minimize the bottle distortion due to high temperatures filling (Selke et al 2004).

Although PET is 100% recyclable (APC 2006), the use of recycled PET in direct food contact has not yet been approved. PET can be recycled into product forms other than food packages, such as carpets.

2.6.3. Aluminum

Aluminum is the most abundant metal and the third most abundant element on earth. The first metallic aluminum was prepared in 1825 by Hans Oersted. Aluminum is a white-silver metal with very low density (2.70 g/cm^3). It is non toxic and very machinable (Shakhashiri 2006)

Aluminum can be found in the packaging world in the form of foil or sheets that are later transformed into containers. Aluminum foil has been available commercially since 1910, and aluminum containers have been available since 1950.

Cans are the most common aluminum containers, coated with a polymeric lining to be used as food grade. Aluminum cans are the second largest material for can production (Second to steel). There has been a decline in aluminum can use from a value of U.S. $4,823 million in 1997 to $ 1,677 million in 2004. Aluminum remains the second largest material for can production (Impact Marketing Consultants Inc 2006). Aluminum cans are mostly used for soft drinks and beer (Impact Marketing Consultants Inc 2006).

Aluminum bottles were first used in Japan and are now used for beverages world-wide. In 2004, a re-closable aluminum bottle was launched by Alcoa and the Pittsburgh Brewing Co. (NBC News 2004). Bottles are formed by the stamping method (CCL Containers 2005). Beer, sport drinks, energy drinks

and juices are now being packed in aluminum bottles. The aluminum provides a very sleek, modern, state-of-the-art look.

No information has been published regarding the stability of products in aluminum bottles. However, aluminum provides a good barrier against light, air and moisture, and does not oxidize like steel, and so aluminum bottles are expected to give good product stability. Unlike steel rust, which exposes the surface to more oxidation, aluminum oxidation creates a white layer that will protect the aluminum from further exposure. Food grade aluminum cans and bottles are coated with various kinds of polymers.

Like PET, aluminum is light weight. It has an even higher recycling value than PET, highest of all packaging materials (The Aluminum Association, Inc. 2001).

2.6.4. Flexible packaging

Multilayer flexible packaging has grown steadily for the past 20 years, reaching 17% of the U.S. $127 billion packaging market in 2006 (Flexible Packaging Association 2006). Flexible materials can be used to pack almost any kind of product. Different combinations of polymers, as well as aluminum and/or paper, are possible. The choice of materials depends on the intrinsic needs and characteristics needed to ensure the protection of the food product. The materials can be laminated, extrusion laminated or co-extruded.

Multilayer materials are not recyclable because they include a combination of materials. Compared to rigid packaging, however, flexible materials can

reduce the volume and the weight of waste (Flexible Packaging Association 2006).

Six benefits of flexible packaging, compared to rigid packaging, are: (1) cost savings, (2) less material used, (3) less space required through transportation and storage, (4) reduced effects on the environment, (5) ease of use and (6) efficiency (Risch 1999).

The main disadvantages are a lack of strength, which may requires an additional of package to support the load when shipping, less convenience for the consumer, and lack of recycling. However the addition of reclosable polypropylene spouts has helped to increase sales in markets where flexible packages compete with rigid packages (Selke et al 2004).

High density polyethylene (HDPE), low density polyethylene (LDPE) or linear low density polyethylene (LLDPE) or another polyolefin is used inside of flexible pouches for food contact and for heat sealing. Polyethylene (PE) is a non-polar, linear thermoplastic with a density ranging from 0.910 to 0.940 g/cm3. The melting temperature is between 128 and 138 °C (Fiadus and Tong 1997). There are many methods to seal the inner polyethylene layer of pouches and choosing one depends on the needs and the machinery available. Bar or thermal sealing is the most common method for stand-up pouches. Thermal sealing is based on two bars that heat press the materials together. Heat causes the sealing materials to fuse. Some pressure and some dwell time[9] are

[9] Dwell time is define as the period during which a dynamic process remains halted in order that another process may occur (ITS 2008)

necessary to develop a complete bond between the seals (Selke et al 2004). Hot tack is the ability of the seal to resist strain while still molten.

Linear low density polyethylene (LLDPE) will be the material in contact with the juice. Since LLDPE is non-polar, it is expected that it will attract volatiles through the material structure causing absorption or scalping. Depending on the levels of absorption, degradation may occur, including swelling and disruption of the polymer molecular structure (Reim 2005).

Multi-layer flexible packaging materials can be laminated or co-extruded. A barrier can be included between layers to provide a better or longer shelf-life (Reim 2005). Most flexible packages for liquid have an aluminum foil barrier for gasses, moisture and UV light, normally ranging from 0.2 to 0.4 mil (Selke 2004).

The outer layer is normally polyethylene terephthalate (PET) due to its glossy properties, giving a more polished look to the flexible package, better gas and moisture barrier and better canvas for printing purposes (Plastic Packaging Innovation news 2005 and Packaging Digest 2004).

III. EXPERIMENTAL DESIGN AND METHODS

The major degradation mechanisms for tart cherry juice are:

- Microbial growth, which is limited by a hot filling high acid process,
- Oxidation, which is exacerbated by UV light, and
- Sorption of flavor, color and aroma by the packaging material.

The package and process must be selected to ensure that these are prevented. With the above in mind, four packages were chosen: glass bottles, aluminum bottles, PET bottles with oxygen scavenger and flexible stand-up pouches with an aluminum foil barrier and central reclosable spout.

The methods used to analyze these degradation mechanisms are based on color change, the pH and solids stability (Brix degrees), microbial growth, and the stability of anthocyanins and flavor. This research is designed to record these changes analytically and by sensory perceptions and consumer preferences. Analytical and organoleptic tests were used to compare the stability of the product in four different packages. During this research the oxidation due to light (photo-oxidation) was not measured directly since the packages were kept in closed cartons during the length of the experiment; but the effect of oxidation was one of the parameters used to evaluate color and flavor due to the singular brown tones and rusty flavor characteristic of this reaction. Figure 9 shows the conceptual model for this project.

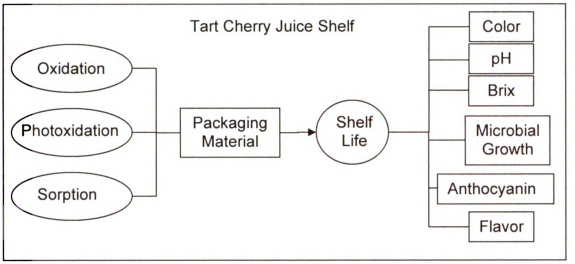

Figure 9. Tart cherry juice shelf-life, conceptual model.

The experiment was a series of tests for a 12 month stability study of hot-filled tart cherry juice in the four package types. At one month intervals, the juice was tested analytically for color, solids contents, acidity, microbial growth and retention of anthocyanins compounds. Flavor, color, texture, and overall quality were qualitatively analyzed with a trained panel as shown in Figure 10.

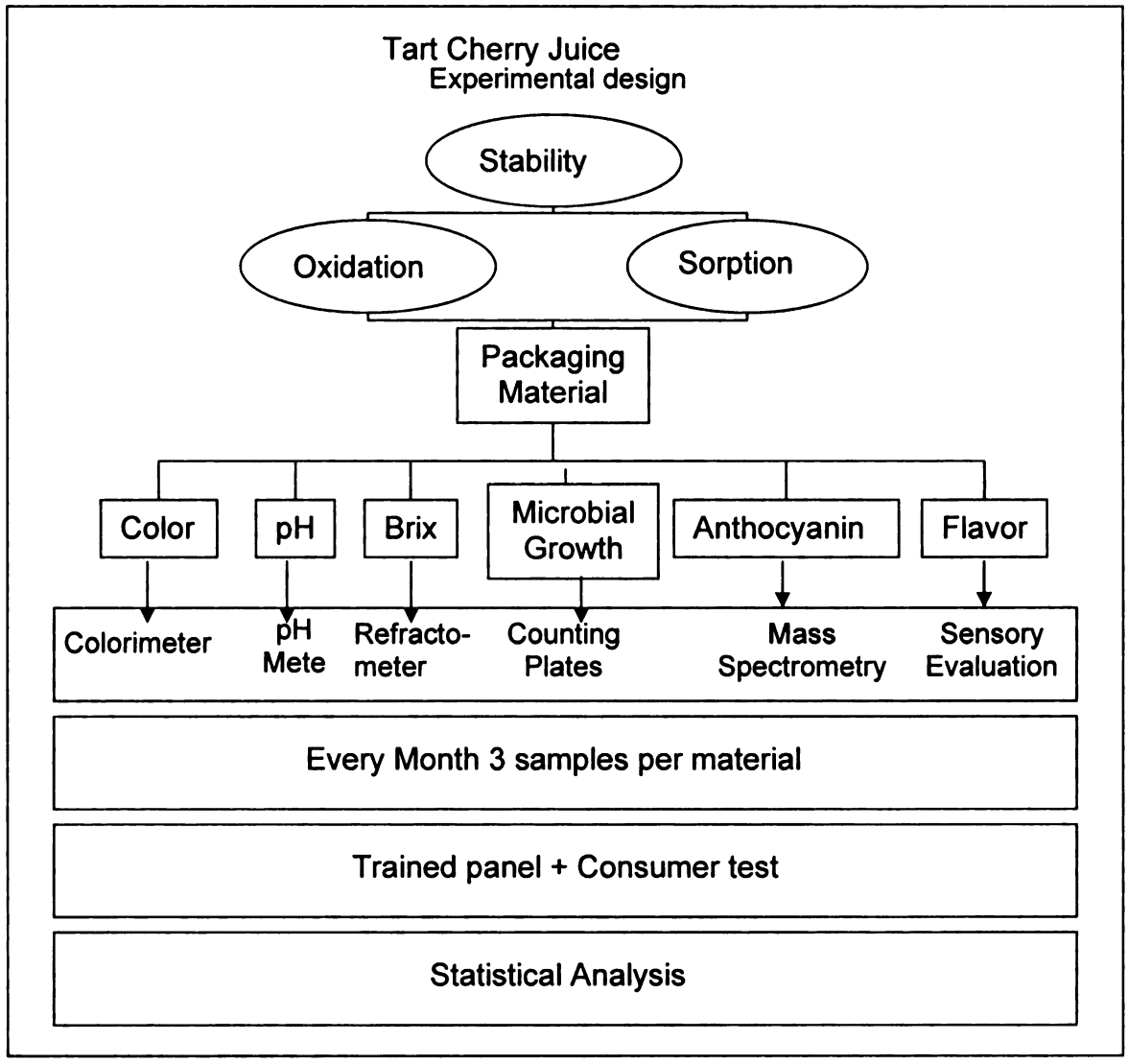

Figure 10. Experimental model scheme.

3.1 Packages and Closures

Four different containers were chosen to evaluate their effect on product quality: glass bottles, PET bottles, aluminum bottles and stand-up pouches with a multi-layer structure. These containers were chosen because of their properties and availability in the market place. They can be obtained easily by tart cherry processors in different quantities. The packages for this research were generously donated by Performing packaging (stand-up pouches), CCL Containers (Aluminum bottles), Toyo Seikan (PET bottles) and St. Gobain (Glass bottles.

3.1.1. Glass bottles

The clear glass bottles (flint) were donated by St. Gobain Inc. The bottle mold code is 4356012B. These bottles are narrow neck and have a capacity of 12.75 fluid ounces. The specification sheet for these bottles details the following dimensions: height 7.719 inches, diameter 2.492 inches and weighs 255.03 g. Figure 10 shows the glass bottle used for the project and drawings of the principal measurements.

The finish designation is 38-400 (continuous thread) and the closures are metal screw caps with plastisol liners. These types of liners are used in hot fill procedures, so the plastisol liner melts during processing and seals to the rim of the bottle. Once the temperature cools, it solidifies creating a tight seal between the container and the closure. The Standard Finish Dimension Tolerances gives minimums and maximums for E (size of the finish without the tread), T (size of the finish including the thread), H (height of the thread part of the finish), S (distance between the first thread and the top of the bottle finish) and I (internal

size of the bottle finish). The 38-400 E has a maximum of 1.476 inches and a minimum of 1.452 inches; T ranges from 1.382 to 1.358 inches; H from 0.418 to 0.388 inches; S from 0.61 to 0.31 inches; and the interior of the bottle finish (I) is 0.987. The GPI[10] finish is 4009.

The metal cap weighs 5.04 g making the total weight of the package 260.07g. Caps can be silver, gold, white and black, and in this study they are gold (Figure 11).

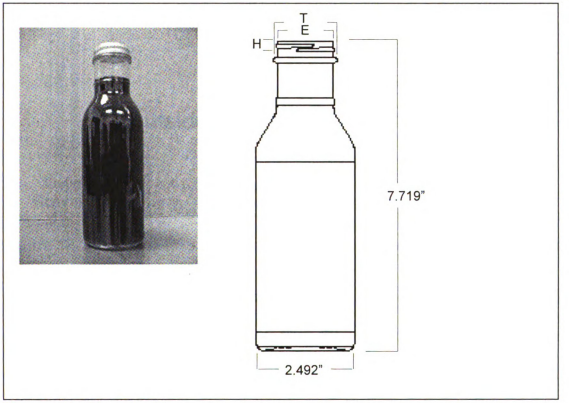

Figure 11. Glass bottle.

3.1.2. Hot fill Polyethylene terephthalate (PET) bottles

The PET bottles were donated by Toyo Seikan. The hot fill PET bottles have a 5 layer structure as follows: 3 layers of PET sandwiched with 2 layers of

[10] GPI stands for Glass Packaging Institute. They have create a volunteer set of standards that allows manufacturers to be interchangeable and matching bottle finishes with caps.

Sirus 101, a Toyo Seikan patented oxygen scavenger material (composition unknown and proprietary). Sirius 101 is an oxygen scavenger that provides a high oxygen barrier by trapping oxygen from the outside and absorbing oxygen present in the product as shown in Figure 12.

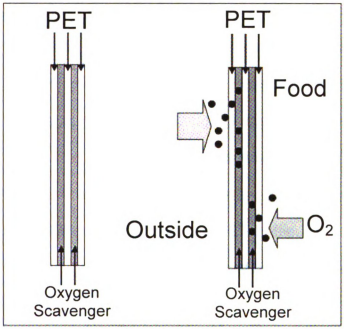

Figure 12. Multilayer PET bottle structure and oxygen scavenger process.

Common weights for hot fill PET bottles range from 33 to 38 grams. Since the bottling temperatures will range from 82 to 95°C PET bottles will suffer distortions because this temperature is above PET glass transition temperature (Tg) (80°C). The packaging industry have found different methods to prevent this PET shrinkage. One method is to "heat-set" bottles, increasing the crystallinity of the bottle walls up to 30 % under stress to maintain the bottle shape. A second method is to first heat the bottle pre-forms (10-15°C) and then blow at higher temperatures (120-140°C) to form the bottles. The base of the bottle is kept cool to prevent the formation of thermally induce crystals, and bottles are cooled with

circulating air for up to one minute to stop the crystallization process. A third option is to include vacuum panel designs (varying from company to company) to allow the filled bottle to compensate for pressure changes in the bottle through the cooling process (Selke et al 2004). Lately the PET industry has found ways to design "panel-free" hot fill bottles to promote a more aesthetically pleasing bottle and ease the labeling process (Food Processing 2004 and Plastics Technology 2005). The bottle walls have a thickness of 16 mil, and the bottle weighs 33.43 grams meeting the requirements to prevent bottle shrinkage.

The finish is a 38-400 continuous tread with a tamper evident band that breaks when opened. The finish measurements are the same as the glass bottle. Figure 13 shows the PET bottle. The cap is made from polypropylene with teflon (PTEF) liners Produced by Toyo Seikan as well.

Figure 13. The 8 oz PET bottle from Toyo Seikan.

3.1.3. Aluminum bottles

The aluminum bottles were produced and donated by CCL containers. The bottle's capacity is 18 fl oz, and it is suitable for hot filled beverages. The

raw material is aluminum with an inside proprietary polymeric coating (HOBA 8280/2).

The shoulder profile is Sonic 66. The outside diameter is 66 mm, the overall height is 206.0 mm. The weight of the empty aluminum bottle is 67.40 g, the cap weight is 4.13 g and so the total weight is 71.53 g.

The bottles are made by stamping aluminum sheets into the shape of the bottle. With this method there are no seams or welding necessary, therefore an extra step is necessary to create a reclosable finish for the aluminum bottle. A polyethylene lug sleeve is applied to the finish during the last spinning to create a slug finish that will match a 38 snap slug shallow aluminum cap. This cap will have a polyethylene liner to ensure a good seal when hot filled. Figure 14 shows the dimensions of the bottle and a picture of the container.

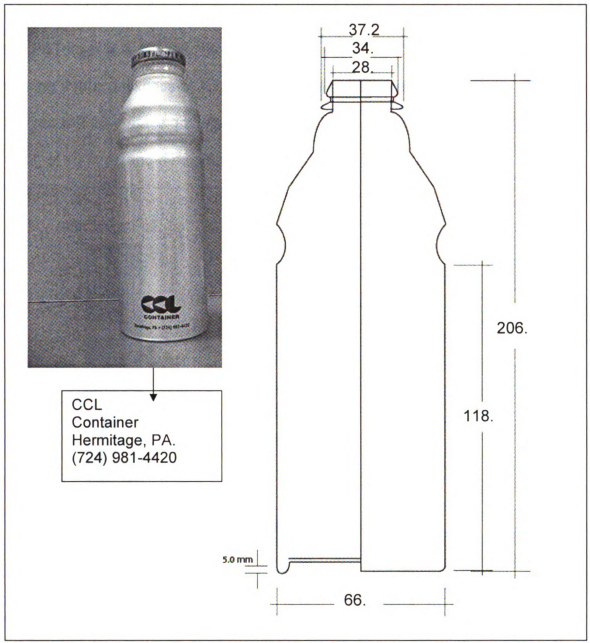

Figure 14. Aluminum bottle (dimensions in mm).

3.1.4. Stand-up pouches

The flexible pouches were donated by Performance Packaging, and they contain 7 fluid ounces, the smallest amount of any of the packages' capacity. The multilayer structure is formed from an inside layer of low linear density polyethylene (LLDPE), an outside layer of polyethylene terephthalate (PET), and

an aluminum foil layer (Al) in between as is shown in Figure 15. The thickness of the PET layer is 0.472 mil, the aluminum foil is 0.28 mil, and the LLDPE is 4 mil making a total thickness of 4.752 mils. The weight of the structure is 11.70 g and the cap is 1.20 g making a total weight of 12.90 g. The stand up pouch has a width of 100 mm and a height of 180 mm.

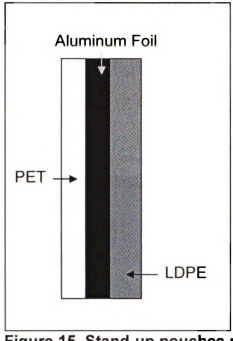

Figure 15. Stand-up pouches multilayer structure.

The stand-up pouch has a centered Polyethylene (PE) cap and spout that makes the flexible package reclosable, as is shown in Figure 16. The opening of the spout is 9 mm.

Special fillers and cappers are necessary to fill this container, since pouches are filled differently than bottles. The equipment varies from manufacturer to manufacturer, though the most common type uses preformed pouches that are received in a Z- stack. The packer/filler inserts the spout and

cap, and the pouches are filled from the bottom.[11] The machine will make the seal, separate the pouches, and then send them to the cooling conveyor.

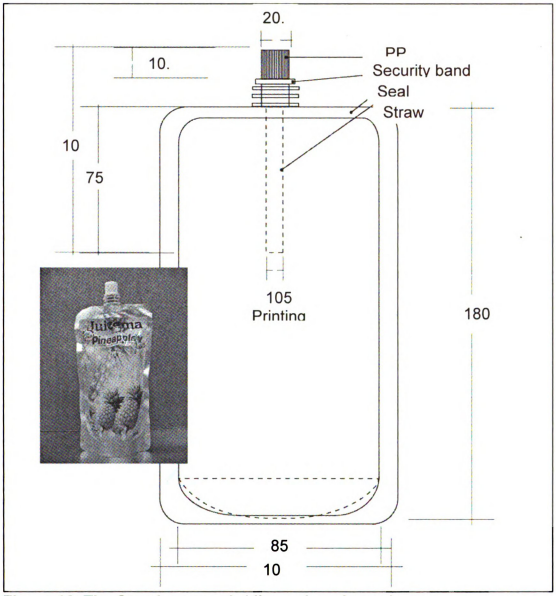

Figure 16. The Stand-up pouch (dimensions in mm).

[11] One manufacturer of equipment like this is Goglio in Italy.

3.2 Packaging technique and facilities

The juice was packed on March 24, 2006 by *Food for Thought*, a small packing facility located in Honor, Michigan. Half of the cost was donated by the company, and they agreed to pack the four different packages.

Food for Thought does not have the technology to pack the pouches. Performance Packaging, sent the stand up pouches formed, sealed and with spout and caps on. This is the reason why they were filled with the smallest nozzle and capped by hand without nitrogen flush.

The company used three cookers with kettles from Savage Brothers in Chicago, a filler with 2 nozzles from EPAK in LaPointe, IN, a SureKap spindle capper from Winder, GA. and a cooling belt, as shown in Figures 17 to 19.

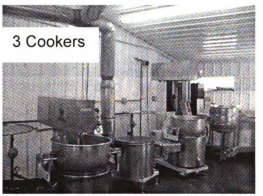

Figure 17. Food for Thought, cookers.

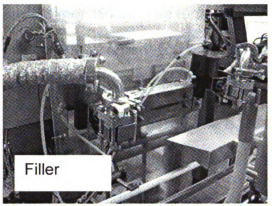

Figure 18. Food For Thought, filler for bottles and jars.

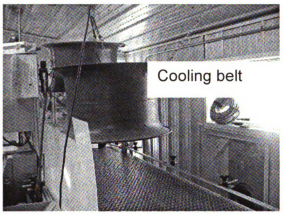

Figure 19. Food For Thought, cooling belt.

The tart cherry juice was reconstituted and heated to a pasteurization temperature of 85°C or 185°F (Hicks 1990) before filling the different packages. Hot fill was chosen over aseptic packaging, because it is the general practice for juice production, especially for high acid juices (below 4.5 pH). The aseptic packing equipment it is more expensive, and it is not available among the tart cherry processors of Michigan.

The temperature was raised over a time period of 15 minutes (time needed by the cookers at the plant to raise the temperature) and held for 30 seconds. This is sufficient to kill all the enzymes and potential pathogens (Paul

and Palmer 1972). The temperature selected was the lowest possible in order to minimize the degradation of anthocyanins present in the tart cherry concentrate. Bottles were heated with lamps for 10 minutes to prevent temperature shock and breakage, and then filled. The headspace was flushed with nitrogen to displace any oxygen, capped and cooled immediately.

One hundred packages were filled with the juice for each of the four types of package. The bottles were closed with 15 lbs/in^2 of torque with an automatic capper. Manual torque was used to apply caps to the pouches since the existing capper could not be adjusted for this kind of package.

3.3. Tests

After packing, the packages were stored at room temperature (23°C and 50%RH) in corrugated fiberboard shipping containers that shield from light exposure. Every month, three replicates from each treatment were evaluated for:

- Color change
- Solids change measuring Brix° degrees
- pH change
- Microbial and pathogen growth
- Anthocyanins stability
- Flavor and taste

The samples were evaluated monthly for a twelve month period. The different tests are described in the following section.

