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EFFECTS OF PROCESSING, STORAGE TEMPERATURE AND  
STORAGE DURATION ON PHYSICO-CHEMICAL, SENSORY  
QUALITY, AND ANTIOXIDANT PROPERTIES OF MICHIGAN  
MONTMORENCY CHERRY JUICE CONCENTRATE

presented by

Claudia Jean Place

has been accepted towards fulfillment  
of the requirements for the

M.S. degree in Food Science

  
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**EFFECTS OF PROCESSING, STORAGE TEMPERATURE AND STORAGE  
DURATION ON PHYSICO-CHEMICAL, SENSORY QUALITY, AND  
ANTIOXIDANT PROPERTIES OF MICHIGAN MONTMORENCY CHERRY  
JUICE CONCENTRATE**

**By**

**Claudia Jean Place**

**A THESIS**

**Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of**

**MASTER OF SCIENCE**

**Food Science**

**2009**



## **ABSTRACT**

### **EFFECTS OF PROCESSING, STORAGE TEMPERATURE AND STORAGE DURATION ON PHYSICO-CHEMICAL, SENSORY QUALITY, AND ANTIOXIDANT PROPERTIES OF MICHIGAN MONTMORENCY CHERRY JUICE CONCENTRATE**

**By**

**Claudia Jean Place**

Unpasteurized and pasteurized tart cherry juice concentrate was stored for 48 weeks at 4, 21 and 38°C. Microbial activity, total soluble solids, pH, titratable acidity, Hunter color, and turbidity were measured over time. Concentrate was analyzed for total antioxidant capacity (Oxygen Radical Absorbance Capacity) total anthocyanins (pH-differential) and total phenolic content (Folin-Ciocalteu). Sensory quality was measured by trained and consumer panels. Results for yeast and mold were found negative. Pasteurization had minimal to no significant effect on all attributes tested. Storage temperature and duration had minimal to no effect on Hunter "L" and "b" values, pH and total phenolics. Total soluble solids and titratable acidity showed significant decrease for ambient and elevated storage conditions. Hunter "a" value, turbidity and anthocyanin content showed significant change over time, showing a significantly higher decrease at ambient and elevated temperature conditions. Storage duration decreased ORAC values significantly; however, no differences were seen among storage conditions. Analytical analysis and sensory panels showed most overall stable concentrate quality for refrigerated temperatures while ambient storage condition showed poor quality after week 12 and elevated storage conditions after week 2.

## **DEDICATION**

**To my family and friends for all their love and support.**

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## INTRODUCTION

In 2008 the United States total tart cherry production was 213 million pounds. Michigan is the United State's leading producer of tart cherries, producing approximately 75% of the overall yield per year. In 2008 Michigan produced 165 million pounds of cherries, valued at 64.7 million dollars (Boriss and others 2009). Though Michigan produces both sweet and tart cherry varieties, in 2007 94.8% of all cherries produced in Michigan were of the tart Montmorency variety (USDA 2007). In 2008 Michigan used 10 million pounds of tart cherries to produce cherry juice and cherry juice concentrate (Schwannecke 2009).

Currently during the concentration processing of 68°Brix cherry juice concentrate, producers do not pasteurize the concentrate due to the product's high acidity, and high Brix. When removing water during the concentration process the product is heated to temperatures as high as 190°F (~88°C). Though the product goes through high heating temperatures when being concentrated it is undetermined if the process of later pasteurization has an effect on the physio-chemical, sensory quality or antioxidant content of the cherry juice concentrate.

Recently tart cherry juice concentrate, along with other tart cherry products, have been gaining support among consumers as a functional food owing to its nutraceutical potential. The high levels of antioxidants and anthocyanins found in tart cherries have been shown to have anti-inflammatory, anti-carcinogenic, and anti-aging properties (Blando and others 2004;

Kirakosyan and others 2009). Food products having high antioxidant activity have been shown to reduce stress (Kirakosyan and others 2009), lower the risk of heart disease (Solway 2009) and have a number of other health benefiting properties.

During storage or long-distance shipment these beneficial compounds of tart cherry juice concentrate are susceptible to deterioration and may not be stable under all conditions. Currently processors do not have sufficient shelf life information to account for these negative changes. Properly researched shelf life data are important to preserve both the nutraceutical and sensory quality of the concentrate. The results from this study will help to provide guidance, not currently available, to the tart cherry industry to improve or lengthen tart cherry juice concentrate shelf life.

Therefore, the objectives of this research were to:

- 1) Determine the effect of pasteurization, storage temperature and storage duration on the physico-chemical (total soluble solids, pH, titratable acidity, color, and turbidity), microbial, and sensory quality of Michigan tart Montmorency cherry juice concentrate.
- 2) Determine the effect of pasteurization, storage temperature and storage duration on the antioxidant properties (total antioxidant, total anthocyanins, and total phenolics) of Michigan tart Montmorency cherry juice concentrate.

This thesis presents the results of one shelf life study in the format of two separate chapters. Each chapter contains a literature review, materials and methods section and a conclusion pertaining to the chapter's topic. Chapter 1 directly addressed objective 1 while chapter 2 directly addresses objective 2.

# **1. EFFECTS OF PROCESSING, STORAGE TEMPERATURE AND STORAGE DURATION ON PHYSICO-CHEMICAL PROPERTIES AND SENSORY QUALITY OF MONTMORENCY CHERRY JUICE CONCENTRATE**

## **1.1 Literature Review**

### **1.1.1 Background of Tart Cherries and Tart Cherry Concentrate**

The state of Michigan is the leading producer of tart cherries (*Prunus cerasus*) within the United States producing over 75% of the country's total output. In 2008 the United States produced 214.4 million pound of tart cherries. It is projected that the 2009 total will be 283.6 million pounds (NASS 2009). Following Michigan in top production is New York, Oregon, Pennsylvania, Utah, Washington, and Wisconsin. The state of Michigan uses approximately 10 million pounds of tart cherries each year to produce cherry juice and cherry juice concentrate (Schwannecke 2009). The majority of the tart cherries grown in Michigan are of the Montmorency variety.

During concentration process cherry juice is pressed to a product with total soluble solid content of 68°Brix and a pH between 3.2 and 3.8 (Cherry Marketing Institute 2007a). In order to achieve this high Brix level the cherry juice goes through a number of heating and filtering steps, where it is heated up to temperatures of 190°F to remove water for concentration. Currently during the processing of the concentrate producers do not pasteurize the product before being packaged and sold.



### **1.1.2 Microbiological Content**

The microbiological content of food is very important for food safety, shelf life and food quality. Fruits have the ability to support the growth of bacteria, mold and yeast. Fruit concentrates such as tart cherry juice concentrate are known for not being a successful medium for bacterial growth. This is due to the concentrate's low acidic pH and its high concentration (Brix) level. However, processors of tart cherry juice concentrate have had problems with mold growth on their product.

There are some varieties of fungi that have the ability to grow and survive in acidic environments. A Filamentous fungus is highly tolerant to high acidity. Also fungi such as *Penicillium*, *Aspergillus*, *Cladosporium* and *Byssoschlamys* spp. have been found to grow as surface colonies on fruit juices and purees (Garza and Sanchis 1998). The heat resistant mold, *Byssoschlamys* type spp. *B. fulva* is common in fruits such as cherries, apples, grapes, and strawberries. This mold type has been known to survive 1 minute at 200°F (Murdock and Hatcher 1976). It is also reported that *Zygosaccharomyces rouxii* and *Z. bailii* are the principle yeasts to cause spoilage in