3.3.1. Color

The change in the color of the juice is related to the quantity of anthocyanins, solids and pH. Color also indicates UV light degradation and oxidation, when the juice has brown tints.

De Ancos et al (1999) recommended using a colorimeter to measure Hunter L*a*b* values for juice. They proposed the use of cylindrical cups for the test measurement.

As an adaptation for this research 15 ml Petri dishes were filled with 15 ml of juice. They were closed and sealed with parafilm® and with black electrical tape to prevent the penetration of light through the sides as shown in Figure 20. A Hunter Lab Colorimeter 45/0 with Color Quest software from Hunter lab in Road Reston, Virginia U.S. was used. The colorimeter was standardized using a black and a white tiles to give the following results: L* value: 94.53, a* value: -0.91 and b* value: 0.94. Samples were placed under the optical reader and readings were taken by the colorimeter. Measurements were taken in triplicate and then averaged for the analysis.

Figure 20. Sample preparation for color measurements.

The L* value is a measure of lightness varying from completely opaque when the value is 0 and to completely transparent when the value reaches 100. The a* value measures the redness and a negative value will measure greenness. The b* value measures the yellowness and the negative value blueness. Figure 21 gives a graphical reference of the 3 dimensions of color in this scale.

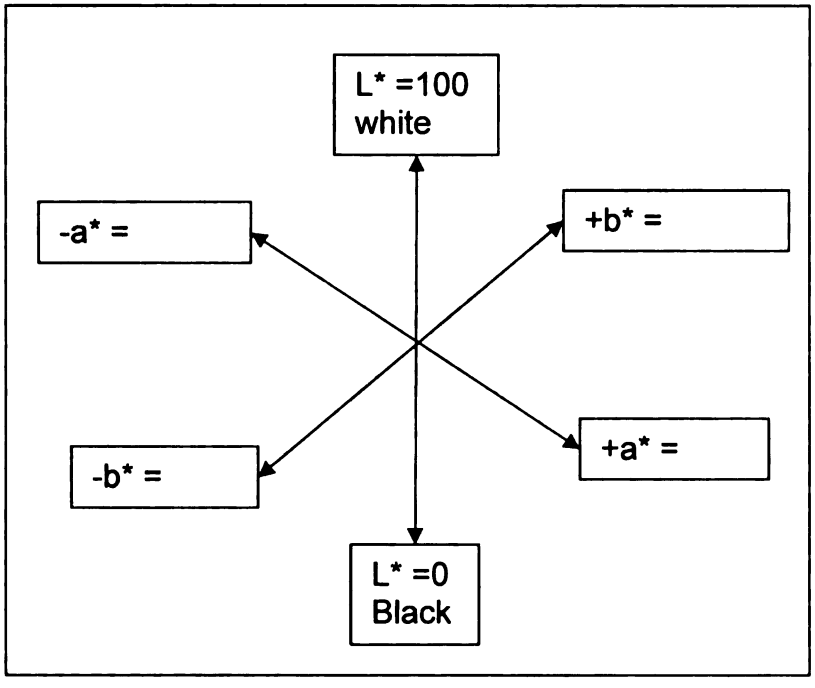

Figure 21. Hunter Lab scale reference.
Adapted from Hunter Lab 1999.

The hue value is the ratio of absorbance of fruit juice at 520 and 420 nm. Lower values of hue represent oxidation and browning of the anthocyanins (Alper et al 2005). The hue angle h is measured using the formula:

$$h = \arctan b^*/a^*.$$

Chrominance is the saturation of a specific color, and the formula is:

$$C = (a^{*2} + b^{*2})^{1/2}$$

Finally, total color difference formula is:

$$\Delta E = \sqrt{\{(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2\}}$$ (Remon et al 2004).

3.3.2. Solids

Brix degrees (°Bx) are the amount of dissolved sugars to water mass ratio of a liquid solution. If a liquid has 30 °Bx means that it has 30 grams of sugar and 70 grams of water. Brix degrees were measured with an Aldrich Z281611 portable refractive index detector from Sigma-Aldrich in St. Louis, MO (Figure 21) at room temperature following standard procedures (Serrano et al 2005; Righetto et al 2005).

Three replicates were analyzed for each type of packaging. For each sample of the juice, one reading was taken by observing the line where color changes on the scale, as shown in Figure 23.

Figure 22. Refractometer used in the tests.

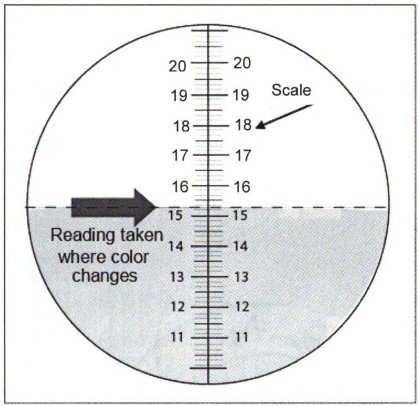

Figure 23. How a reading looks in the refractometer visor.

3.3.3. pH levels.

The pH measurement is used to determine the acidity of a product. Acidity provides a general inhibition of pathogens (4.6 and lower pH) and the data collected was used to assess the overall quality of the juice (Raso et all 1998).

The samples were tested using a probe, (KS701 ISFET) pH Tester with Non-Glass Advance Electrode from Pulse Instruments from Bas Inc. in Tokyo, Japan (Figure 24). The pH meter was calibrated each month before use with three buffers: acid (4.0), neutral (7.0) and alkaline (10.0), as recommended by the pH meter manufacturer and literature. Then the probe was submerged in the tart cherry juice until a stable reading was obtained, and the stable reading

sign appeared in the pH meter panel. After every reading the probe was rinsed with water.

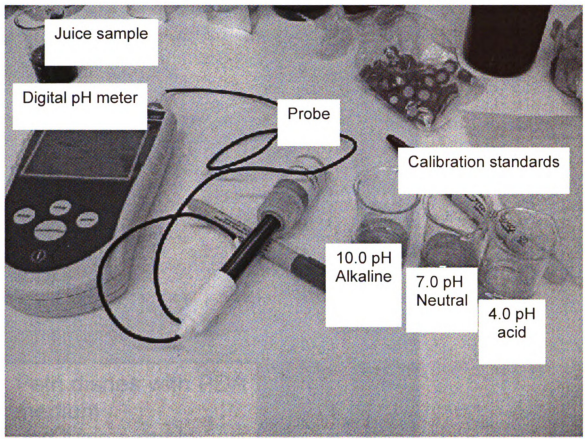

Figure 24. Digital pH meter and buffers for calibration.

3.3.4. Microbial growth

The growth of yeast and mold was evaluated with general counting plates containing potato dextrose agar (PDA). The juice was diluted with sterile distilled water to 10^{-1}, 10^{-2} and 10^{-3}.[12] Then 10 µl of the solutions were dispersed into the medium in triplicate for each sample. After the dishes were stored at room temperature (23 °C and 50% RH) for two weeks, the number of visible colonies was counted (Figure 25).

[12] 10 µl of juice diluted 100 µl of distilled water gives 10^{-1},10 µl of 10^{-1} dilution with 100 µl of distilled water gives 10^{-2}, finally 10 µl of 10^{-2} dilution with 100 µl of distilled water gives 10^{-3}

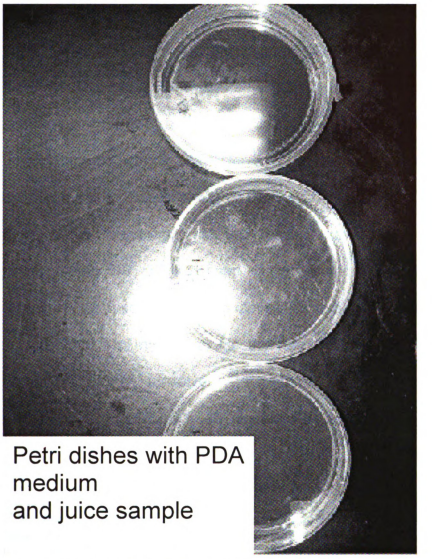

Figure 25. Counting plates with PDA medium and juice sample.

3.3.5. Antioxidants compounds

There is general agreement among researchers to use high pressure liquid chromatography (HPLC) techniques to determine anthocyanin content and the intensity of flavor compounds in juices (Kim et al 2005; Bauer-Christoph et al 2005; Mattheis et al 1997; Girard and Kopp 1996; Serrano et al 2005 and Meheriuk et al 1995). Typically C 18 columns are used with two solvent systems, typically 99 % water as solvent A and 1% formic acid and acetonitrile mixture as solvent B.

For this research the results obtained by HPLC[13] methods were not consistent due to some calibration and technical problems with the equipment at the time. This experiment was substituted by liquid chromatography/mass spectrometry (LC/MS) at the Mass Spectrometry Laboratory in the Biochemical Department at Michigan State University. Samples were evaluated by the technicians of that department. A Waters LCT Premier XE mass spectrometer[14] from Waters Corporation in Milford Massachusetts, U.S., with a Shimadzu HPLC from Portland, OR, U.S was used. Data was acquired and managed with MassLynx® MS software. A Novapack (Waters) C18 Column was used with a flow rate of 0.8 ml min^{-1} at an injection rate of 20 µl at 25 °C. The mobile phase was HCOOH (10%) as solvent A and ethanol (50%) as solvent B. Solvent A had a gradient from 5 to 22 % in 40 minutes. (Esti et al. 2002)

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[13] The central problem with the current generation of HPLC detectors is that there is a broad trade-off between sensitivity and specificity. Highly sensitive detectors, such as those utilizing ultra-violet detection, tend only to be able to detect a certain range of compounds, while those detectors that can detect a broad range of compounds, such as those utilizing refractive index detection, and tend not to be very sensitive (Detectors 2008).

[14] The LCT Premier™ XE mass spectrometer delivers high sensitivity, resolution, and exact mass (Waters 2008).Features:

- High MS resolution for the selectivity needed to separate analyte spectra from isobaric interferences and background chemical noise
- High sensitivity for achieving very low detection limits
- High linear dynamic range, which allows experiments to be carried out across a range of concentration levels
- Exact mass MS measurements, which give elemental composition information that can be used to identify analyze compounds; you can obtain exact mass on molecular and fragment ions, simplifying the process of spectrum interpretation
- Versatile ionization options that apply to a range of compound classes
- Versatile software options for a variety of applications

3.3.6. Sensory evaluation

Sensory evaluation gained extra impetus during the 1940s and 1950s thanks to the U.S Army which tested different food products for the military. Sensory evaluation was then an emerging science, and several other institutions started developing new tests to obtain more accurate results to evaluate their food products (Stone and Sidel 2004).

Various different sensory tests can be used with food and beverages such as descriptive analysis or quantitative measurements using nominal, ordinal, interval, or ratio scales (Meilgaard et al 1999). Tests can also vary depending on the number of panelists and the number of samples to be tested. The data can be qualitative, quantitative or both (van Oirschot and Tomlins 2005).

In sensory evaluation more than fifty subjects may be used when testing for consumer preferences. A trained panel with six to twelve subjects who have learned to recognize the specific product characteristics and deterioration mechanisms is another approach. For this research both types were used: consumer and trained panels.

The consumers' preferences were evaluated at the end of the experiment. The consumer's test at the end of the experiment was used to establish ranking and preferences for the juice stored after 12 months in the two better performing packages (glass and aluminum) compared to freshly make tart cherry juice from concentrate. These two better performing packages were those selected by the trained panel based on their assessment of the juice at the end of the twelve months of storage. The consumer's test was done in the sensory lab at Michigan State University in the department of Food Science and Human Nutrition. It was

done under controlled light and setting to minimize errors. One hundred panelists participated. The panelists were recruited via flyers posted on the Michigan Sate University campus and via e-mail. This panelists were people that like to drink juice. The consumer's test had two parts: Preferences and ranking. In the preference part they had to evaluate the fresh juice and one of the stored juices with a hedonic scale. The second part was to rank the two juices in order to know which one was preferred.

For the trained panel sensory evaluation, a panel of 15 subjects was identified in a screening test for this sensory evaluation (Stone and Sidel 2004; Esti et al 2002; Romero et al 2008 and Ross and Weller 2008). Potential participants were asked to identify different aromas such as coffee, vanilla, allspice, nutmeg, cloves, lemon, onion and garlic. They were asked to identify different degrees of sourness and sweetness by ranking samples in ascendant order for each set of samples. The selection criteria were based on the percentage of correct answers.

Subjects who scored over 60 percent correct were selected for the panel. Once selected, panelists were trained to identity specific tart cherry juice characteristics such as taste, color, texture, off flavor and acidity, as part of the overall expected quality.

For the training sessions, panelists were introduced to different forms of cherry flavor. For taste and aroma they tasted and smelled different products with benzaldehyde (cherry flavor). As was mentioned in the literature review, benzaldehyde is one of the most concentrated volatile compounds in tart cherry

juice. Benzaldehyde is responsible for the overall cherry/almond flavor and aroma that many commercial products use to replicate or characterize cherry flavor (e.g. Jell-o, cherry coke, maraschino cherries). Acetaldehyde, on the other hand, is responsible for the fresh-like aroma and taste. During the training sessions panelists were exposed to these two compounds in different products to learn to characterize them and later to identify quantity changes in juice.

The panel training for descriptive analysis (DA) began in March 2006, when juice was bottled. Four months later data started to be collected. The moderator collected the panel's impressions in addition to their written and individual evaluations (Stone and Sidel 1999).

Panelists were presented, at each session, with four samples (glass, aluminum and PET bottles and stand-up pouches) in transparent cups with a three digit random code in random order for the first two sessions and three sample cups (glass, aluminum and PET bottles) for the rest of the sessions.

The panel evaluated color, aroma, texture, flavor, off-flavor, overall quality and quality adequate for consumption using semantic differential scales. These were 15-point bipolar scales with words in the extremes that are antonyms:

-"too sweet" and "not too sweet" for sweetness

- "too tart" and "not too tart" for acidity

- "too dark" and "too light" for color

- "smooth" and "grainy" for texture,

- "fresh" and "not fresh" for fresh cherry juice aroma

- "light" and "strong" for off flavor good and bad for overall quality

A copy of the trained panel survey is attached in the Appendix. The results were tabulated and analyzed using Quantitative Descriptive Analysis (QDA). QDA was used with the subjects because it provides qualitative and quantitative data and allows for the evaluation of different characteristics. Additionally, the four different packages can be simultaneously tested in one session. The QDA test recommends that 6 to 12 subjects be trained on the specific properties of the product and the objectives of the project. Not all the panelists were available for all the meetings and a minimum of six is acceptable therefore there were always more than six trained panelists assisting to the sessions and six of them where consistent through the research. For this test language needed to be simple and easy to understand to translate the results to the objectives of the project. With the use of a graphic rating scale, such as the semantic differential scales, the qualitative results could be interpreted as quantitative results (Stone and Sidel 1999).

3.3.7. Statistical analysis

The laboratory data was collected monthly for a twelve month period with three replications of each treatment. Treatment refers to the different containers analyzed in this study (glass, aluminum, PET bottles and stand-up pouches). It was recorded and analyzed with SPSS statistics software. A two-way analysis of variance (ANOVA) was used to identify the difference and interaction between the different treatments and time at a ninety-five % confidence interval as previous researches have done (Esti et al 2002; Mattheis et al 1997).

For the ANOVA analysis the full model was used. If interference between treatment and month was found (significant statistical difference in the treatment*month analysis), then a custom model was run to avoid the interference. The full model can be defined by the following formula:

Design =Intercept + treatment + month+(Treatment *Month)+ εl + εt + εm + ε(t.m)

The custom model is then defined as:

Design = Intercept + treatment+ month + εl + εt + εm

The p-value obtained in the two factor ANOVA test without replication determined the significance of the results and how much evidence was found against the null hypothesis H_1 (all packages behave equally). The significance level $\alpha = 0.05$ means that is not statistically significant at a five % confidence level.

Correlation was done between different sets of data to understand how they relate to each other.

IV. RESULTS AND DISCUSSION

The juice packed in the four different packages was tested over the course of twelve months, and the data was analyzed. This chapter reports results regarding the changes in color, solids, acidity, microbial growth, antioxidant content, and flavor of juice packed glass, aluminum and PET bottles. Results for the stand-up pouch are presented in Appendix 5.

4.1 Stand-up pouch test was compromised

Unfortunately, after eight months the stand-up pouches were disqualified from this research because microbial growth was found (yeast) and there was significant reduction in Brix degrees, pH, and color. Two months earlier the trained panel had identified an off-flavor, a decrease in color, sweetness, tartness and aroma, and they did not think the quality was adequate for consumer purchase.

It is possible that the microbial contamination had occurred during filling and manually closing the pouches' spout. The plant where the juice was packed only had the capacity to pack bottles, and it did not have the special machine needed to pack stand-up pouches in a sterile manner. The special filler grips the inverted pouches which already have the spout and cap applied, fills them from the bottom, flushes the headspace with nitrogen, and then they are impulse sealed along the bottom. Another possibility was that the pouches might be contaminated during shipping and storage.

The reason for testing the stand up pouches was due to the novelty of the container in the beverage market segment in the United States. This type of

container is very popular in other countries in Europe and Latin America and is becoming more popular in the United States.

But in our case the pre-sealed stand-up pouches had to be filled through the small spout, held and capped by hand with no standard torque application and without flushing the headspace with nitrogen. When handling these containers cross-contamination may have occurred. Since the caps were screwed by hand, unequal closure torque may have been applied, and so the seal quality may have been compromised. These are the most probable reasons for the failure of the package, and so the decision was made to eliminate them from the experiment and the statistical analysis.

For the statistical analysis it was necessary for all the variables to have the same number of data points. The stand-up pouches only had eight months of data and so they could not be evaluated in the statistical analysis with the other containers. Therefore, results obtained for the stand-up pouches are offered in Appendix 5.

Only data generated during twelve months from the glass, aluminum and PET bottles were considered for analysis. These results are presented in the following sections.

4.2. Changes in color

Color change in tart cherry juice can be due to: (1) oxidation, (2) change in pH, (3) physico-chemical reactions and (4) material contact. In this research L*a* b* values were measured monthly in triplicate and hue, chrominance and ΔE were calculated. Then, all the results were analyzed with ANOVA.

A statistically significant difference between juice transparency (L* values), b* values and hue was not found among in the different packages or over time. However the a* values and chrominance were significantly different. These results are explained in detail in the following sections.

4.2.1. Changes in transparency (L* values)

The L* value measures the luminosity or transparency and the darkness of the juice. Changes in transparency during the twelve months of storage were not statistically significant (Figure 26).

A change in transparency was expected because change in solids and polymerization of anthocyanins over time has been associated in other studies with a loss of transparency in different juices. Cloudiness also has been found due to the presence of precipitates (Von Elbe and Schwarts 1996; de Freitas and Mateus 2006; Hernandez et al 1999; Gomez-Cordovés 2004; Eglinton et al 2004). Additionally, differences in mouth feel can be detected by consumers (Paul and Palmer 1972). But the results of transparency testing were similar for the juice in all of the three packages.

With the ANOVA full model, the treatment variable resulted in a p-value of 0.21 and the month*treatment variable resulted in a p-value of 0.53 (Table 10 in Appendix 1). Since both values were higher than α=0.05, there was no statistical significance for either variable. Therefore it was not necessary to run an ANOVA custom model. The changes in the juice transparency were not found to be significantly different between packages. There was, however a significant difference between months for the first 10 months. The last two months were not

significantly different with a p-value of 0.164 (see Table 50 in Appendix 2). Although the data showed a minor difference in transparency values, this difference was not statistically significant enough to consider a difference between time and treatment. Hypothesis 1: The container systems will perform equally regarding the stability of the juice with respect to transparency was not rejected, and it was concluded that the juice in the three packages is equally transparent through the twelve months of storage.

The plotted statistical means of the L* values, for each of the three containers, followed a similar parallel pattern (see Figure 26). After the first month the mean L*value was 10. After the second month of storage the mean L* values for all containers increased to 15, and for the next two months there was a steep decline. Between month 4 and 9, transparency ranged between 7.5 and 5. All these results were significant different between months.

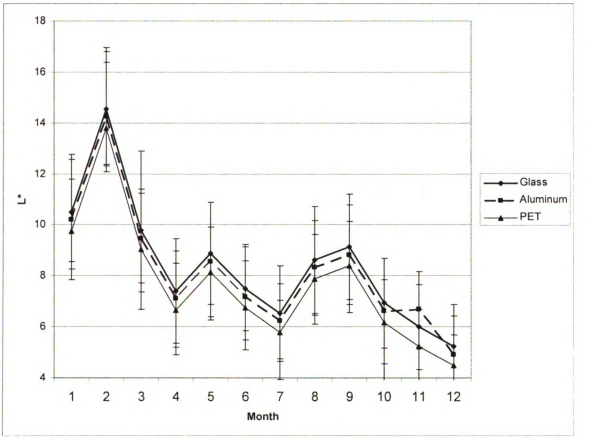


Figure 26. Change in transparency (L* values) of tart cherry juice stored at room temperature (23°C, 50%RH) for twelve months.

A possible explanation for these variations may be due to the anthocyanin polymerization due to acetaldehyde. Gómez-Plaza et al 2004, described that a decrease on monomeric anthocyanins in wine will not be reflected in the intensity

or saturation of the color, but in the transparency of the wines. When monomeric anthocyanins polymerize due to reactions with pyruvic acid or acetaldehyde, they create precipitation of solids and variations in transparency of the wine (Saucier et al 2004). It has been reported that acetaldehyde and benzaldehyde are present in cherries (lapin variety) at 59.2 x 10^5 ion count and 72.9 x 10^5 ion count respectively (Meheriuk et al 1994). Thus the presence of acetaldehyde may have caused polymerization of the anthocyanins which could result in precipitation of solids.

The presence of precipitate was also noticed in the sensory evaluation by the trained panel and the consumers. The trained panel and consumers were able to sense some particles at the bottom of the glass and detected a less transparent juice for each of the three different packages after the sixth month. There is positive correlation of 0.556 between the L* values and the question about mouth feeling to the trained panel (see Figure 27).

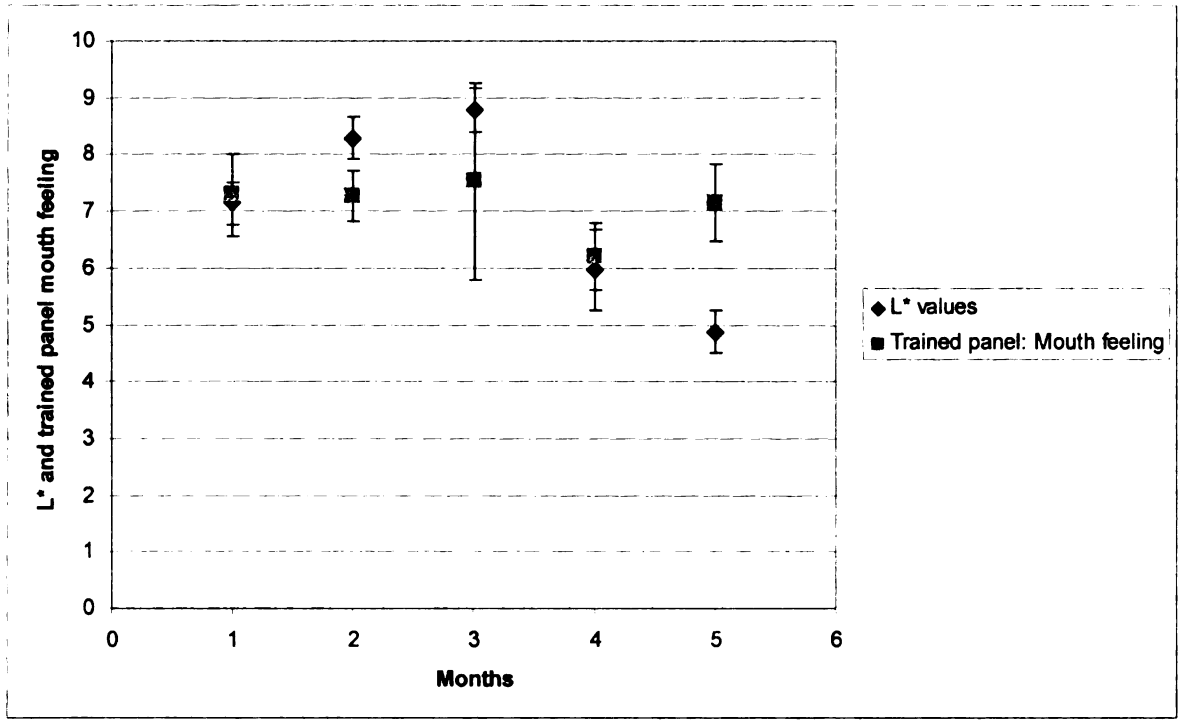


Figure 27. Correlation between L* values and trained panel mouth feel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

4.2.2. Changes in redness (a* values)

Any variation in the bright red color associated with tart cherries is probably a result of oxidation or changes in acidity or solids. Changes in oxidation are not reversible and can be measured by a constant and steady drop in redness. The red color takes on a brown hue as the pigments degrade, oxidize and polymerize (Kearsley and Rodriguez 1981; Constela and Lozano 2005; Marquez et al 2003; Turker and Erdoğdu 2005).

On the other hand, color loss due to changes in acidity is reversible (Scaman 2005; Jiang et al 2004; Turker and Erdoğdu 2005). Pigment color can change as pH values vary. Jiang et al (2004) described how the red color of litchi fruit was manipulated by modifying the pH.

In this project a full ANOVA model was run to evaluate the a* values for the month*treatment variable. This test resulted in a significant difference (p-value below α=0.05) which indicated an interaction between the two variables. Next a custom model was run to compare the two variables, month and treatment. This custom ANOVA test resulted in a statistical significance for both the month (Table 51 in Appendix 2) and treatment variables (Table 11 and 12 in Appendix 1). These results suggest that at least one of the three packages behaved differently throughout the twelve months of storage. Therefore hypothesis 1 was rejected on the basis of a* values.

The statistical means of the red a* values for all of the three containers followed a similar trend over time as shown in Figure 29. Between the first and third months the overall mean values declined dramatically for the juice all three containers. During months four and five, the mean values rose and then declined between the sixth and seventh month. In the eighth month, the mean values rose again to values similar to those in the sixth month. Between months ten and twelve, there was yet another decline to the lowest value recorded in the study.

Although the mean values for juice in both the glass and the aluminum bottle values were almost equal throughout the duration of the study, the results for the PET bottles were significantly lower. Correlation results between a* values and pH or Brix degrees have negative values of (-0.43346) and (-0.26) respectively.

These results indicate that the PET bottle is the package type behaving differently over time (Figure 29). On direct observation, the inner layer of the bottle was found to have a red tint, which is possibly explained by migration of the pigment to the bottle walls. Additionally, the walls of the bottle were observed to easily delaminate when bottles were crushed. The supplier's explanation is that the oxygen scavenger may have been consumed over time, which could cause the ties between layers to loosen and compromise the bottle structure, as shown in Figure 28 (Personal communication 2006).

Figure 28. Picture of the PET bottle delaminating with tart cherry juice stored at room temperature (23°C, 50% Rh) after six months of storage.

The up and down pattern is not correlated to changes in the pH and solids in the juice (Figure 29). But, the changes in a* values are correlated (r=0.46) with the color change perceived by the trained panel during the course of the study.

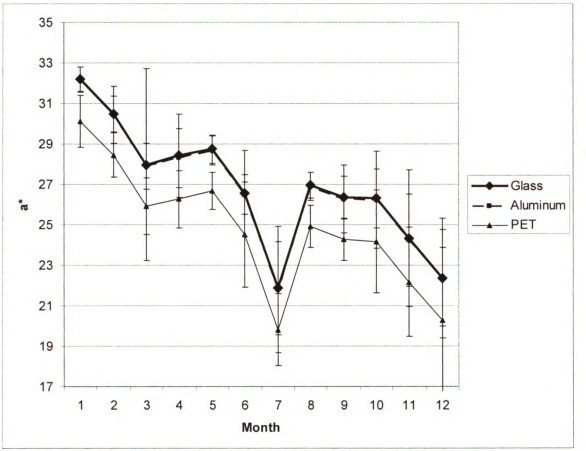


Figure 29. Changes in a* values (redness) of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.2.3. Changes in yellowness (b* values)

Oxidation would be evidenced by large amounts of yellow in the juice (Scaman 2005; Jiang et al 2004; Turker and Erdoğdu 2005). The b* value is a measure of the amount of yellow or blue in the tart cherry juice. No significant difference in mean b* values was found in any of the three packages.

The full ANOVA model showed that there was not an interaction between the month*treatment variable for the mean b* values represented by a p-value of 0.164 (Table 13 in Appendix 1). Additionally, there was no significant difference

between the mean a* values of the month*treatment variables for any of three the packages. Therefore, the hypothesis 1 was not rejected on the basis of yellowness.

The statistical means of the b* values for each of the three containers followed a trend that was similar to the L* values (Figure 30). At the second month the first increase in the mean b* values was observed. The mean b* values decreased during the following two months. At the fifth month the b* mean values rose to similar levels as the third month. Then the most significant decrease was found during the seventh month. From the eighth to ninth months, the mean values rose again to values similar to those in the fifth month. Between months ten and twelve, there was yet another decline until the values reached levels that were similar to those at the seventh month. But, throughout the duration of the study the mean values for the juice in three packages were almost equal. There was significant differences between months with the exception of month 5 and 6 and month 11 and 12, where the p-values were 0.940 and 0.307 respectively (Table 52 in Appendix 2).

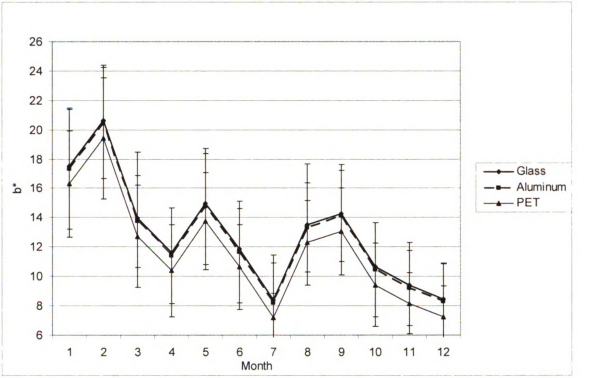


Figure 30. Changes in b* values (yellowness) of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

These results indicate that there were no traces of yellowness or oxidation. The juice was moving towards a more blue pigmentation and moving away from the yellow scale, which would have indicated oxidation (Fulcrand et al 2004). When red anthocyanins like cyanidin, pelargonidin or peonidin are co-pigmented with tannins they tend to turn more into the blue tones and this could be an explanation of why the tart cherry juice was turning more into the blue tones during the length of the study (de Freitas and Mateus 2006, Gomez-Cordovés 2004 and Eglinton et all 2004). These results were confirmed by the trained panel which identified that the juice had a more "wine-like", color or looked more purple or blue tending to a burgundy color, at the end of the twelve

months of storage. The correlation between b* values and the color question to the trained panel has a very weak positive relationship (r= 0.055).

In Figure 31 the positive relationship within these three parameters (L*a*b*) is shown. The correlation results are: L* and a* values r= 0.946, b*and a* values r= 0.832 and finally L*b* values= 0.783 showing a strong positive relationship between these three parameters.

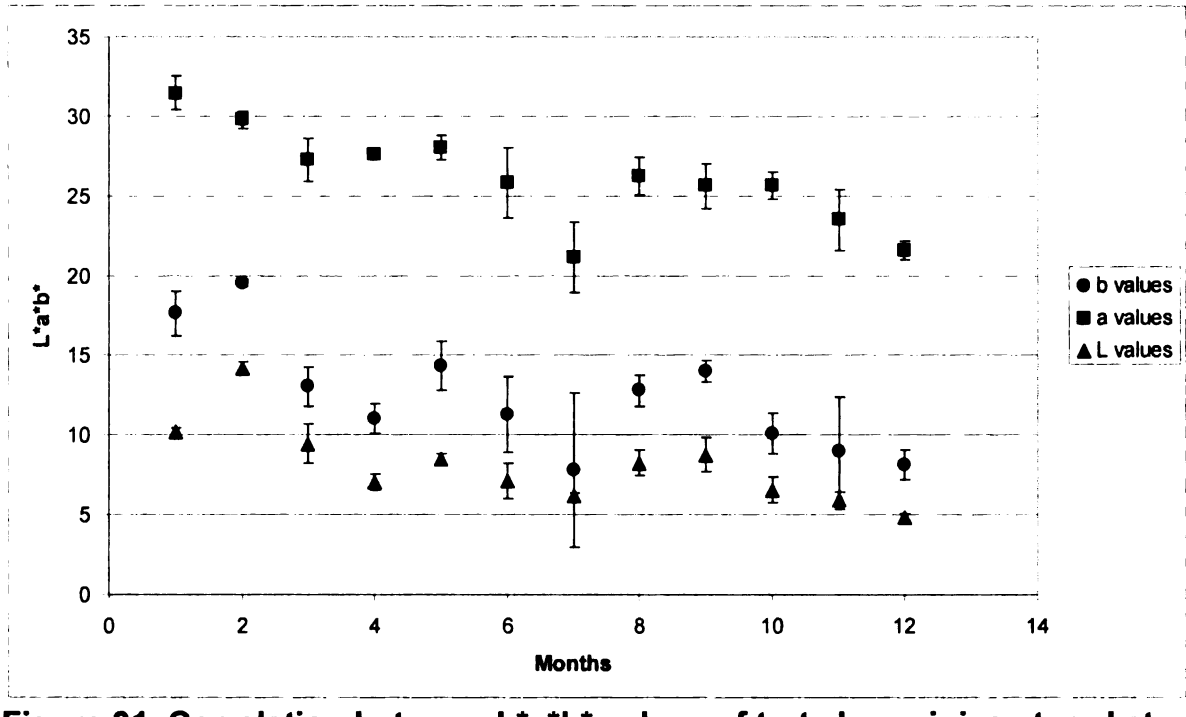


Figure 31. Correlation between L*a*b* values of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.2.4. Changes in chrominance

Chrominance is defined as the color attributes that include hue (predominant color) and saturation (amounts of color) (PC Magazine 2008). In other words chrominance is the judgment of the color compared to the brightness of the surroundings (MacDougall 2002). In this test the amount of red was evaluated to determine the development of any traces of brown hues as

evidence of oxidation. In this test, the PET package had a lower performance than the glass and aluminum bottles.

After calculating the chrominance with the formula $C= (a^{*2} + b^{*2})^{1/2}$, an ANOVA custom model was run, and both treatment and month variables were found to be statistically significant different with p-values below $\alpha= 0.05$. Additionally, no interaction was detected between the month*treatment variable. This significance meant that at least one of the containers was behaving differently, resulting in rejection of hypothesis 1 for chrominance (Table 14 in Appendix 1). ANOVA analysis was also run for the month variable determining that almost all the month were significantly different with the exception of month seven (p-value 0.97) and eleven (p-value 0.26) as shown in Table 30 in Appendix 2.

The statistical means of the chrominance values for each of the three containers followed the same trend found for the L* and b* values as shown in Figure 32. At the end of the second month the first increase in the chrominance mean values was observed. The mean chrominance decreased during the following two months and at the fifth month the chrominance values rose to similar levels as those during the third month. Then the most significant decrease was found during the seventh month. From eight to nine months, the mean values rose again to values similar to those in the fifth month. Between months ten and twelve, there was yet another decline until the valued reached a level similar to month seven. Throughout the year, the mean values for the three

packages values, were parallel to each other, but, the PET bottle had a lower performance.

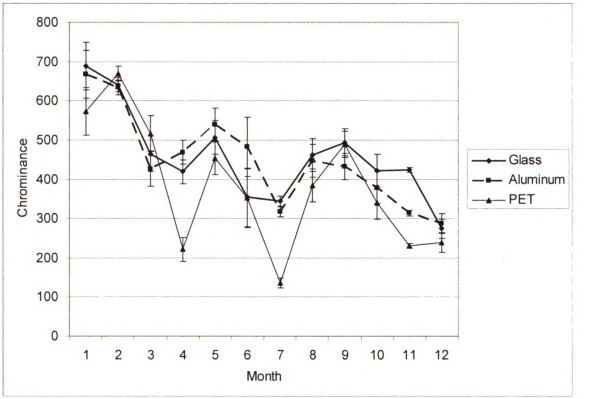


Figure 32. Changes in chrominance of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

The zigzagging pattern the curves followed can also be explained by the fact that the pH and solids in the juice were changing over time. However there was no evidence of oxidation, since the mean chrominance values returned to previous values at month five, eight and nine; and there was no consistent drop in these same values (Figure 32). These results were confirmed by the outcomes of pH, solids and anthocyanins tests and also by the trained panel.

Remón et al (2004) reported that cherries with CO_2 –enriched atmospheres have higher chrominance values than the ones with not modified

atmosphere and that the chrominance numbers can go up during the storage time. This might be a reason for the changes in chrominance.

There is a positive relationship between L* (r= 0.63), a*(r=0.5542), b* (r= 0.5195) values, hue (r= 0.4706) and ΔE (r= 0.2848), although, the relationship still not really strong.

4.2.5. Changes in hue

Hue represents the saturation of a specific color. Saturation is a measurement of colorfulness compared to its own brightness and purity. A pure color (with high hue) has only one wave length and in high amounts. Only pure colors can achieve purity; all other colors are a combination of them (Schanda 2007). It has been reported that the color intensity of pomegranate can increase intensity during storage thanks to the anthocyanin activity (Artés et al 2002). This research found that the tart cherry juice became darker because the red color turned into a more blue-red but with similar levels of saturation and chrominance. Fulcrand et al (2004) explained that anthocyanins which are co-pigmented with help of acetaldehyde turn wine into more of a blue-red color than the original red. Will and Dietrich (2006) find a similar reaction between anthocyanins and aldehydes creating a more blue-red color plum juices, and oxidized juices showed a distinct browning tendency. Freitas and Mateus (2006) describe how the interaction between acetaldehyde and benzaldehyde were responsible for the blue-red wine color. Bauer-Christoph et al (1997) reported 2.6 mg/100 ml of benzaldehyde in cherries, Mattheis et al (1997) explained how benzaldehyde results from the hydrolysis of amygdalins contained in the cherry

pits. Finally, Girard and Kopp (1998) characterized volatile constituents in cherries finding acetaldehyde and benzaldehyde present in cherries. Since anthocyanins and acetaldehyde and benzaldehyde are present in the juice it can explain the change from bright to a blue-red color. No browning was detected; therefore it can be concluded that probably there no oxidation or very slow oxidation rates were happening to the stored tart cherry juice.

In this project a full ANOVA model was run to evaluate the hue values for the month*treatment variable. This test resulted in a significant difference which indicated an interaction between the two variables. Then a custom model was run between the two variables, month and treatment. This custom ANOVA test resulted in no statistical significance for the treatment variables. There was not enough evidence to reject the hypothesis 1 on the basis of hue.

There was no statistical significant difference been containers in the hue measurements meaning that the juice in the three containers behaves similarly and had similar hue results (Table 16 in Appendix 1). Freitas and Mateus (2006) explained that the nature of the sugars (glucose, arabinos, rutinose) are also important to influence the hue pigments. But, the packaging material is not an influence to promote these changes since the three different materials have similar behavior. Therefore the ANOVA results for the month variable were not significantly different for months four (p-value 0.214), seven (p-value 0.141), ten (p-value 0.293) and eleven (p-values 0.817) as shown in Table 31 in Appendix 2.

The statistical means of the resulting hue values for each of the three containers (Figure 33) followed a trend similar to the L* and chrominance values.

At the second month the first increase in mean hue value was observed. The mean hue values decreased during the following two months and at the fifth month the mean hue values rose to levels similar to the third month. The most significant decrease was found during the seventh month. From the eighth to ninth months, the mean values rose again to values similar to those in the fifth month. Between months ten and twelve, there was yet another decline until the values reached a level similar to those at the seventh month. Throughout the duration of the study, the mean values for the glass and aluminum bottles were almost equal to each other (Figure 33).

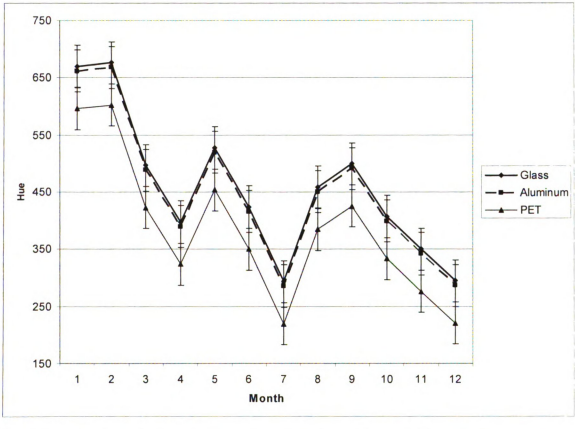


Figure 33. Changes in hue of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

Two possible reasons for changes in hue are: changes in anthocyanins sugar moieties and changes in acidity, both of which will be discussed later in this chapter.

The trained panel and consumers also noticed that the color was darker but no brown hues were identified. They were more inclined to describe the juice as blue-red.

4.2.6. Color change (ΔE)

Color change (ΔE) is expressed as the correlates of lightness (L*values), hue and chrominance (McDougall, 2002). Color change is a relationship between these three components and gives a general idea of how the color has changed through time. In all the data from L*a*b* values, hue and chrominance it can be observed that the lower point in the data set was always at month seven. In ΔE month seven represents the one of the highest amount of change in the color of the juice (Figure 34). ΔE has a positive relationship with correlation of L* (r=0.34), a*(r=0.266), b* (r= 0.198), Chrominance (r=0.28) and finally with hue (r=0.22). The strongest correlation of the change is with regard of the luminosity or transparency that suffers changes during the study.

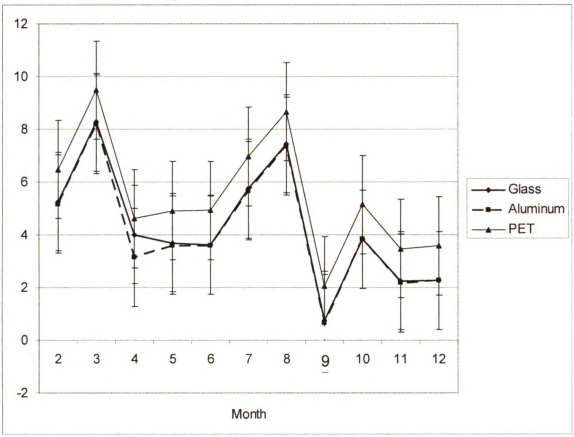


Figure 34. Color change (ΔE) of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.3. Changes in Solids

Sugar moieties can change forms through time, and it has been found that antioxidants play a very important role in changing soluble sugars. Sugars are reorganized and attach to the antioxidants under different types of glycosils (glucose, arabinose, rutinose) (Freitas and Mateus 2006). Hernandez et al (1999) described how sugars in pomegranate juice changed during storage and attributed these changes to the degradation in pectin. When pectin degrades soluble sugars can increase. Von Elbe and Schwartz (1996) describe how

fructose, arabinose, lactose and sorbose at low concentrations are less stable than glucose, sucrose and maltose, and they can change over time.

The soluble solids in the tart cherry juice changed throughout the twelve months of storage, and results show a significant difference between containers and time (Table 16 and 17 in Appendix 1 and Table 33 in Appendix 2).

With the full ANOVA model the variable month*treatment had a p-value below α=0.05, meaning there was a significant difference and the hypothesis 1 was rejected on the basis of solids (Table 34 in Appendix 2). Then a custom model was applied and the treatment was found to have a p-value below α=0.05, and month of 0.193 (Table 35); again the null hypothesis was rejected on the basis of solids.

The results from each container followed a similar trend, but a different level, as shown in Figure 35. The juice in the PET bottles had a higher amount of solids, in the range of 15.90 to 16.20 degrees Brix. The second highest was the juice in aluminum bottles which had values between 15.30 and 15.60 degrees Brix. Finally the juice in the glass containers was between 15 and 15.30 degrees Brix. The three containers were in a range of .30 degrees Brix. The largest decrease in Brix for the three containers was at the sixth month, but it then rose back to the original levels at the ninth month (Figure 35).

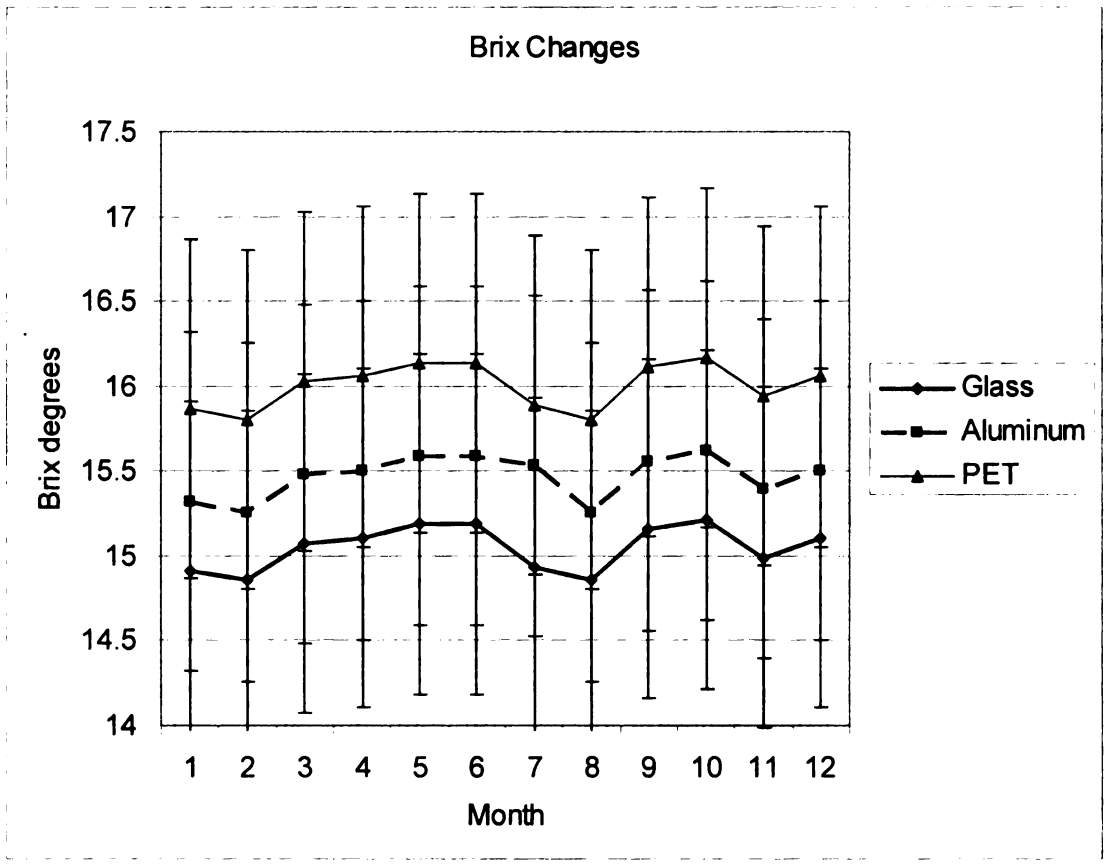


Figure 35. Changes in solids of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

The changes in solids overtime were similar to the changes in color which were discussed above, and with the changes taking place with the anthocyanins, which will be discussed later. However, there is negative statistical correlation between the different color parameters and the changes in Brix degrees.

It could be concluded that sugars were suffering changes in their sugar moieties, since they were going up and down in a zigzag - type curve (Paul and Palmer 1972). Von Elbe and Schwartz (1996) described that cyanidin 3-glucosyl-rutinoside is less stable and shorter life than cyanidin 3-rutinoside.

Freitas and Mateus (2006) in their description of physical-chemical properties explain that anthocyanins can have self-association or co-pigmentation resulting in different pH values and different sugars attached to the glycosil. The trained panel confirmed these results. They found that the tart cherry juice was becoming less tart and more sweet through time, but the correlation with the Brix degree show a weak relationship between the variables (Brix degrees and sweetness r= 0.169 and Brix degrees and tartness r= 0.138).

4.4. Changes in pH

The pH levels of the tart cherry juiced also changed during the twelve months of storage, but the tart cherry juice packed in the three containers behaved similarly over time.

As with the other tests, the pH data was analyzed with a full ANOVA model. The p-value for treatment*month was 0.170, greater than 0.05, and provided no statistical evidence to reject the hypothesis 1 in the basis of pH levels (Table 18 in Appendix 1). Therefore, the changes in pH levels were not statistically different for the three different containers (Table 18 in Appendix 1). For the month variable a consistent p-value for every month below α=0.05 was found when running the ANOVA model (Table 34 in Appendix 2). This statistical significance gives enough evidence to reject hypothesis 1 on the basis of the pH levels.

The data on acidity shows that the containers were behaving similarly, since the curves were very close to each other. But the changes over time were more dramatic. At the end of three months of storage, the tart cherry juice

suffered the most dramatic decrease in pH. At six months the pH value rebounded to almost the same values found at the beginning of the experiment, and at nine months the pH increased to the highest level during the evaluation time.

This trend was similar to the redness values and the solids curves providing a clue to the relationship between the three characteristics in the behavior of the tart cherry juice (Figure 36). Even though, the changes in the pH levels are not significantly different, it cannot be ruled out that change in the pH levels contributed to the changes in color. The relationship of anthocyanins with other components is very complex and difficult to describe in single parameters. The changes in pH could benefit the co-pigmentation of anthocyanins and change the colors (Will and Dietrich 2006, Freitas and Mateus 2006). Since there is not a significant difference between the different containers it can be inferred that the contact material plays no role in promoting the changes in pH levels.

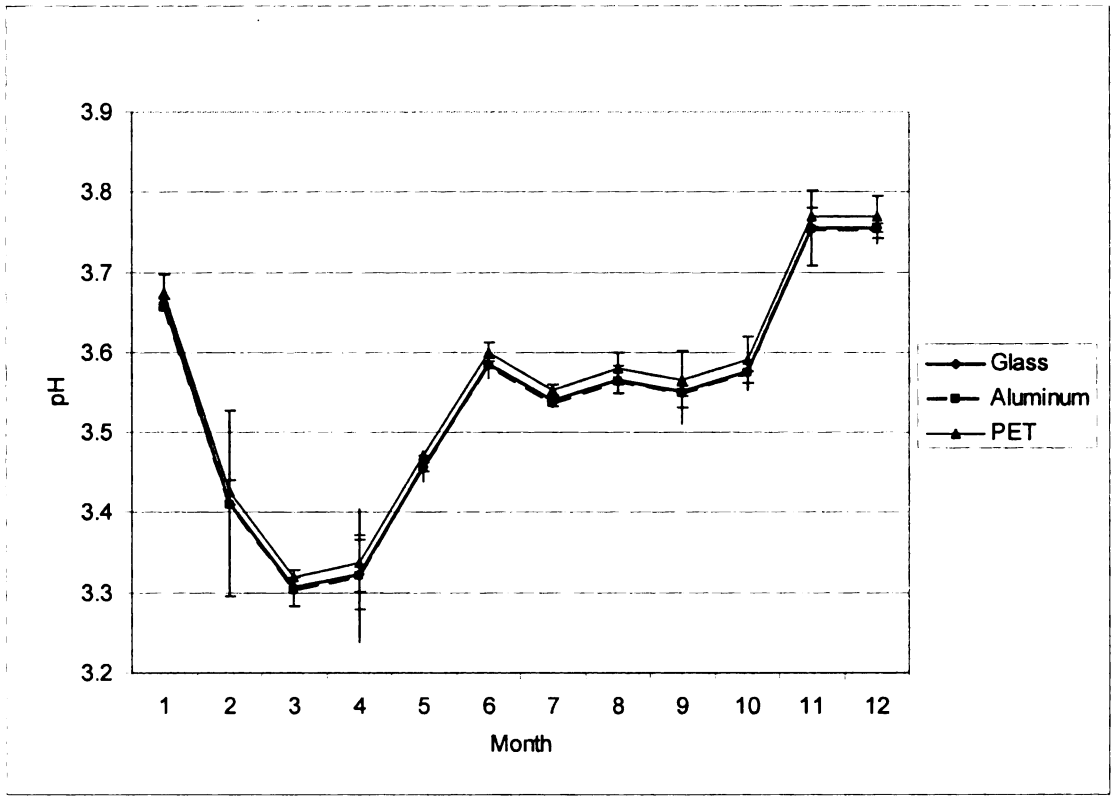


Figure 36. pH changes of tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.5. Changes in microbial growth

During the twelve months of storage no microbial growth occurred in any of the three bottles. High acid foods like tart cherry juice inhibit mold and yeast growth, and anaerobic environments like that in the sealed packages inhibit the pathogen growth most likely to occur in tart cherry juice. Since the pH level was always below 4.6 the tart cherry juice was considered a high acid food. The hot fill process plus the nitrogen flush done before capping the bottles were good methods for keeping the headspace oxygen-free. This not only created the correct anaerobic environment where most of the aerobic bacteria could grow, but also helped to prevent oxidation of the juice, as shown in the color evaluation.

The plates with glass, aluminum and PET bottle samples were always clean, with no traces of microbial growth.

However, the plates with samples from the stand-up pouch had yeast and mold beginning at the end of the six months. Probably this microbial growth started before but only appeared at the end of the end of the sixth month of storage. After eight months, stand-up pouch was suspended. This is the reason that this container was not included in the analysis. As mentioned previously the standing pouches could have been compromised by the lack of technology to process this type of packages, the lack of ability to flush with nitrogen and the possibility of being previously contaminated.

4.6. Changes in anthocyanin compounds

Anthocyanin content decreased in general during the twelve months of storage, confirming findings by Freitas and Mateus (2006), Will and Dietrich (2006) and Gómez-Cordovés (2004). The glass bottles retained the anthocyanin content of the tart cherry juice best, followed by the aluminum and finally the PET bottles. Changes in the anthocyanin content were correlated with other changes in color, solids and pH noted previously.

The anthocyanins most present in fresh tart cherries were expected to be: cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside, peonidin 3-rutinoside and pelargonidin 3-rutinoside with the largest concentration of cyanidin 3-rutinoside (Esti et al, 2002). Kim et al (2000) reported the presence of cyanidin 3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside in tart cherries.

Wang et al (1997) reported cyanidin and peonidin in tart cherries. Hong and Wrolstad (1990) reported only the presence of cyanidin in cherries.

In this research the tart cherry juice was found to contain the following anthocyanins: cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, delphinidin 3-glucosylrutinoside, delphinidin 3-rutinoside, peonidin 3-rutinoside, pelargonidin 3-rutinoside and petunidin 3-rutinoside. Cyanidin 3-glucosylrutinoside was the one found in the highest concentration in the tart cherry juice at the beginning of the experiment.

Cyanidin is responsible for the crimson color, pelargonidin for the scarlet-orange tones at low pH and the peonidin for the cherry red at low pH (Paul and Palmer 1972). The following section analyzes the levels of these anthocyanins present in the juice over time.

4.6.1 Changes in cyanidin

Cyanidin 3-glucosylrutinoside was the anthocyanin with the highest initial concentration in the juice but the levels fell dramatically over time as reported by Esti et al (2002); Kim et al (2000); Hong and Wrolstad (1990) and Wang et al (1997). Cyanidin 3-glucosylrutinoside had a retention time (RT) at 7.90 minutes. The glass bottles were found to preserve higher levels throughout the duration of the project, followed by aluminum and finally by the PET bottles, as shown in Figure 37.

There was an average decrease of 26 % for the three different packages during the twelve months of the experiment. The p-values for treatment and month alone were below $\alpha=0.05$ for most of the entries suggesting that at least

one of the packages did not behave as the other two (Table 19 in Appendix 1 and Table 35 in Appendix 2). Although, cyanidin 3-glycosilrutinoside decline was similar for all three containers, PET bottles retained the lowest amounts during the twelve months of the experiment.

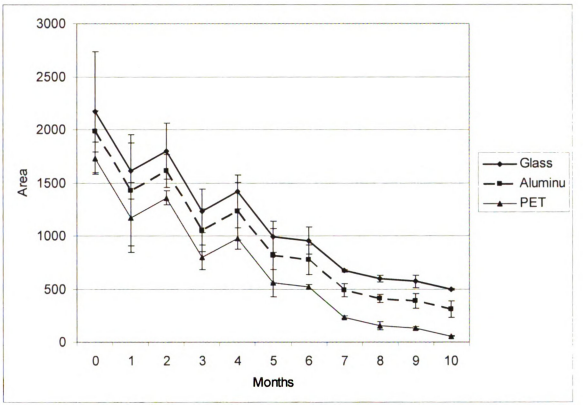


Figure 37. Changes in cyanidin 3-glucosylrutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

On the other hand, as cyanidin 3-glucosylrutinoside decreased, cyanidin 3-rutinoside (RT 7.98 minutes) increased by 33 percent as shown in Figure 38, which was seven percent more than the decrease in cyanidin 3-glucosyrutinoside.

These changes can be explained by the theory that over time antioxidants will change their glycosil attachments and change their attachment to different sugar moieties soluble in the juice (Von Elbe and Schwartz 1996 and Freitas and Mateus 2006). It can be assumed that the cyanidin lost the glucose moiety attachment and retained the rutinose moiety.

The ANOVA analysis of the mean results for cyanidin 3-glucosylrutinoside showed that there was no interaction between month and treatment with a p-value of 0.190 (Table 20 Appendix 1). Therefore, treatment and month separately had both p-values below $\alpha = 0.05$, which provides enough evidence to reject the hypothesis 1, because the juice in PET had consistently the lowest values of both kinds of cyanidin retention. The ANOVA analysis for each month resulted in a significant difference between the second, third and fourth month. All the other month came out with a p-value higher than 0.05 (Table 36 Appendix 2).

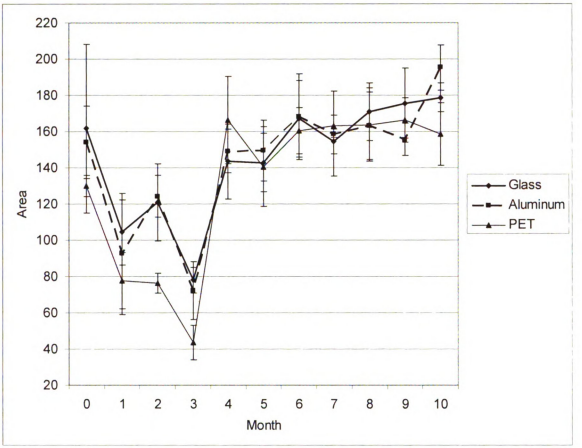


Figure 38. Changes cyanidin 3-rutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage..

Correlation analysis between the two types of cyanidin was done with strong negative relationship between the two of them (r=-0.56), meaning that when one was declining the other one was increasing, confirming the possibility of changes in the sugar moieties described previously, and shown in Figure 39.

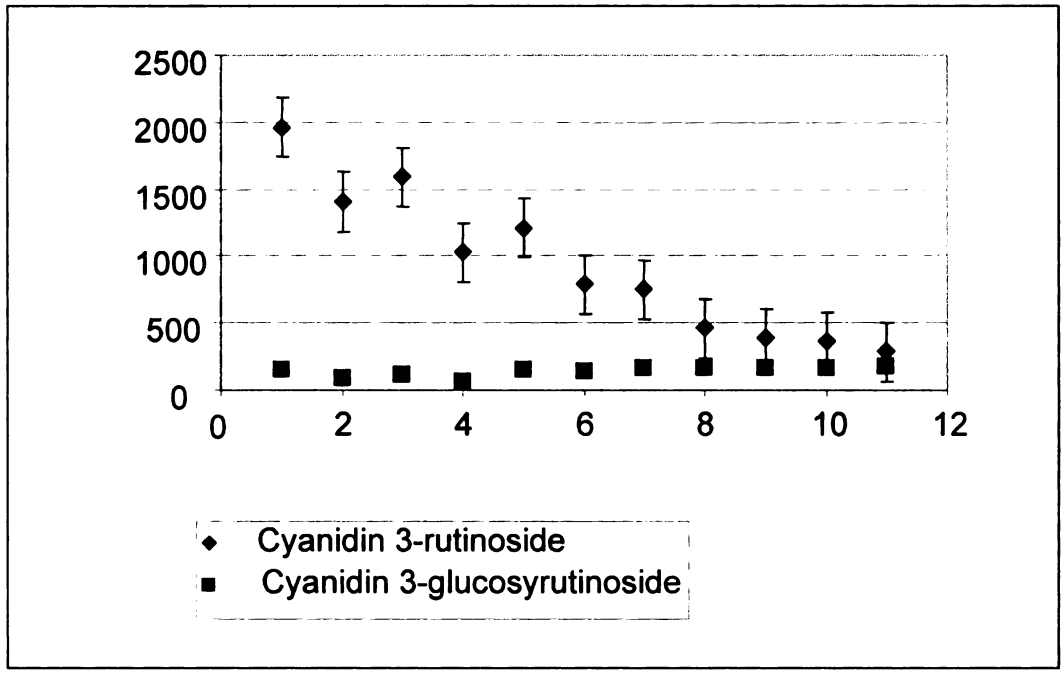


Figure 39. Correlation between the two types of cyanidin in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.6.2. Changes in delphinidin

For this compound the juice in all three containers behaved similarly. Delphinidin has the same two glycosil attachments (3-glucosilrutinoside and 3-rutinoside) as cyanidin, and had a similar behavior as well.

Delphinidin causes the blue-red tones in wines such as cabernet sauvignon, creating a more burgundy hue. This is different from the bright crimson caused by cyanidin. Contrarily to cyanidin, delphinidin 3-glucosylrutinoside was found in lower quantities than delphinidin 3-rutinoside, and while one decreased the other one increased in ways similar to the cyanidin (Figures 38 and 39). In the case of this compound, with both of the glycosil residues, there was no statistical significance between packages over time, and so the null hypothesis cannot be rejected. The p-values for month*treatment

were 0.447, and 0.653 for the treatment alone, as shown in Table 21 in Appendix 1. The ANOVA results for the month variable show that there was no statistically significant difference between months with p-values below $\alpha = 0.05$ as shown in Table 38 in Appendix 2.

Delphinidin 3-glucosylrutinoside increased by 68% during the first four months but then had a drastic loss of forty eight percent at the fourth month. From the sixth month it increased steadily to 46 % of the total value and stayed at similar levels to those at which it started (Figure 40).

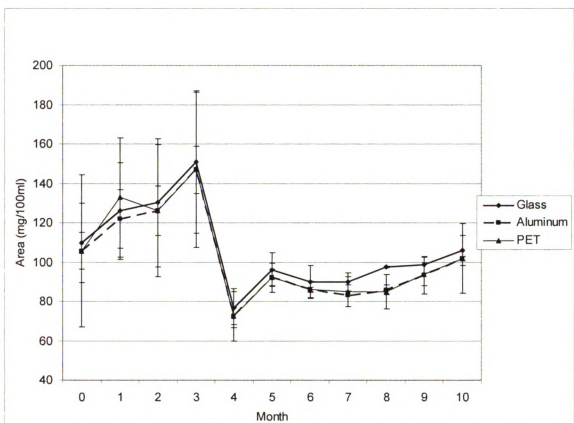


Figure 40. Changes in delphinidin 3-glucosylrutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

Delphinidin 3-rutinoside, on the other hand, was the compound with higher concentration in the delphinidin family, and its behavior was equally dramatic. In the first five months the compound almost doubled. At the sixth month a considerable drop was apparent when approximately 50 percent of the compound decreased. At the seventh month the compound regained about 50 percent decreased previously, and at the end of the storage time with a total decrease of 25 percent (Figure 41).

The results of the ANOVA full model analysis showed that there was no statistical significance between treatment*month and treatment alone, showing that for this compound the packaging material was not a reason for the changes in concentration (Table 22 in Appendix 1). The p-values obtained for treatment variable were 0.933 and 0.967 for the month*treatment (Table 22 in Appendix 1). For the month variable the ANOVA gave as results a significant difference for the first two months with p-values below $\alpha = 0.05$. For the other months all the p-values obtained were above 0.05 (Table 38 in Appendix 2).

The higher levels of delphinidin 3-rutinoside at the end of the storage time can also explain why the color of the tart cherry juice changed to a more burgundy (wine-like color) compared to the bright crimson color of the fresh juice. Like cyanidin, delphinidin 3-glucosilrutinoside lost the glucose moiety and kept the rutinose.

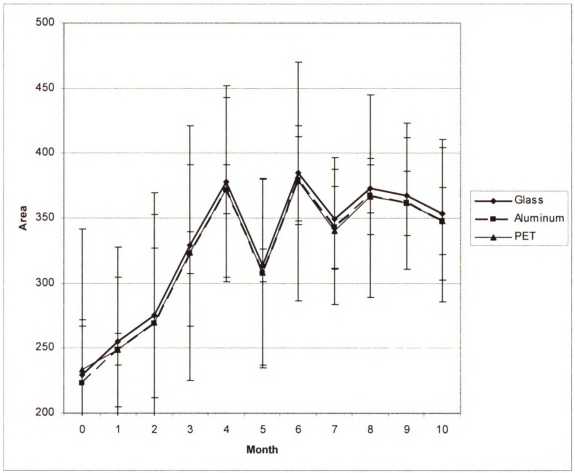


Figure 41. Changes in delphinidin 3-rutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

There is a negative relationship between delphinidin 3-glucosylrutinoside and delphinidin 3-rutinoside (r= -0.56) showing that when one decreased the other one increased correlated (Figure 42).

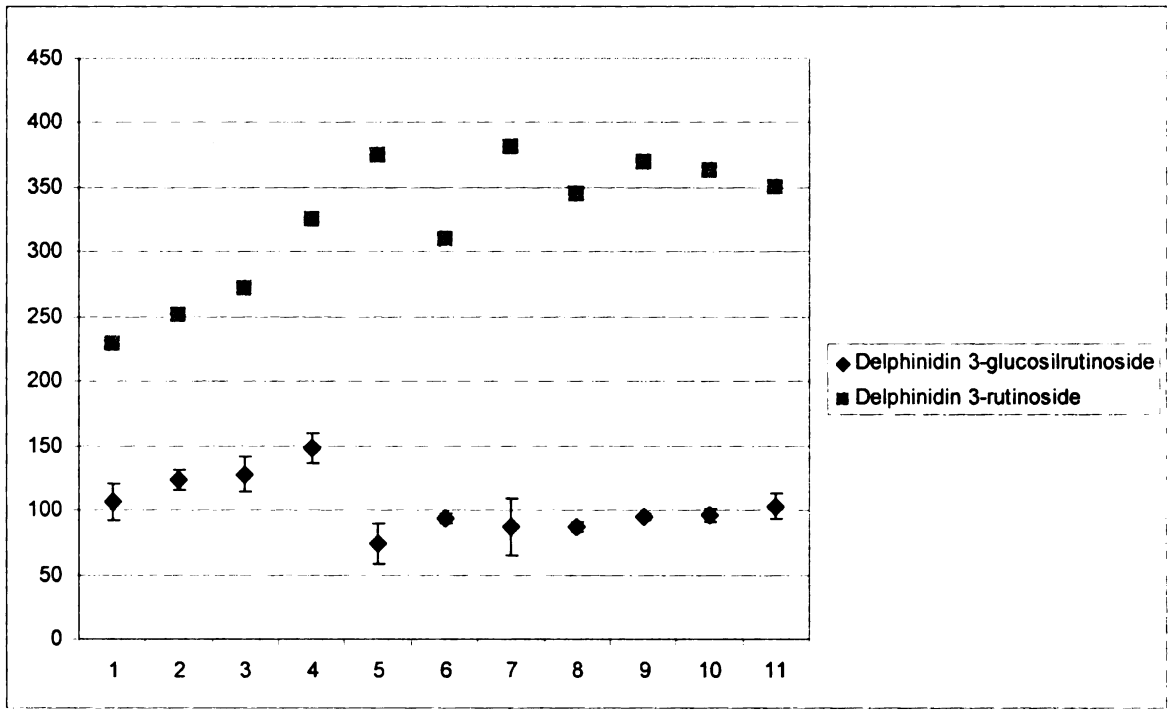


Figure 42. Correlation between the two types of delphinidin in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.6.3. Changes in peonidin 3-rutinoside

Peonidin is a blue pigment that can be found in flowers like morning glories and in blueberries. The juice in all three packages behaved similarly with respect to peonidin 3-rutinoside. The outcome of the ANOVA full model analysis found that there was no statistical significance between the different containers (Table 23 in Appendix 1). The p-value for the treatment*month interaction was 1.000 and 0.860 for the treatment. The p-value for the month variable was all superior to 0.05 meaning that there was not significant difference between months (Table 39 in Appendix 2).

The peonidin values increased dramatically after the forth month. This result correlates with the drop in redness (b* value) in the fourth month. In the next month it oscillated between 100,000 and 125,000 mg/100 g, maintaining a blue tint or a wine-type color for the juice (Figure 43).

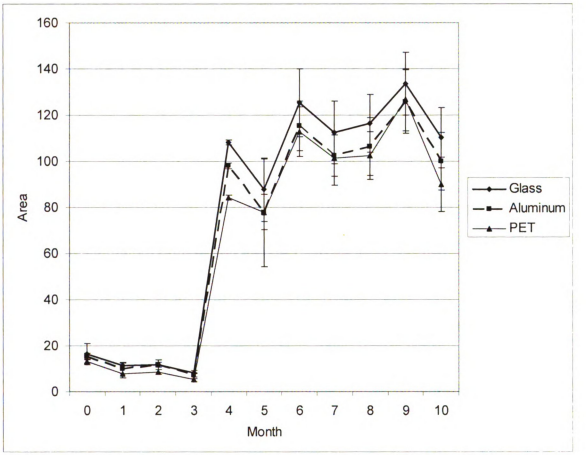


Figure 43. Changes in peonidin 3-rutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.6.4. Changes in pelargonidin 3-rutinoside

Glass bottles had better performance maintaining the pelargonidin compound in the tart cherry juice, followed by aluminum bottles and finally the

PET bottles. Pelargonidin is responsible for the orange tones in the juice, similar to red geraniums, red strawberries and tart cherries.

The changes in this compound were statistical significant. Treatment (different containers) was also significantly different, since the month*treatment interaction had a p-value of .001 in the full model (Table 24 in Appendix 1). In the custom model both month and treatment had p-values below $\alpha = 0.05$ implying that they are statistically different and that at least one container were behaving differently to the others (Table 25 in Appendix 1). The p-values obtained for the month variable were above 0.05 resulting as no significant difference between months (Table 40 in Appendix 2).

The glass bottles best maintained pelargonidin in the tart cherry juice, followed by aluminum bottles and the PET bottles shown in Figure 44. At the end of the fifth month, the compound tripled for the juice in three containers and oscillated between 125 mg/100ml and 175 mg/100ml until the end of the experiment (Figure 44). Pelargonidin followed the same trend as the other anthocyanins in that the compound values increased in the fifth month.

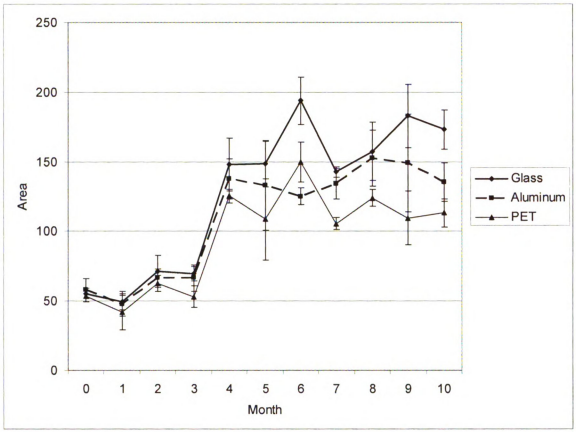


Figure 44. Changes in pelargonidin 3-rutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.6.5. Changes in petunidin 3-rutinoside

The three different containers behaved similarly for retaining the petunidin compound. Petunidin 3-rutinoside, as pelargonidin 3-rutinoside, peonidin 3-rutinoside and delphinidin 3-rutinoside, increased by one 100 percent in the fifth month and stabilized at the eighth month (Figure 45). Petunidin provides a bluish tint to the tart cherry juice, and its increase correlates with the color changes. The juice turned to a more wine-like color due to the increase of the blue pigments and the decrease in the red color.

This compound was not found to be affected by the type of package, since the differences between packages was not statistically significant. The p-values

obtained with the ANOVA full model were 0.116 for month*treatment and 0.505 for treatment (Table 26 in Appendix 1).

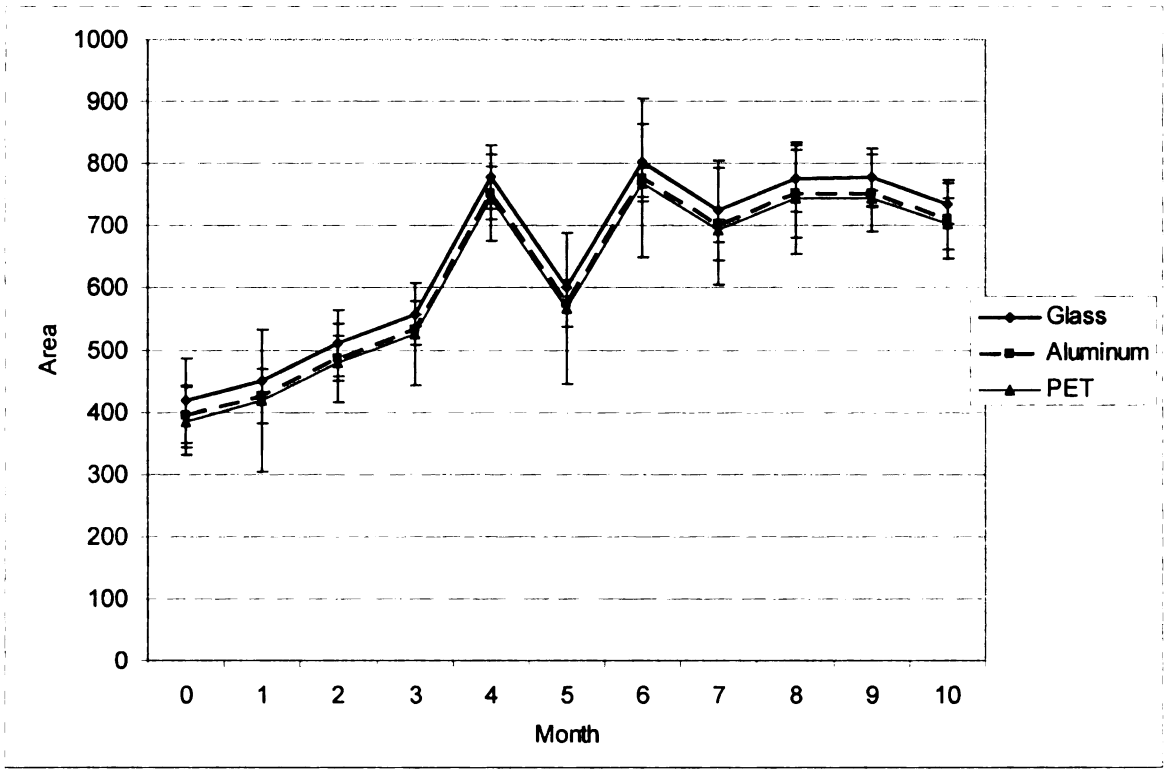


Figure 45. Changes in petunidin 3-rutinoside in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.6.6. Anthocyanin result summary

As it can be observed all the decreases or increases in anthocyanin content began to happen after two months of storage. This confirms Will and Dietrich (2006) findings that anthocyanin changes occurred during storage and more drastically after six months of storage. They describe that this change might be caused by other reactions happening in the juice. They also explain how new anthocyanins and anthocyanins attachments are created during aging or storage due to polymerization.

Changes in color were noticeable, but very little traces of oxidation are present since the anthocyanins were not eliminated or used up in free radical reactions. In the color section it was discussed how the color of the juice changed to a more blue or wine-like color, and this phenomenon is consistent with the changes in anthocyanins. Cyanidin was responsible for the total decrease of bright red color (considering cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside) which decreased by seventy five %, retaining only twenty five % of the original combined amount. Both types of delphinidin, peonidin 3-rutinoside, pelargonidin 3-rutinoside and petunidin 3-rutinoside significantly increased in the fifth month. Delphinidin combined increased by 39 percent; peonidin 3-rutinoside and pelargonidin 3-rutinoside increased 500 percent. Finally, petunidin was found to have a 200 percent increased.

Since the red-orange colored anthocyanins are less stable than blue-purple ones, and both categories are present in tart cherry juice, this can explain the shift from bright red from fresh juice to a blue-red color of the stored juice (Von Elbe and Schwartz 1996). After averaging, red and blue anthocyanins they were correlated with an r-value of 0.07 and as shown in Figure 46 when the red decreased the blue increase.

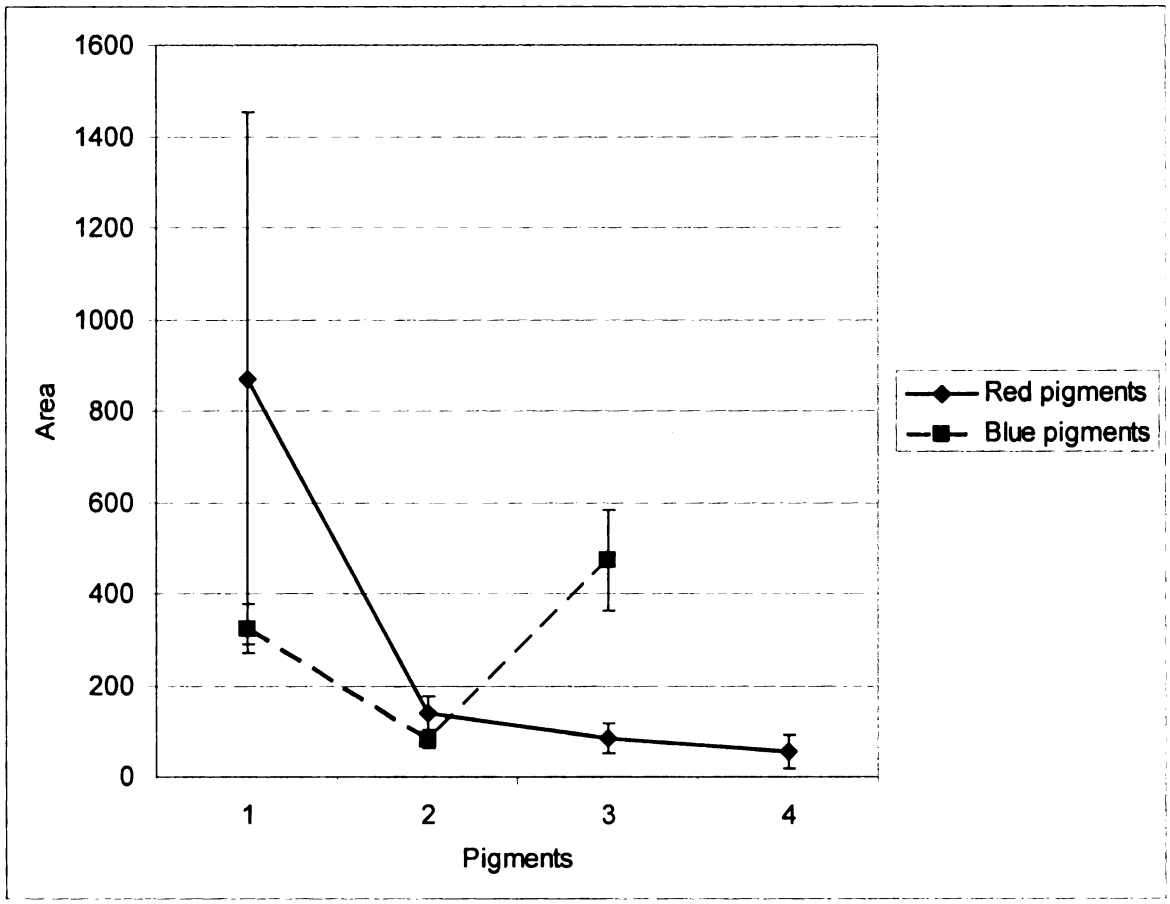


Figure 46. Correlation between red and blue anthocyanins found in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

Anthocyanins can polymerize or co-pigment in the presence of acetaldehyde or benzaldehyde. Both volatile compounds are present in tart cherry juice (Freitas and Mateus 2006, Gomez-Cordoves 2004) and they will develop a blue-red tint in juices containing anthocyanins. This polymerization is then supported by changes in the sugar moieties attached to the different anthocyanins.

Not all of the anthocyanins analyzed had a significant difference related to the packaging material. Only cyanidin and pelargonidin changes were correlated

by the different packaging materials used in the experiment. And, both of these are responsible for the bright red tones. The other anthocyanins which are responsible for the blue tones in the juice did not have an interaction with the packaging materials.

The amount of total anthocyanins at zero, six and twelve months is presented in Figure 47. There is no statistical difference between the different times.

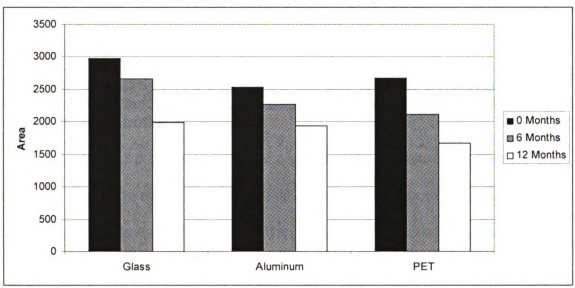


Figure 47. Accumulation of all anthocyanins in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

4.7. Trained panel results

The trained panel stated that there was not significant difference and that the overall quality of the juice was good for consumption throughout the year. The color was always perceived as good, as long as it was not compared with a fresh sample. The aroma was still perceived as "fresh" at the end of the twelve months of storage. The panel also agreed that the sweetness increased through time and the tartness decreased. There is a positive correlation between the changes in the sweetness and tartness perception with r= 0.44 (Figure 48).

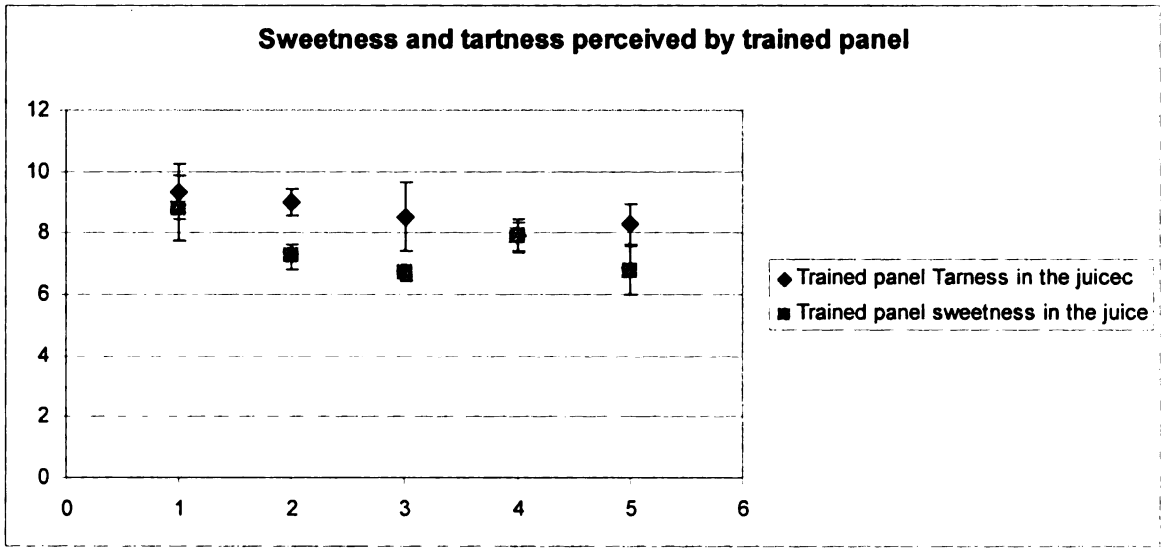


Figure 48. Correlation between sweetness and tartness perceived by the trained panel in tart cherry juice stored at room temperature (23°C, 50% RH) during twelve months of storage.

The trained panel was asked at each session, in different order of samples, about the same characteristics: color, aroma, mouth feel, sweetness, tartness, cherry flavor and overall quality. A copy of the survey is attached in Appendix 3.

4.7.1. Color perception

At the end of twelve months of storage, despite the changes in color, the trained panel agreed that the juice color remained pleasant and not too dark. First, the panel evaluated the color of the juice, where 0 was too light and 15 was too dark. They were asked to evaluate the juice by color and to compare it with the fresh juice on which they had been trained.

The p-value obtained for month and treatment were below the $\alpha=0.05$, meaning that there was a significant difference and enough evidence to reject hypothesis 1. The three containers behaved differently, with aluminum being the

option that best preserved color, followed by glass and then PET bottles from the trained panel observation (Table 8). Since this is a panel perception (qualitative variable) it can be different from the analytical results that are exact measurements. The analytical results showed that glass maintained better color followed by aluminum and PET was the last one.

Table 8. Color perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	2.3810	0.8120	2.9323	0.0041	yes
October-06	1.0648	0.7623	1.3968	0.1652	no
November-06	-0.4278	0.7623	-0.5611	0.5758	no
January-06	0.6429	0.8120	0.7917	0.4302	no
February-07	0.0000	.	.	.	
Glass	1.9325	0.6076	3.1805	0.0019	yes
Aluminum	2.4950	0.6076	4.1062	0.0001	yes
PET	0.0000	0.6076	3.2436	0.0005	yes

4.7.2. Aroma perception

The trained panel found that the aroma of the tart cherry juice softened over time. The second question regarded the aroma of the juice. The panelists were asked to sniff the samples and to evaluate the aroma of the juice on a 15-point scale, where 0 was too soft and 15 too strong.

The p-values for month and treatment were below the α=0.05 when running an ANOVA full model analysis. Both numbers were smaller than α= 0.05 which provides enough evidence to reject hypothesis 1. At least one of the packages behaved differently than the others. Glass and aluminum had very similar curve trends and numbers. But the PET bottles retained less of the

aroma compounds in the cherry juice and might be due to the polarity difference between PET and the aroma compounds.

The aroma began in the strong range and remained there only decreasing to a medium level at the end of storage for all 3 packages. Even after a year it was still in the higher end of medium, and so the juice was not bland or without any aroma (Table 9).

Table 9. Aroma perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	3.2810	0.7653	2.9870	0.0003	yes
October-06	1.0988	0.8764	1.4569	0.0020	yes
November-06	0.3456	0.9654	0.4999	0.0499	yes
January-06	0.6429	0.7659	0.8760	0.0444	yes
February-07	0.0043		.	.	
Glass	1.8970	0.6076	3.4561	0.0017	yes
Aluminum	2.6543	0.6076	2.0987	0.0002	yes
PET	0.0000	0.6076	3.3323	0.0003	yes

4.7.3. Mouth feeling perception

The panelists found that at the end of the twelve month of storage that the juice had some precipitate or sediments at the bottom of the glass similar to those found in wine.

Feeling in the mouth was used to indicate the smoothness or graininess of the juice. Clarified (juice with no pulp) tart cherry was used for all of the experiments, therefore residues formed at the end of storage due to the changes in antioxidants, pH and solids. The main reason for the sediment in the juice is the polymerization of the antioxidants (Alper et al 2004).

There was no statistically significant evidence to reject hypothesis 1, since the p-values for time and treatment were 0.692 and 0.874 respectively. These numbers provide enough confidence to assume that all three packages behaved similarly and that the observed changes were not different statistically.

The graininess trend for juice in the three containers followed a very similar pattern with one exception: the graininess increased greatly in the aluminum bottles during the ninth month of storage, only to drop below the others by the end of the experiment. However, the increase in panel perception results was not statistically significant (Table 10). Since these are perceptions from the trained panel it might be the reason for the variation.

Table 10. Mouth feel perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	0.1399	0.9952	0.1406	0.0490	yes
October-06	0.1319	0.9344	0.1412	0.0089	yes
November-06	-0.7292	0.9344	-0.7804	0.4368	no
January-06	-0.9435	0.9952	-0.9480	0.0340	yes
February-07	0	.	.	.	
Glass	-0.3187	0.7448	-0.4280	0.0007	yes
Aluminum	-0.3500	0.7448	-0.4700	0.0007	Yes
PET	0.0000	0.7448	-0.4700	0.0007	.yes

It is interesting to observe that the values for the mouth feel always remained at upper levels of graininess, and the juice was not considered smooth, but not considered unpleasant to the taste, it was more referred as "body" like in wine.

4.7.4. Sweetness perception

Predictably for tart juice, the trained panel found that the juice was in the "not sweet" ranges. They also found that the sweetness levels changed through time. At the end of the experiment, the juice was perceived as less sweet than the fresh tart cherry juice.

One of the major constraints of tart cherries is the low degree of sweetness, with which some consumers are not familiar. All major juices in the American market tend to be sweet (some think overly sweet) to please consumers. The juice used in this research was 100% tart cherry juice, with no sweetener additive or sugar, which is why this question was critical.

When the ANOVA full model statistical analysis was run with the data obtained from the trained panel, a p-value of 0.431 for treatment and 0.046 for month were found. These results reveal that there was no perceptible difference between juices packed in the different packaging materials, and that all three packages maintained a similar pattern of behavior for the sweetness of the juice.

It can be observed that the behavior of the three packages appears close to each other in range of sweetness of the scale but through time the perceived sweetness of the juice decreased (Table 11) since it started in the scale, at ten and ended at six.

These results can be correlated with the changes observed in the changes of the solids and in the anthocyanins, especially for cyanidin and delphinidin which changed their glycosil attachment after five months of storage and can result in different perception than with fresh tart cherry juice.

Table 11. Sweetness perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	2.0893	0.8269	2.5268	0.0129	yes
October-06	0.4491	0.7763	0.5785	0.5641	no
November-06	-0.1620	0.7763	-0.2087	0.8350	no
January-06	1.1012	0.8269	1.3318	0.1856	no
February-07	0.0000	0.8269	1.3318	0.1856	no
Glass	-0.7250	0.6188	-1.1717	0.2438	no
Aluminum	-0.0187	0.6188	-0.0303	0.9759	no
PET	0.0000	0.6188	-0.0303	0.9759	no

4.7.5. Tartness perception

The trained panel found that the tart cherry juice became less tart by the end of the experiment.

Similar to the sweetness of the juice, it was important to measure the degree of tartness of the juice for reasons of consumer preferences. If the juice was too tart consumers would not buy it nor find it pleasurable to drink. The right balance between sweetness and tartness if desirable since this activates more gustative cells in the tongue and mouth.

ANOVA results show that the treatment had a p-value of 0.439 and month of 0.335. These values were not significantly different and there was not enough evidence to reject the null hypothesis: all three packages behaved similarly with regards to the tartness in the juice over time. The pH results were similar to these findings, since the packaging materials behaved similarly and did not interact with the tart cherry juice.

The mean curves plotted show a change in acidity in patterns that were very similar for all three packages (Table 12), although the juice in glass started

with the higher levels of acidity and ended with the least tart samples. PET and aluminum bottles behaved similarly and very close to each other.

Table 12. Tartness perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	2.0893	0.8269	2.5268	0.0129	yes
October-06	0.4491	0.7763	0.5785	0.5641	no
November-06	-0.1620	0.7763	-0.2087	0.8350	no
January-06	1.1012	0.8269	1.3318	0.0186	yes
February-07	0.0000	0.8269	1.3318	0.0186	yes
Glass	-0.7250	0.6188	-1.1717	0.2438	no
Aluminum	-0.0187	0.6188	-0.0303	0.9759	no
PET	0.0000	0.6188	-0.0303	0.9759	no

4.7.6. Fresh cherry flavor perception

The trained panel found that the juice lost some of its fresh cherry flavor. Fresh cherry flavor is characterized by a benzaldehyde compound that is the cherry or almond flavor most commonly used for artificial flavoring. Benzaldehyde generally decreases and loses power over time and can be transformed into acetaldehyde which will release some fermented notes to the cherry flavor due to lower levels of oxidation.

The p-values obtained for treatment were 0.205 and 0.390 for month. These differences were not statistically significant, and so the null hypothesis cannot be rejected. All three containers behaved similarly in maintaining the fresh cherry flavor, although aluminum was found to slightly better retain the fresh cherry flavor, followed by glass and then the PET bottles (Table 13).

Table 13. "Fresh cherry flavor" perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	0.9259	0.8705	1.0637	0.2897	no
October-06	0.7407	0.8705	0.8510	0.3966	no
November-06	0.2857	0.9271	0.3082	0.7585	no
January-06	0.0000	0.8765	0.4567	0.6543	no
February-07	0.4938	0.6938	0.7117	0.4781	no
Glass	0.9563	0.6938	1.3783	0.1708	no
Aluminum	0.0000	0.3498	1.2450	0.1567	no
PET	0.0000	0.3498	1.7654	0.2345	no

4.7.7. Off-flavor perception

In general the trained panel did not found any strong off-flavor from any of the containers. Off-flavor is always a very important parameter to evaluate when developing a new product because it can be bad for human consumption or will create a bad taste which consumers will reject. Glass, being an inert material, was not expected to produce any off-flavor. However, the other two packages (aluminum and PET) might produce an off-flavor or react with any chemical component from the juice. Some panelists identified some different flavor more often in aluminum and PET bottles than in glass bottles (Table 14).

Aluminum bottles have a polymer base coating that may be released and give an off flavor to the juice. Also if there are pinholes in the polymeric coat, the aluminum can chelate with the acidity of the cherry juice and add a metallic off flavor to the juice. Straws (2005) reported the interaction of aluminum containers with polymeric liner with fruit juices to cause flavor loss and chelating.

For the PET bottles, an off flavor can come from different possibilities -- from sorption of any PET residues, from the oxygen scavenger, plasticizers or adhesives.

Finally the off-flavor may have come from the changes in the flavor notes of the cherry juice itself. The changes from benzaldehyde to acetaldehyde can create an off-flavor as well.

Table 14. Off-flavor perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	Aug.06		Oct. 06		Nov.06		Jan.07		Feb.07	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Glass	2	5	2	6	2	6	3	3	3	4
Aluminum	2	5	2	6	5	3	3	3	5	2
PET	2	5	6	2	5	3	4	2	2	5
Stand-up Pouch	3	4	6	2						
n=		7		8		8		6		7

4.7.8. Overall flavor perception

The trained panel found that the juice retained the overall expected flavor and was pleasant to their palate throughout the year of testing.

The overall flavor is the total impression of the product and how it is perceived by consumers. For this question the trained panelists evaluated the quality of the juice.

An ANOVA full model analysis produced the following results for this question: p-values were 0.164 for month and 0.174 for treatment. This numbers were greater than $\alpha = 0.05$, and so there is no statistical significance to reject the null hypothesis.

All three packages behaved similarly and end results were above the middle point in the scale (Table 15). One of the limitations is that the trained

panel was trained with the fresh cherry juice, and panelists were asked only to report changes perceived in the product. They were not asked about their preferences. Questions about preferences were posed in the consumer's preferences test, and results will be discussed in section 4.8.

Table 15. Overall flavor perception by trained panel of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months.

	B	Std. Error	t	P-value	Sig
August-06	0.8976	0.8234	1.0798	0.3450	no
October-06	0.7890	0.8456	0.8976	0.3678	no
November-06	0.3456	0.9876	0.2897	0.8965	no
January-06	0.0000	.	.	.	no
February-07	0.5432	0.7498	0.7654	0.5632	no
Glass	0.9654	0.7435	1.4567	0.2345	no
Aluminum	0.8754	0.4567	1.2435	0.3456	no
PET	0.0010	0.4567	1.8934	0.2367	no

4.7.9. Purchasing appropriateness perception

About half of the trained panel reported that the juice was still good enough to purchase after twelve month of storage and half believed the opposite. This last question addressed whether or not the trained panel considered that the juice had retained enough characteristics for consumers to enjoy the juice.

Of course, it is important to remember that the panel had been trained with the fresh cherry juice and had been evaluating the juice throughout the duration of the project. Therefore this last question does not reflect general consumer's preferences or purchasing intentions. Further work is needed it to evaluate this criteria, and other parameters of the marketing mix (product, place, price and promotion) will influence the decisions to purchase or not.

Again for this question the panel tended to like the juice packed in the aluminum bottles the best, with glass and PET following directly in that order. The least preferred juice was packed in the PET bottle. Contrarily to the initial concept that glass would be the least inert and would better protect the juice and retain better quality, glass fell below the values for the aluminum bottle (Table 16).

Table 16. Is this sample's of tart cherry juice stored at room temperature (23°C, 50% RH) for twelve months, quality appropriate for consumers to purchase the drink?

	Aug.06		Oct. 06		Nov.06		Jan.07		Feb.07	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Glass	5	2	7	1	8	0	5	1	4	3
Aluminum	5	2	7	1	6	2	6	0	5	2
PET	6	1	6	2	6	2	3	3	4	3
Standing Pouch	2	5	1	7						
n=		7		8		8		6		7

4.8. Consumer preferences results

Surprisingly, the non-trained consumers preferred the juice stored for twelve months over the fresh tart cherry juice. But, they preferred the color and texture of the fresh tart cherry juice. Aroma, flavor, overall flavor and overall acceptability were equal for both samples.

In a preference test, over one hundred participants compared fresh juice to juice that had been stored for twelve months. Six major factors were analyzed: appearance, aroma, flavor, texture, overall flavor and overall acceptability.

The consumers' data showed no statistical perceptible difference in aroma, flavor, overall flavor and overall quality. The scores were very similar (Table 17). Appearance and texture were perceived better for the fresh juice

than for the stored juice when compared directly, with p-values of 0.0007 and 0.0016 respectively. Consumers commented that they would not be disliked by the color and texture of the juice that was stored for twelve months if they did not compare it directly with the fresh juice.

There was no statistically significant difference in perceived aroma, flavor, overall flavor and overall acceptability of the juice, and no strong difference between fresh and stored juice (Table 18). Appearance and texture scores were greater for the fresh juice; the difference with the twelve month old juice was statistically significant at alpha level 0.05 (Table 17).

Finally, the consumer rankings show that the twelve month old juice was preferred to the fresh juice, as shown in Table 8, with a statistically significant difference. It seems that with all of the changes in the juice during the storage time, flavors developed and aged in a way that seemed to be more pleasant to consumers.

Table 17. Means of consumers' preferences results of fresh tart cherry juice and twelve month old juice in glass and aluminum containers.

	12 month old stored tart cherry juice	Fresh tart cherry juice	P-Value	Sig
Appearance	7.29	7.72	0.0007	yes
Aroma	6.35	6.35	1	no
flavor	6.34	6.74	0.0746	no
Texture	5.9	6.6	0.0016	yes
Overall flavor	6.34	6.52	0.4457	no
Overall acceptability	7.05	7.02	0.8692	no

Table 18. Ranking results results of fresh tart cherry juice and twelve month old juice in glass and aluminum containers.

	12 month old stored tart cherry juice	Fresh tart cherry juice	P-Value	Sig
Ranking	1.64	1.36	0.0051	yes

IV. CONCLUSIONS

Since human nutrition has received more emphasis, the stability of functional food components, like the anthocyanins in tart cherry juice, are becoming more and more important for consumers and for the industry to understand. This is particularly true for food manufacturers who seek to identify the level of such beneficial compounds on food package labels.

Packaging is a very important part of every new product, and it must be understood from several perspectives to minimize the risks of launching or keeping a product in the market. It will be very important to analyze packaging from all the possible interactions: product/package, product-package/consumer, product-package/distributor, product-package/retailer, product-package/environment, and product-package/transport. These last two interactions are becoming more important every day. This research aimed to show how important it is to consider all of these points of view when developing a new product. Tart cherry juice undergoes many changes during twelve months of storage. Therefore, it is important to understand these changes in order to choose the best package to commercialize the product.

It is important to conclude as well, that the link between academia and industry was successfully attached since the results were well accepted by the target group. The research provided the industry with clue elements to help them minimize the risks when bottling tart cherry juice.

Not all the packages used provided the same result, even though each was chosen using the same criteria. They are good barriers against humidity and

oxygen, and have good reclosability and commercial availability. But it has been shown that the properties of the cherry juice will go through different slightly patterns of change in each of the different containers.

Some of the changes observed in the juice were not related to the type of container, since they took place in all of the packages at the same levels: transparency, yellowness, hue, and the changes in the anthocyanins with a blue tint such as petunidin, peonidin and delphinidin.

On the other hand, the changes in redness and the anthocyanins with red tints (cyanidin and pelargonidin) were related more to the container in which the juice was packed. Glass and aluminum were the containers that better kept the juice characteristics. However, glass presents disadvantages since it is a material that requires high energy for production and transport, and it breaks easily.

In focus groups conducted it was found that consumers preferred glass packages for premium juices or functional beverages in all of the tested age ranges. Only the groups of mothers with children below twelve years old preferred the PET bottles for their children's juice. However, these same mothers chose glass containers for their own juice since glass containers were perceived to have better quality and well-being. In conclusion, consumers preferred packages that were transparent and recyclable (Whaling 2007).

Processors do not like to use glass containers and are trying to shift to other possible containers such as those used for milk, baby food, baby juices. There are several reasons for this: (1) the transport costs and the carbon

footprint are very high, (2) glass breaks and production lines have to been shut down for cleaning, (3) it is heavy to manipulate at the warehouses, (4) retailers prefer juices to be packaged in other containers than glass.

PET bottles are widely used to pack juices because they have good barrier, are transparent, are less heavy and transport costs can be reduced. They are less breakable and molds are less expensive than for the glass bottles. PET is easier to mold into proprietary shape; if generic bottles are used, the marketing mix will not have the full benefit. It is much more effective to develop a unique package as was done for Pom-Wonderful (pomegranate juice) in a glass bottle shaped as two fruits, one on top of the other. Later the company switched from the glass bottle to the same bottle made in PET. Other examples companies like Simply Orange® are using clear PET packages for their gently pasteurized juices.

The use of PET bottles is rapidly growing in the market. PET is desired more by the beverage industry due to its light weight, ease of handling and lower energy levels needed for its production and transport compared to glass. It is also recyclable (where PET recycling operations exist) although FDA restricts the use of recycled material in direct contact with food products (CFSAN/Office of Food Additive Safety 2006).

However, this study shows that PET bottles are the least protective package for tart cherry juice due to the migration, and the loss of antioxidants and general characteristics over time. If the industry wants to use this kind of package, one option is to acknowledge a shorter shelf-life (around 6 months) to

avoid the major decay of the quality, anthocyanin content and taste properties of the tart cherry juice. This is the approach that the soft drink industry has taken.

Aluminum bottles are a great barrier against light which could trigger photo-oxidation; and the performance for preserving the antioxidants was similar to glass. Aluminum bottles are becoming more popular and they are reclosable and recyclable as well. Aluminum bottles provide a large printing area, and so attractive graphics labels can be printed directed on the bottle. This could be a good option for the industry, a middle point between glass and PET. One of the disadvantages with aluminum bottles is the lack of transparency, a characteristic that Whaling (2007) found critical for functional foods, since the majority of the panelists participating in the focus groups agreed that transparency was one of the major preferences for a functional juice. Transparency is also a sign of quality.

It is unfortunate that the stand-up pouch tests could not be completed. But Whaling's focus groups considered them to reflect a very low quality product, to the point that the juice inside was not considered to be natural (Whaling 2007). But this perception may change over time, as graphic, shapes and applications increase.

More and more products are packed by retailers in stand-up multi-layer packages due to their versatility. Some of these products are tuna fish, crackers, cookies, baby finger foods, candy, chocolates, etc. The flexible packaging provides versatility since no molds are required. Transportation costs are also low since the material comes in rolls, and the packages are light weight.

It can be also observed that pasteurization at low temperatures inhibits pathogen growth and does destroy anthocyanins present in tart cherry juice. With this type of gentle pasteurization the bottles of juice can be stored at room temperature and in closed boxes for a period of twelve months without any pathogen growth. It is important to not rely on a cold chain since it is difficult and expensive to maintain through all of the supply chain. The packaging method of hot fill and nitrogen flush before capping was successful in preventing the microbial growth and the browning of oxidation.

Anthocyanins suffered losses in varying degrees, a fact that it is very important to know the limits for labeling purposes. There is no authorized health claim for antioxidants, but it is important to remember that labels can be a way to communicate and educate consumers about the health benefits and strength of the product. Since the front and information panel have very strict regulations of contents (FDA CFR 21) the other panels can be used to inform and educate the consumers. They can write how much antioxidant power there is in the bottle (always using the end of the shelf life data to prevent liability), how it might help, what is an antioxidant and benefits. Then consumers will be able to recognize the different products with this power. Graphics and design need to be related to the trend.

The anthocyanins that suffered the most were those that are red: cyanidin and pelargonidin. These also apparently migrated into the PET bottle walls. The blue anthocyanins, petunidin, peonidin and delphinidin, increased through time, and there is no evidence that they were influenced by the type of container used.

The changes in sugars and pH, the co-pigmentation thanks to acetaldehyde and benzaldehyde were linked to the changes in anthocyanins and this resulted in color changes. There was no evidence of oxidation in the juice, since the color only became darker but did not turn brown. The trained panel never identified oxidation flavors in the juice during the different sessions.

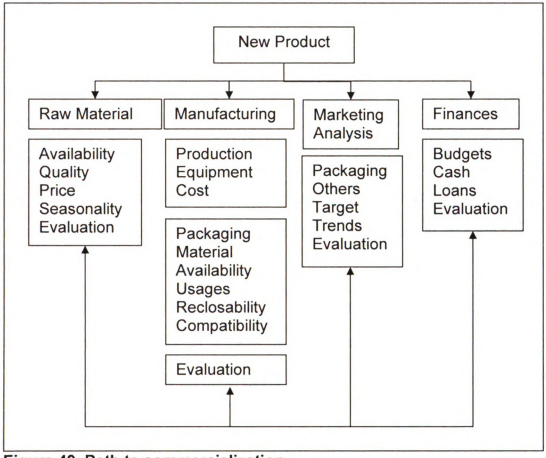


Figure 49. Path to commercialization
Adapted from Adelaja 2004.

The tart cherry industry will have to make decisions about the future of juice and packaging. It will continue to evaluate the product, packaging material and processes. Tart cherries are considered as "super fruits" (fruits with antioxidant power" but the cherry flavor did not make it on the top ten flavors of 2008 (Halliday 2007). The top flavors for super fruits were acai berry and

mangosteen. Maybe a possibility for the tart cherry industry will be to find blends and alliances to find a market outlet for their juice.

The industry will have to evaluate the technical results presented in this study and continue to evaluate consumer's preferences. They will need to determine if consumers are willing to pay higher price for their juice packaged in glass or if they should switch to a PET bottle with a shorter shelf-life (around six months). They might also rethink the aluminum possibility and more closely evaluate consumer's perception closer related to this innovative new packaging. Flexible packaging will need more years to catch up with the perceptions of quality.

These results have shown that the existing heat processing equipment can be used without making great investments. Alternatively, aseptic packaging technology may be adopted.

These results are a guide for the tart cherry industry to make decisions about the implementation of value-added products. This research is a link to support and consolidate future projects between Michigan tart cherry industry and Michigan State University.

V. FUTURE RESEARCH

Since this research used tart cherry juice concentrate, it would be very interesting to compare these findings to single strength juice with no previous concentration. The limitations will be the seasonality of the tart cherry crop, since the juicec will need to be packaged immediately after harvesting. Concentrating the juice up to 65° Brix might have damaged the anthocyanin content and color.

They are many other packages that might be interesting to evaluate for future research like biodegradable polymers and Tetra-pack™ for example. These packaging systems are not as readily available in the market and will require further analysis or investment from the industry point of view.

Third, the industry now uses very basic heat processing equipment and does not have plants with state of the art fillers. It does not have any aseptic filling systems which are becoming more and more popular in the juice industry. due that it minimizes the heat processes. It will be very interesting to analyze the difference between the different possible packaging lines to see which produces a better taste and anthocyanin outcome that could justify the investment in equipment.

Fourth, It will be very interesting to see if the standing pouch could be a viable packaging source since it produces less post-consumer waste and uses are growing significantly around the world. The appropriate filling and closing equipment is needed in order to evaluate this packaging possibility.

Finally, consumer and supply chain research could better identify target markets and marketing strategies to better serve the tart cherry industry.

APPENDIX 1: ANOVA Tables (treatment analysis)

Table 19. ANOVA full model (L* value).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2011.226(a)	35	57.464	14.782	.000
Intercept	21106.924	1	21106.924	5429.431	.000
Month	1846.600	11	167.873	43.183	.000
Treatment	30.528	2	15.264	3.926	**.021**
Month * Treatment	134.099	22	6.095	1.568	**.053**
Error	1119.601	288	3.888		
Total	24237.751	324			
Corrected Total	3130.827	323			

Table 20. ANOVA full model analysis of a* values.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3656.618(a)	35	104.475	25.847	.000
Intercept	221492.858	1	221492.858	54798.131	.000
Month	2740.395	11	249.127	61.635	.000
Treatment	294.045	2	147.023	36.374	**.000**
Month * Treatment	622.178	22	28.281	6.997	**.000**
Error	1164.090	288	4.042		
Total	226313.566	324			
Corrected Total	4820.708	323			

Table 21. ANOVA custom model analysis of a* values.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3034.441(a)	13	233.419	40.509	.000
Intercept	221492.858	1	221492.858	38439.256	.000
Month	2740.395	11	249.127	43.235	**.000**
Treatment	294.045	2	147.023	25.515	**.000**
Error	1786.267	310	5.762		
Total	226313.566	324			
Corrected Total	4820.708	323			

Table 22. ANOVA full model analysis of b* values.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4409.574(a)	35	125.988	12.040	.000
Intercept	50416.964	1	50416.964	4817.890	.000
Month	4015.607	11	365.055	34.885	.000
Treatment	92.981	2	46.490	4.443	**.013**
Month * Treatment	300.986	22	13.681	1.307	**.164**
Error	3013.785	288	10.465		
Total	57840.323	324			
Corrected Total	7423.359	323			

Table 23. ANOVA custom model analysis of chrominance.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	559305.631(a)	13	43023.510	12.380	.000
Intercept	6675085.845	1	6675085.845	1920.736	.000
Treatment	39290.446	2	19645.223	5.653	**.010**
month	520015.185	11	47274.108	13.603	**.000**
Error	76456.057	22	3475.275		
Total	7310847.533	36			
Corrected Total	635761.688	35			

Table 24. ANOVA custom model analysis of chrominance.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.169(a)	13	.013	16.894	.000
Intercept	6.922	1	6.922	9014.338	.000
month	.168	11	.015	19.937	.000
Treatment	.000	2	.000	.157	.855
Error	.017	22	.001		
Total	7.108	36			
Corrected Total	.186	35			

Table 25. ANOVA full model analysis of solids change.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24.876(a)	35	.711	12.326	.000
Intercept	25948.550	1	25948.550	450010.984	.000
treatment	16.470	2	8.235	142.817	.000
month	1.750	11	.159	2.759	**.005**
treatment * month	6.655	22	.303	5.246	**.000**
Error	4.152	72	.058		
Total	25977.578	108			
Corrected Total	29.027	107			

Table 26. ANOVA custom model analysis of solids change.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18.220(a)	13	1.402	12.191	.000
Intercept	25948.550	1	25948.550	225701.429	.000
month	1.750	11	.159	1.384	**.193**
treatment	16.470	2	8.235	71.629	**.000**
Error	10.807	94	.115		
Total	25977.578	108			
Corrected Total	29.027	107			

Table 27. ANOVA full model analysis of pH.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.162(a)	35	.062	64.894	.000
Intercept	1356.884	1	1356.884	1425520.390	.000
Treatment	.006	2	.003	2.989	.057
Month	2.128	11	.193	203.235	**.000**
Treatment * Month	.028	22	.001	1.352	**.170**
Error	.069	72	.001		
Total	1359.115	108			
Corrected Total	2.230	107			

Table 28. ANOVA full model analysis for cyanidin 3-glucosylrutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31960339.286(a)	32	998760.603	25.296	.000
Intercept	85703755.135	1	85703755.135	2170.6	.000
Month	28012231.842	10	2801223.184	70.949	**.000**
treatment	3189248.900	2	1594624.450	40.388	**.000**
Month * treatment	758858.544	20	37942.927	0.961	**.518**
Error	2605831.691	66	39482.298		
Total	120269926.111	99			
Corrected Total	34566170.977	98			

Table 29. ANOVA full model analysis for cyanidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	130969.959(a)	32	4092.811	11.861	.000
Intercept	1947098.611	1	1947098.611	5642.77	.000
Month	117947.058	10	11794.706	34.182	**.000**
treatment	3812.406	2	1906.203	5.524	**.006**
Month * treatment	9210.496	20	460.525	1.335	**.190**
Error	22773.999	66	345.061		
Total	2100842.570	99			
Corrected Total	153743.959	98			

Table 30. ANOVA full model analysis for delphinidin 3-glucosylrutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	50575.788(a)	32	1580.493	3.744	.000
Intercept	1066412.982	1	1066412.982	2526.459	.000
Month	41555.218	10	4155.522	9.845	**.000**
treatment	362.214	2	181.107	.429	**.653**
Month * treatment	8658.356	20	432.918	1.026	**.447**
Error	27858.461	66	422.098		
Total	1144847.230	99			
Corrected Total	78434.249	98			

Table 31.ANOVA full model analysis for delphinidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	309133.805(a)	32	9660.431	1.754	.028
Intercept	10397826.147	1	10397826.147	1887.365	.000
Month	255955.433	10	25595.543	4.646	**.000**
treatment	762.650	2	381.325	.069	**.933**
Month * treatment	52415.722	20	2620.786	.476	**.967**
Error	363605.618	66	5509.176		
Total	11070565.570	99			
Corrected Total	672739.423	98			

Table 32. ANOVA full model analysis for peonidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	240084.438(a)	32	7502.639	.851	.687
Intercept	505631.669	1	505631.669	57.352	.000
Month	220846.401	10	22084.640	2.505	**.013**
treatment	2658.660	2	1329.330	.151	**.860**
Month * treatment	16579.377	20	828.969	.094	**1.000**
Error	581870.715	66	8816.223		
Total	1327586.822	99			
Corrected Total	821955.153	98			

Table 33. ANOVA full model analysis for pelargonidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	192138.771(a)	32	6004.337	25.709	.000
Intercept	1208970.118	1	1208970.118	5176.508	.000
Month	163157.944	10	16315.794	69.860	**.000**
treatment	16271.743	2	8135.872	34.836	**.000**
Month * treatment	12709.083	20	635.454	2.721	**.001**
Error	15414.258	66	233.549		
Total	1416523.147	99			
Corrected Total	207553.029	98			

Table 34. ANOVA custom model analysis for pelargonidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	179429.687(a)	12	14952.474	45.724	.000
Intercept	1208970.118	1	1208970.118	3696.980	.000
month	163157.944	10	16315.794	49.893	**.000**
treatment	16271.743	2	8135.872	24.879	**.000**
Error	28123.341	86	327.016		
Total	1416523.147	99			
Corrected Total	207553.029	98			

Table 35. ANOVA full model analysis for petunidin 3-rutinoside changes.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2302240.290(a)	32	71945.009	5.246	.000
Intercept	39195897.009	1	39195897.009	2858.110	.000
Month	1875247.971	10	187524.797	13.674	**.000**
Treatment	18935.498	2	9467.749	.690	**.505**
Month * treatment	408056.821	20	20402.841	1.488	**.116**
Error	905118.885	66	13713.922		
Total	42403256.184	99			
Corrected Total	3207359.174	98			

APPENDIX 2: ANOVA tables (month analysis)

Table 36. L* value month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	4.481	0.418	10.719	0.000	3.658	5.304
[Treatment=1]	0.748	0.274	2.734	0.007	0.210	1.287
[Treatment=2]	0.439	0.274	1.604	0.110	-0.100	0.977
[Treatment=3]	0.000	.	.	.	.	.
[Month=1]	5.285	0.547	9.656	0.000	4.208	6.362
[Month=2]	9.321	0.547	17.031	0.000	8.245	10.398
[Month=3]	4.563	0.547	8.337	0.000	3.486	5.640
[Month=4]	2.179	0.547	3.980	0.000	1.102	3.255
[Month=5]	3.650	0.547	6.669	0.000	2.573	4.727
[Month=6]	2.263	0.547	4.135	0.000	1.186	3.340
[Month=7]	1.301	0.547	2.377	0.018	0.224	2.378
[Month=8]	3.403	0.547	6.218	0.000	2.326	4.480
[Month=9]	3.910	0.547	7.143	0.000	2.833	4.987
[Month=10]	1.696	0.547	3.099	0.002	0.619	2.773
[Month=11]	0.763	0.547	1.394	0.164	-0.314	1.840

Table 37. a* values month ANOVA analysis.

c	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	20.269	0.499	40.621	0.000	19.287	21.251
[Month=1]	9.851	0.653	15.079	0.000	8.566	11.137
[Month=2]	8.179	0.653	12.518	0.000	6.893	9.464
[Month=3]	5.649	0.653	8.646	0.000	4.363	6.934
[Month=4]	5.991	0.653	9.171	0.000	4.706	7.277
[Month=5]	6.424	0.653	9.832	0.000	5.138	7.709
[Month=6]	4.241	0.653	6.492	0.000	2.956	5.527
[Month=7]	-0.449	0.653	-0.687	0.493	-1.734	0.837
[Month=8]	4.639	0.653	7.101	0.000	3.354	5.925
[Month=9]	4.017	0.653	6.148	0.000	2.731	5.302
[Month=10]	3.906	0.653	5.978	0.000	2.620	5.191
[Month=11]	1.914	0.653	2.930	0.004	0.629	3.200
[Month=12]	0.000	.	.	.	.	.
[Treatment=1]	2.051	0.327	6.280	0.000	1.409	2.694
[Treatment=2]	1.989	0.327	6.089	0.000	1.346	2.632
[Treatment=3]	0.000	.	.	.	.	.

Table 38. b values month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	7.264	0.680	10.687	0.000	5.927	8.602
[Treatment=1]	1.220	0.445	2.741	0.006	0.344	2.095
[Treatment=2]	1.029	0.445	2.313	0.021	0.153	1.905
[Treatment=3]	0.000	.	.	.	.	.
[Month=1]	9.051	0.890	10.170	0.000	7.300	10.802
[Month=2]	12.154	0.890	13.657	0.000	10.403	13.905
[Month=3]	5.470	0.890	6.146	0.000	3.718	7.221
[Month=4]	3.125	0.890	3.511	0.001	1.374	4.876
[Month=5]	6.481	0.890	7.283	0.000	4.730	8.233
[Month=6]	3.382	0.890	3.800	0.000	1.631	5.133
[Month=7]	-0.067	0.890	-0.076	0.940	-1.819	1.684
[Month=8]	5.049	0.890	5.673	0.000	3.297	6.800
[Month=9]	5.806	0.890	6.524	0.000	4.055	7.557
[Month=10]	2.166	0.890	2.434	0.016	0.415	3.917
[Month=11]	0.911	0.890	1.024	0.307	-0.840	2.663
[Month=12]	0.000	.	.	.	.	.

Table 39. Chrominance month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	220.975	36.7627	6.010848	4.75E-06	144.73	297.21
[Treatment=1]	73.77376	24.06684	3.06537	0.005665	23.86	123.68
[Treatment=2]	65.68584	24.06684	2.72931	0.012245	15.77	115.59
[Treatment=3]	0	.	.	.	.	.
[month=1]	375.3896	48.13367	7.798898	8.98E-08	275.56	475.21
[month=2]	381.31	48.13367	7.921897	6.94E-08	281.48	481.13
[month=3]	201.6422	48.13367	4.189212	0.00038	101.81	301.46
[month=4]	102.7445	48.13367	2.134567	0.044186	2.92	202.56
[month=5]	232.7258	48.13367	4.83499	7.86E-05	132.90	332.54
[month=6]	128.9227	48.13367	2.678431	0.013726	29.09	228.74
[month=7]	-1.28813	48.13367	-0.02676	0.978891	-101.11	98.53
[month=8]	163.6884	48.13367	3.400705	0.002567	63.86	263.51
[month=9]	204.6694	48.13367	4.252105	0.000326	104.84	304.49
[month=10]	112.4787	48.13367	2.336799	0.028958	12.65	212.30
[month=11]	55.41583	48.13367	1.15129	0.261972	-44.40	155.23
[month=12]	0	.	.	.	.	.

Table 40. Hue values month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
						Upper Bound
Intercept	0.353	0.017	20.412	0.000	0.317	0.389
[month=1]	0.142	0.023	6.270	0.000	0.095	0.189
[month=2]	0.240	0.023	10.618	0.000	0.193	0.287
[month=3]	0.104	0.023	4.597	0.000	0.057	0.151
[month=4]	0.029	0.023	1.281	0.214	-0.018	0.076
[month=5]	0.123	0.023	5.434	0.000	0.076	0.170
[month=6]	0.058	0.023	2.561	0.018	0.011	0.105
[month=7]	0.035	0.023	1.526	0.141	-0.012	0.081
[month=8]	0.106	0.023	4.694	0.000	0.059	0.153
[month=9]	0.139	0.023	6.136	0.000	0.092	0.186
[month=10]	0.024	0.023	1.077	0.293	-0.023	0.071
[month=11]	0.005	0.023	0.235	0.817	-0.042	0.052
[month=12]	0.000	.	.	.	.	.
[Treatment=1]	0.006	0.011	0.499	0.623	-0.018	0.029
[Treatment=2]	0.000	0.011	0.028	0.978	-0.023	0.024
[Treatment=3]	0.000	.	.	.	.	.

Table 41. ΔE values month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.580391	1.4309503	2.502107	0.021138	0.595480966	6.565300852
[Treatment=1]	-1.30512	0.9721393	-1.34252	0.194468	-3.33296517	0.722728805
[Treatment=2]	-1.21905	0.9721393	-1.25399	0.224305	-3.24690153	0.808792441
[Treatment=3]	0	.	.	.	.	.
[month=1]	2.901667	1.861505	1.558775	0.134734	-0.98136468	6.784698018
[month=2]	5.915	1.861505	3.177536	0.004733	2.031968649	9.798031351
[month=3]	1.039333	1.861505	0.55833	0.582817	-2.84369802	4.922364684
[month=4]	1.333667	1.861505	0.716445	0.481999	-2.54936468	5.216698018
[month=5]	1.349	1.861505	0.724682	0.47704	-2.53403135	5.232031351
[month=6]	3.390333	1.861505	1.821286	0.083557	-0.49269802	7.273364684
[month=7]	5.079433	1.861505	2.72867	0.012939	1.196401982	8.962464684
[month=8]	-1.51847	1.861505	-0.81572	0.424269	-5.40149802	2.364564684
[month=9]	1.564333	1.861505	0.840359	0.410641	-2.31869802	5.447364684
[month=10]	-0.102	1.861505	-0.05479	0.956846	-3.98503135	3.781031351
[month=11]	0	.	.	.	.	.

Table 42. Change in solids month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
		Upper Bound	Lower Bound	Upper Bound	Lower Bound	Upper Bound
Intercept	16.056	0.122	131.525	0.000	15.814	16.299
[month=1.00]	-0.189	0.160	-1.182	0.240	-0.506	0.128
[month=2.00]	-0.250	0.160	-1.564	0.121	-0.567	0.067
[month=3.00]	-0.028	0.160	-0.174	0.862	-0.345	0.290
[month=4.00]	0.000	0.160	0.000	1.000	-0.317	0.317
[month=5.00]	0.083	0.160	0.521	0.603	-0.234	0.401
[month=6.00]	0.083	0.160	0.521	0.603	-0.234	0.401
[month=7.00]	-0.167	0.160	-1.043	0.300	-0.484	0.151
[month=8.00]	-0.250	0.160	-1.564	0.121	-0.567	0.067
[month=9.00]	0.056	0.160	0.348	0.729	-0.262	0.373
[month=10.00]	0.111	0.160	0.695	0.489	-0.206	0.428
[month=11.00]	-0.111	0.160	-0.695	0.489	-0.428	0.206
[month=12.00]	0.000	.	.	.	.	.
[treatment=1.00]	-0.953	0.080	-11.922	0.000	-1.111	-0.794
[treatment=2.00]	-0.550	0.080	-6.882	0.000	-0.709	-0.391
[treatment=3.00]	0.000	.	.	.	.	.

Table 43. Change in pH values month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.769074	0.011556	326.1469	0.000	3.74	3.79
[Month=1]	-0.09444	0.015131	-6.24185	0.000	-0.12	-0.06
[Month=2]	-0.34333	0.015131	-22.691	0.000	-0.37	-0.31
[Month=3]	-0.44889	0.015131	-29.6672	0.000	-0.47	-0.418
[Month=4]	-0.43222	0.015131	-28.5656	0.000	-0.46	-0.40
[Month=5]	-0.29778	0.015131	-19.6802	0.000	-0.32	-0.26
[Month=6]	-0.16889	0.015131	-11.1619	0.000	-0.19	-0.13
[Month=7]	-0.21556	0.015131	-14.2461	0.000	-0.24	-0.18
[Month=8]	-0.18889	0.015131	-12.4837	0.000	-0.21	-0.158
[Month=9]	-0.20333	0.015131	-13.4383	0.000	-0.23	-0.173
[Month=10]	-0.17889	0.015131	-11.8228	0.000	-0.20	-0.1483
[Month=11]	-1.2E-14	0.015131	-8.1E-13	1.000	-0.030042636	0.030042636
[Month=12]	0	.	.	.	.	.
[Treatment=1]	-0.01417	0.007565	-1.87256	0.064	-0.029187985	0.000854651
[Treatment=2]	-0.01639	0.007565	-2.16629	0.033	-0.031410207	-0.001367571
[Treatment=3]	0	.	.	.	.	.

Table 44. Changes cyanidin 3-glucosylrutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	56.29848	71.6766	0.785451	0.434	-86.1898804	198.7868501
[Month=1]	1674.892	93.243177	17.96262	0.000	1489.530477	1860.253078
[Month=2]	1117.511	93.243177	11.98491	0.000	932.1501439	1302.872745
[Month=3]	1304.01	93.243177	13.98504	0.000	1118.648255	1489.370856
[Month=4]	741.9682	93.243177	7.957346	0.000	556.6069216	927.3295228
[Month=5]	921.511	93.243177	9.882879	0.000	736.1496994	1106.872301
[Month=6]	498.7858	93.243177	5.349301	0.000	313.4244772	684.1470784
[Month=7]	461.7108	93.243177	4.951684	0.000	276.3494772	647.0720784
[Month=8]	176.7644	93.243177	1.895736	0.061	-8.59685614	362.125745
[Month=9]	101.367	93.243177	1.087125	0.280	-83.9943006	286.7283006
[Month=10]	76.99833	93.243177	0.82578	0.411	-108.362967	262.3596339
[Month=11]	0	.	.	.	.	.
[treatment=1]	437.651	48.694661	8.987659	0.000	340.8492179	534.4527821
[treatment=2]	255.0479	48.694661	5.237697	0.000	158.2460967	351.8496609
[treatment=3]	0	.	.	.	.	.

Table 45. Changes cyanidin 3-rutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	158.435	10.725	14.773	0.000	137.023	179.848
[Month=1]	-28.452	15.167	-1.876	0.065	-58.734	1.830
[Month=2]	-80.754	15.167	-5.324	0.000	-111.036	-50.472
[Month=3]	-82.083	15.167	-5.412	0.000	-112.365	-51.801
[Month=4]	-114.828	15.167	-7.571	0.000	-145.110	-84.546
[Month=5]	7.888	15.167	0.520	0.605	-22.394	38.170
[Month=6]	-17.938	15.167	-1.183	0.241	-48.220	12.344
[Month=7]	1.920	15.167	0.127	0.900	-28.362	32.202
[Month=8]	4.694	15.167	0.309	0.758	-25.588	34.976
[Month=9]	5.292	15.167	0.349	0.728	-24.990	35.574
[Month=10]	8.011	15.167	0.528	0.599	-22.271	38.293
[Month=11]	0.000	.	.	.	.	.

Table 46. Changes delphinidin 3-glucosylrutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	93.24967	11.86167	7.861426	0.000	69.56707158	116.9322618
[Month=1]	28.07733	16.77494	1.673767	0.099	-5.41491383	61.5695805
[Month=2]	21.50367	16.77494	1.281892	0.204	-11.9885805	54.99591383
[Month=3]	18.45367	16.77494	1.100074	0.275	-15.0385805	51.94591383
[Month=4]	49.58167	16.77494	2.955699	0.004	16.0894195	83.07391383
[Month=5]	-13.1827	16.77494	-0.78585	0.435	-46.67491383	20.3095805
[Month=6]	4.291667	16.77494	0.255838	0.799	-29.2005805	37.78391383
[Month=7]	-0.94933	16.77494	-0.05659	0.955	-34.4415805	32.54291383
[Month=8]	-4.653	16.77494	-0.27738	0.782	-38.14524716	28.83924716
[Month=9]	-0.62867	16.77494	-0.03748	0.970	-34.12091383	32.8635805
[Month=10]	-1.651	16.77494	-0.09842	0.922	-35.14324716	31.84124716
[Month=11]	0	.	.	.	.	.

Table 47 Changes delphinidin 3-rutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	347.587	25.203605	13.79116	0.000	297.4839065	397.6901218
[treatment=1]	6.113827	17.122478	0.357064	0.722	-27.9245316	40.15218617
[treatment=2]	0.481697	17.122478	0.028132	0.978	-33.5566619	34.52005587
[treatment=3]	0	.	.	.	.	.
[Month=1]	-124.486	32.787048	-3.7968	0.000	-189.664439	-59.30744942
[Month=2]	-98.9176	32.787048	-3.01697	0.003	-164.096062	-33.73907164
[Month=3]	-78.2947	32.787048	-2.38798	0.019	-143.473151	-13.11616053
[Month=4]	-24.5059	32.787048	-0.74743	0.457	-89.6843728	40.67261724
[Month=5]	24.29646	32.787048	0.741038	0.461	-40.8820395	89.47495058
[Month=6]	-39.8061	32.787048	-1.21408	0.228	-104.984595	25.37239502
[Month=7]	30.93599	32.787048	0.943543	0.348	-34.2425061	96.11448391
[Month=8]	-4.47806	32.787048	-0.13658	0.892	-69.6565506	60.70043947
[Month=9]	18.99994	32.787048	0.579495	0.564	-46.1785506	84.17843947
[Month=10]	13.50696	32.787048	0.41196	0.681	-51.6715395	78.68545058
[Month=11]	0	.	.	.	.	.

Table 48. Changes peonidin 3-rutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	98.523	30.229	3.259	0.002	38.430	158.615
[Treatment=1]	11.703	20.536	0.570	0.570	-29.122	52.528
[Treatment=2]	1.594	20.536	0.078	0.938	-39.231	42.419
[Treatment=3]	0.000	.	.	.	.	.
[month=1]	-87.938	39.324	-2.236	0.028	-166.112	-9.764
[month=2]	-93.089	39.324	-2.367	0.020	-171.263	-14.915
[month=3]	-92.263	39.324	-2.346	0.021	-170.437	-14.090
[month=4]	-95.954	39.324	-2.440	0.017	-174.128	-17.780
[month=5]	-1.933	39.324	-0.049	0.961	-80.107	76.241
[month=6]	-22.409	39.324	-0.570	0.570	-100.583	55.765
[month=7]	15.203	39.324	0.387	0.700	-62.970	93.377
[month=8]	2.361	39.324	0.060	0.952	-75.813	80.534
[month=9]	6.292	39.324	0.160	0.873	-71.881	84.466
[month=10]	23.349	39.324	0.594	0.554	-54.825	101.523
[month=11]	0.000	.	.	.	.	.

Table 49. Changes pelargonidin 3-rutinoside month ANOVA analysis.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	16.05648	0.122079	131.5252	0.000	15.81409045	16.29887252
[month=1.00]	-0.18889	0.159839	-1.18174	0.240	-0.50625325	0.128475472
[month=2.00]	-0.25	0.159839	-1.56407	0.121	-0.567364361	0.067364361
[month=3.00]	-0.02778	0.159839	-0.17379	0.862	-0.345142139	0.289586583
[month=4.00]	6.45E-15	0.159839	4.04E-14	1.000	-0.317364361	0.317364361
[month=5.00]	0.083333	0.159839	0.521357	0.603	-0.234031028	0.400697694
[month=6.00]	0.083333	0.159839	0.521357	0.603	-0.234031028	0.400697694
[month=7.00]	-0.16667	0.159839	-1.04271	0.300	-0.484031028	0.150697694
[month=8.00]	-0.25	0.159839	-1.56407	0.121	-0.567364361	0.067364361
[month=9.00]	0.055556	0.159839	0.347572	0.729	-0.261808805	0.372919916
[month=10.00]	0.111111	0.159839	0.695143	0.489	-0.20625325	0.428475472
[month=11.00]	-0.11111	0.159839	-0.69514	0.489	-0.428475472	0.20625325
[month=12.00]	0	.	.	.	.	.

Table 50. Changes in petunidin 3-rutinoside.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	702.6743	44.778163	15.69234	0.000	613.6582409	791.6903248
[treatment=1]	32.39176	30.420771	1.064791	0.290	-28.0827344	92.86624954
[treatment=2]	7.606061	30.420771	0.250029	0.803	-52.8684314	68.08055257
[treatment=3]	0	.	.	.	.	.
[Month=1]	-317.277	58.251342	-5.44669	0.000	-433.077058	-201.4773863
[Month=2]	-284.879	58.251342	-4.89051	0.000	-400.678391	-169.0787197
[Month=3]	-223.104	58.251342	-3.83003	0.000	-338.90428	-107.3046086
[Month=4]	-177.309	58.251342	-3.04386	0.003	-293.108725	-61.50905302
[Month=5]	42.63867	58.251342	0.731977	0.466	-73.1611692	158.4385025
[Month=6]	-135.224	58.251342	-2.32138	0.023	-251.023391	-19.42371968
[Month=7]	66.87644	58.251342	1.148067	0.254	-48.9233914	182.6762803
[Month=8]	-10.0344	58.251342	-0.17226	0.864	-125.83428	105.7653914
[Month=9]	41.03589	58.251342	0.704463	0.483	-74.763947	156.8357248
[Month=10]	42.62333	58.251342	0.731714	0.466	-73.1765025	158.4231692
[Month=11]	0	.	.	.	.	.

APPENDIX 3: Consumer Consent Form and Trained panel survey

Consent Form
"Tart Cherry Juice consumers testing'

Sample: Tart (sour) cherry juice

Before you decide to sign this consent form and continue to participate in our study, please read carefully and thoroughly the reverse side form for the sample ingredients and preparation information, purpose and procedure of this study, potential risks and benefits from your participation, our assurance of your privacy, your right as human subject in our study, etc.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principal investigator. Feel free to contact Dr. Suzanne Thornsbury, the principal investigator of this study., via phone at (517) 432-5418 (211 Agriculture Hall, Michigan State University, East Lansing MI 48823) or Maria-Paz Gonzalez at 517-353-8628. You cacn also reachc us via email at thornsbu@msu.edu or gonza221@msu.edu for any inquiry you might have due to your participation in our study.

In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Director of Human research Protections by phone: (517) 355-2180, fax (517) 432-4503, email: irb@ms.edu or regular mail: 202 Olds Hall, East Lansing MI, 48824.

If you have read all the information we offer to you in this consent form and decide to participate in our study an give us your valuable response to our questionnaire, you can go ahead and sign this form now. Otherwise, you can stop here and feel free to discontinue participation in our study without any penalty.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARLY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE AN ADECQUATE OPPORTUNITY TO DISCUSS THE STUDY WITH THE PRINCIPAL INVESTIGATOR AND HAVE ALL THE QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON REQUEST

SIGNED: _____ DATE _____
You indicate your voluntary agreement to participate by signing above.

Consent Form
"Tart Cherry Juice consumers testing'
Agricultural Economics Department and School of Packaging, Michigan State University.

Invitation to Participate:
You are invited to participate in this study that asses the quality attributes of tart (sour) cherry juice from concentrate.

Purpose of this study:
This study is intended to study the quality of tart (sour) cherry juice from concentrate and consumer acceptability. Appearance and flavor characteristics of tart (sour) cheery juice from concentrate will be evaluated.

Procedure of this study:
Each participant will be presented with tart (sour) cherry juice from concentrate. They will be asked to evaluate visually and flavor, score the attributes as presented on the score sheet for each sample. Samples will be presented using three digit random codes. Consumer marketing questions will also be asked.

Sample Ingredients and sample preparation:
All the ingredients used in our samples are food-grad and FDA approved for foods. The ingredients are: 100% tart cherry juice from concentrate, water and no sugar added or chemical added.

Potential risks:
Because all ingredients we use in this study are food grade and FDA approved for food applications, these samples pose no adverse health risk, provided the subject has not been identified as being susceptible to an allergic reaction to the previously listed sample ingredients. If you believe there is a potential of an allergic reaction upon sniffing and tasting notify the on-site sensory evaluation coordinator and/or principal investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covers or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort are not available. That does not mean that you are giving up any legal rights you may have. You may contact Suzanne Thornsbury with any questions (517) 432-5418.

Potential benefits:
There are no benefits gained directly from your participation in this study. However, your participation and response ill provide us valuable data, which can be use to identify shelf life for tart cherry juice in different containers and help keep these growers profitable.

Assurance of confidentiality:
Any information obtained in connection with this study that could identify you will be kept confidential by ensuring that all consent forms are securely stored. All data collected and analyzed will be reported in an aggregate format that will not permit associating subjects with specific responses or findings. Your privacy will be protected to the maximum extent allowable by the law.

Withdrawal from this study:
Participation in this study is voluntary. Your decision to refuse participation or discontinue participation during this study will be honored promptly and unconditionally.

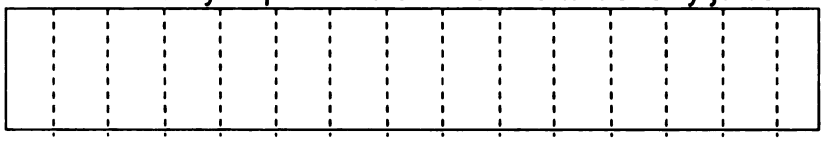
Trained panel survey

Name _____Date_____Code___

Please answer all the question for each of the 4 samples presented to you

Sample 358

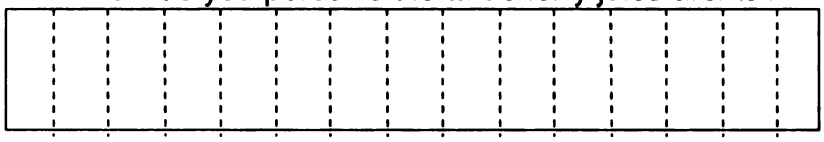
1. How do you perceive color of the tart cherry juice?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Very good Very bad

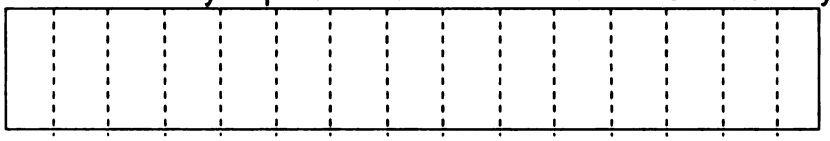
2. How do you perceive the tart cherry juice aroma?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Very good Very bad

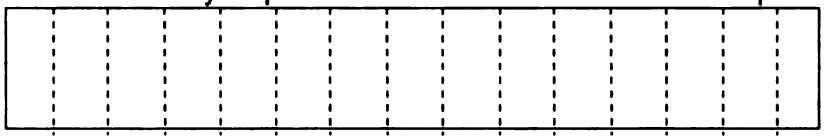
3. How do you perceive the mouth feel of the tart cherry juice?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

smooth grainy

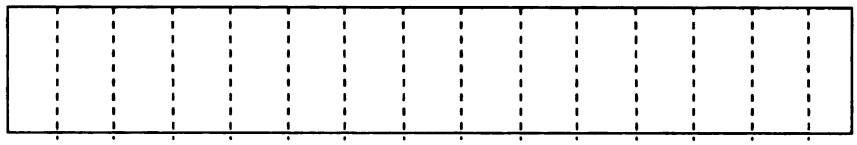
4. How do you perceive the sweetness in this sample?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Not sweet enough Too sweet

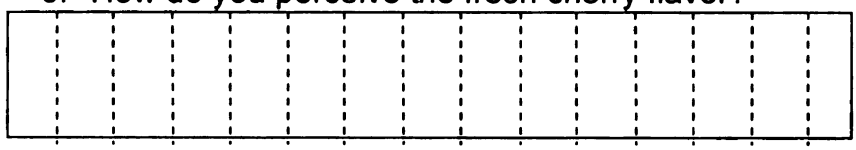
5. How do you perceive the sample's tartness?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Not tart enough Too tart

6. How do you perceive the fresh cherry flavor?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Very good Very bad

7. Do you perceive any off-flavor?

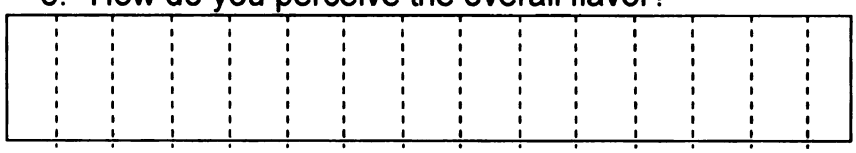
Yes _____ No _____ Which? Comments

_____

_____

_____

8. How do you perceive the overall flavor?



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Very bad Very good

9. Is this sample's quality appropriate for consumers to purchase the drink?

Yes _____ No _____

Comments

_____

_____

_____

APPENDIX 4: Stand-up pouch results

Color results

Figure 50. Changes in L* values.

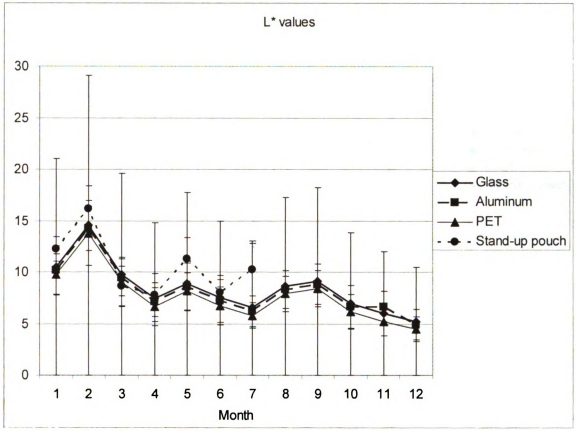


Figure 51. Changes in a* values.

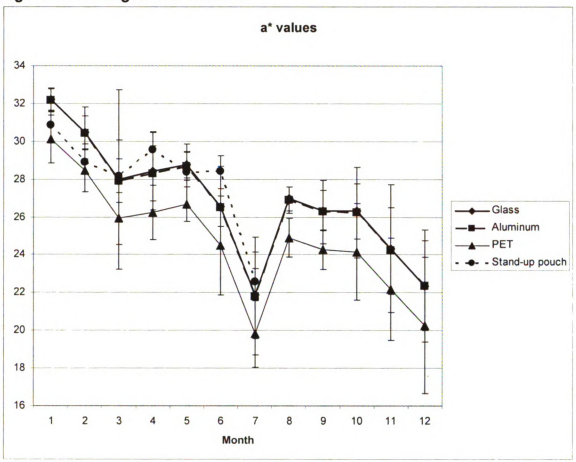
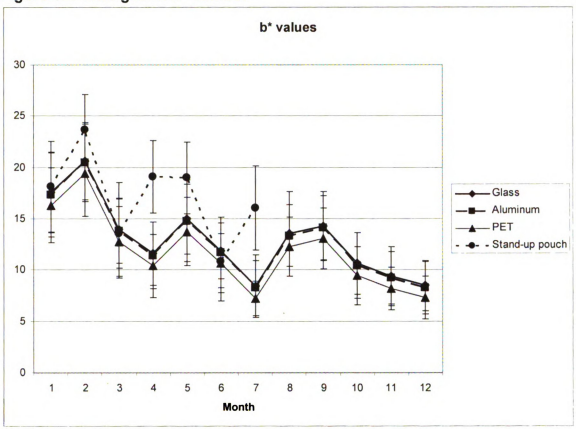


Figure 52. Changes in b* values.



Solids and PH results.

Figure 53 Changes in solids.

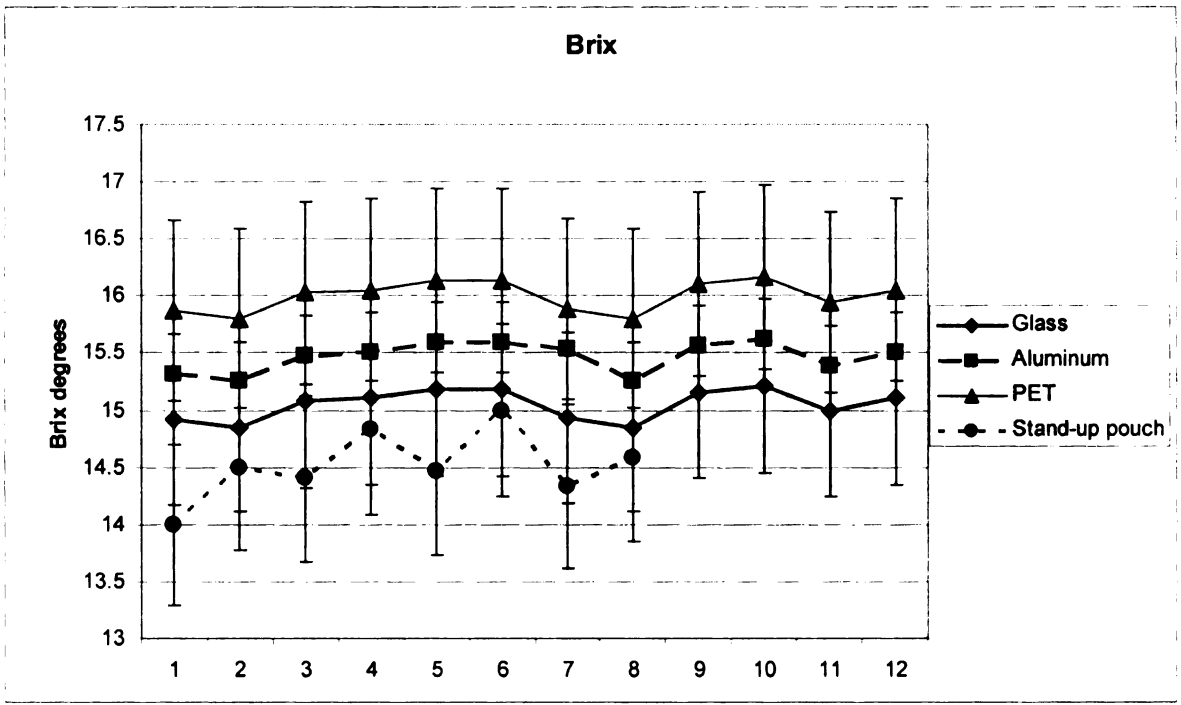
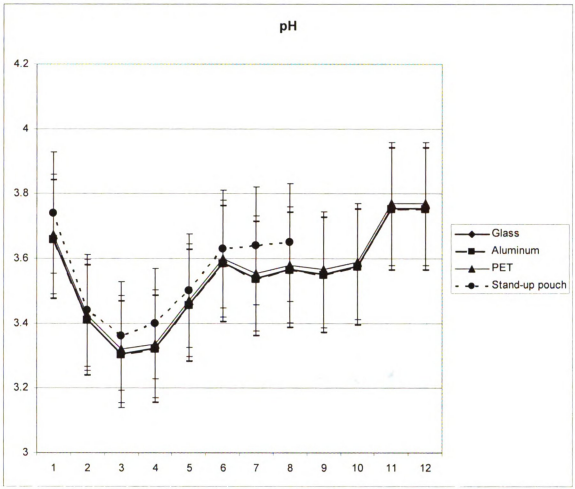


Figure 54. Changes in pH.



Anthocyanins results.

Figure 55. Changes in cyanidin 3-glucosylrutinoside.

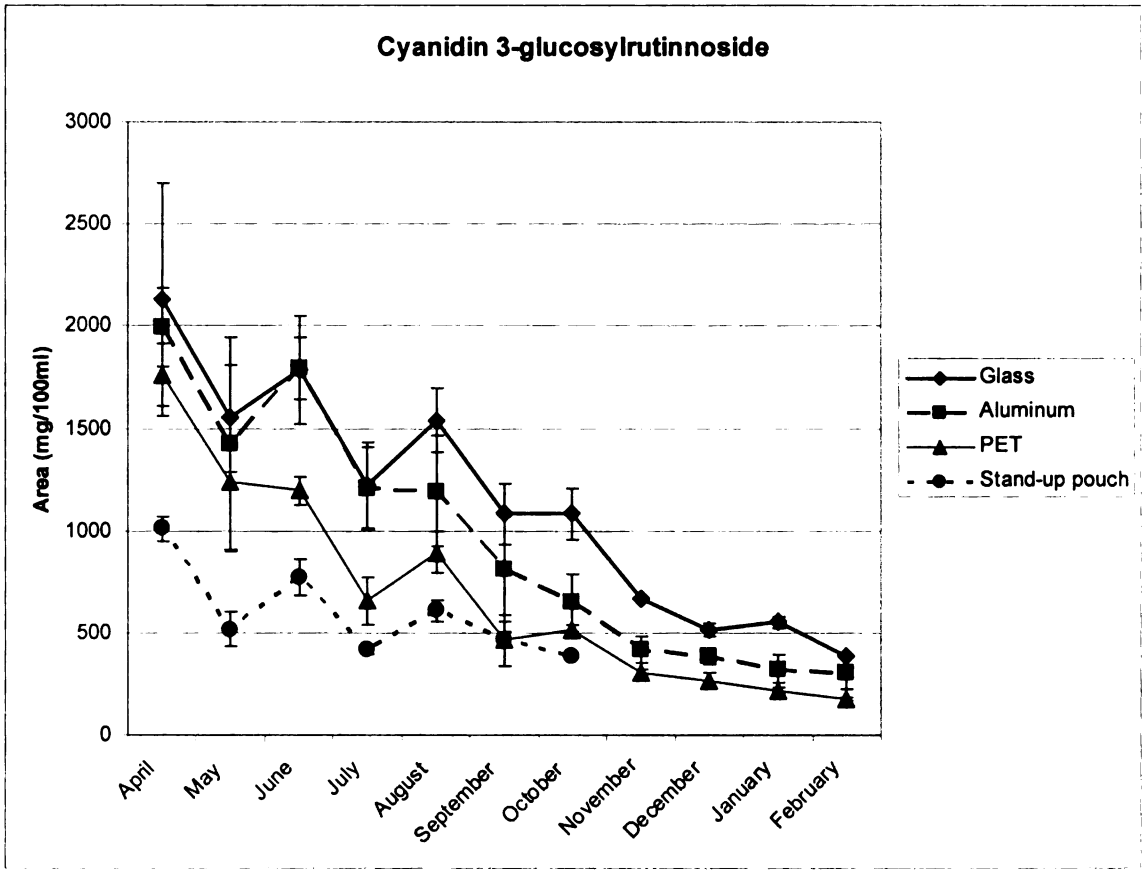


Figure 56. Changes in cyanidin 3-rutinoside.

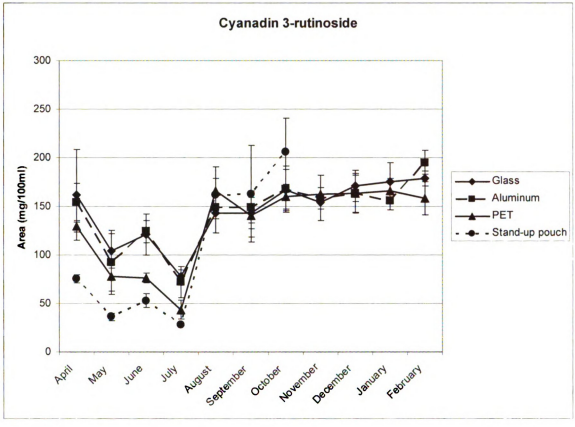


Figure 57. Changes in delphinidin 3-glucosylrutinoside.

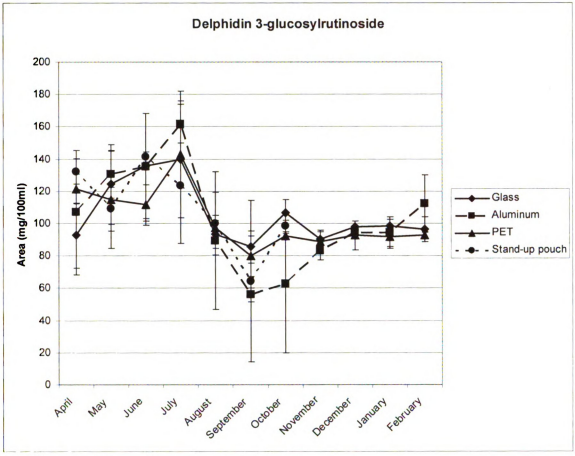


Figure 58. Changes in delphinidin 3-rutinoside.

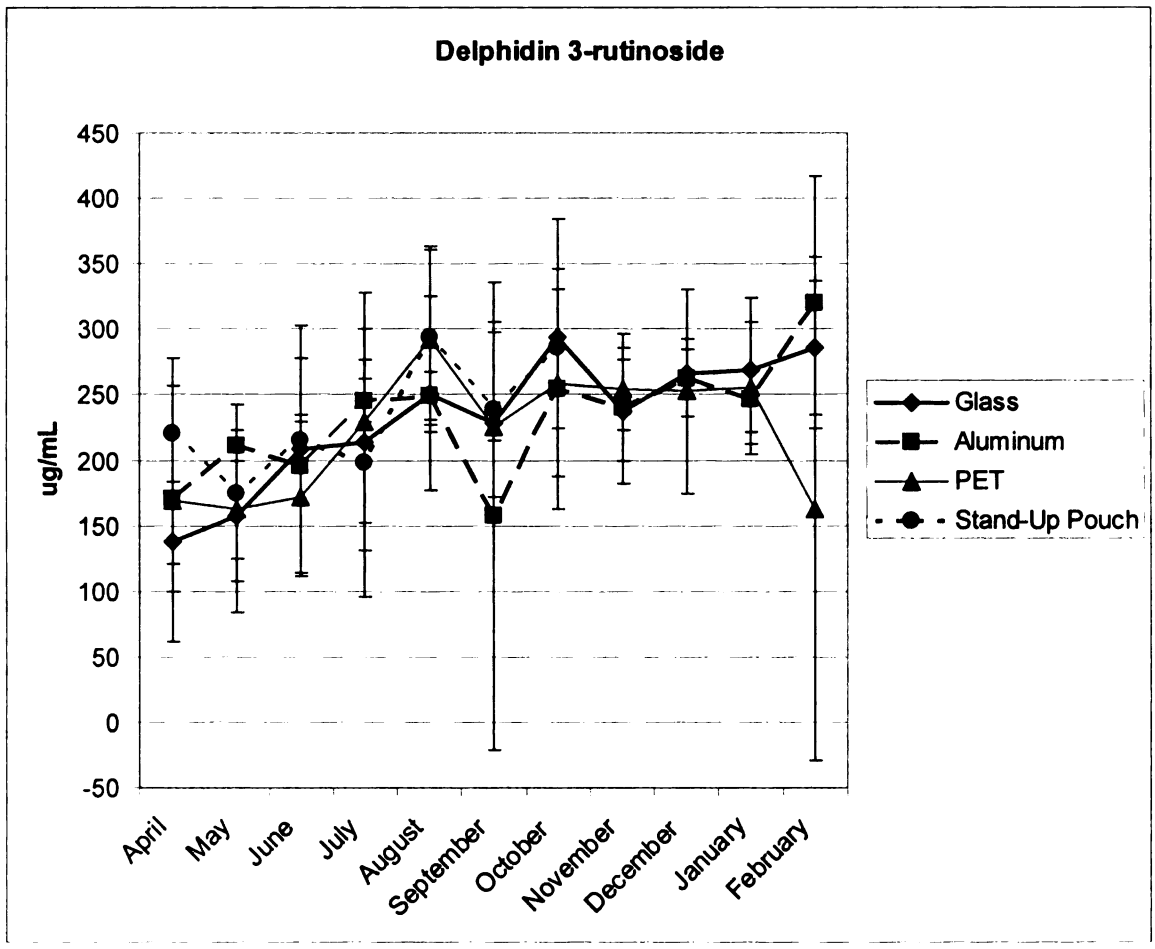


Figure 59. Changes in peonidin 3-rutinoside.

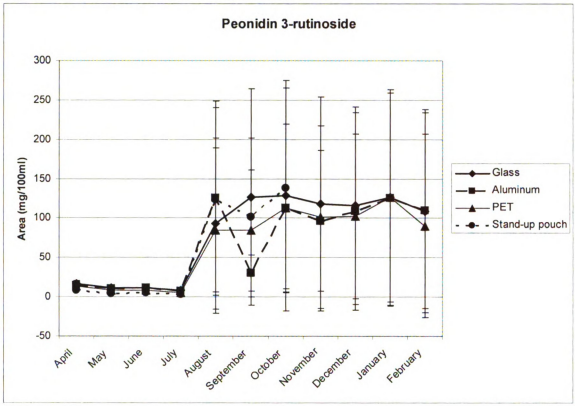


Figure 60. Changes in pelargonidin 3-rutinoside.

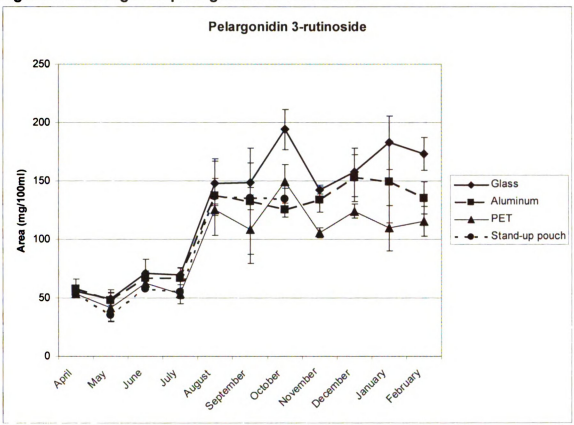
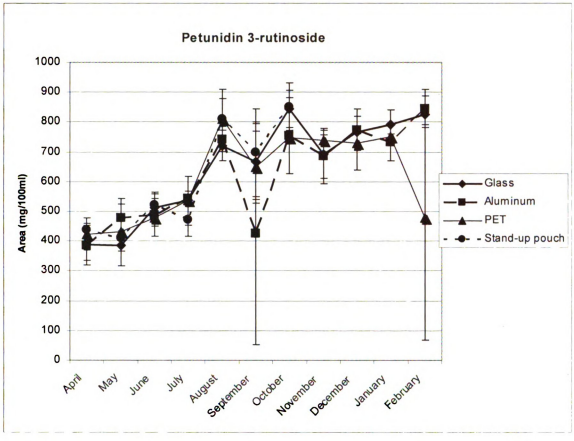


Figure 61. Changes in petunidin 3-rutinoside.



APPENDIX 5: Calibration curves

For cyanidin 3-glucosilrutinoside a step calibration is offered in Figure 62. The lineal model is A= 15552.6 * Concentration

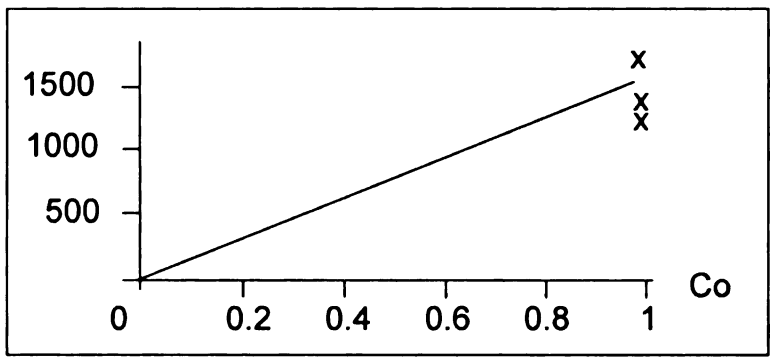


Figure 62. Step Calibration for cyanidin 3-glucosilrutinoside

For cyanidin 3-arabinorutinoside a calibration curve is offered in Figure 63. The lineal model is A= 0.0125938 * Concentration + 0.00551505

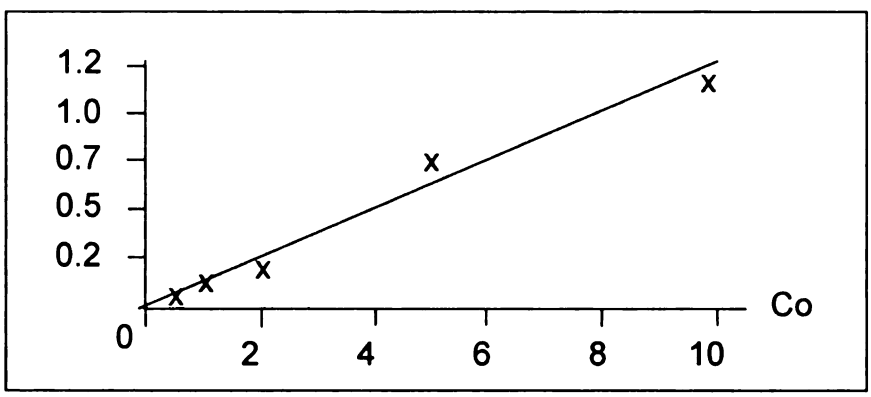


Figure 63. Calibration curve for cyanidin 3-arabinoserutinoside

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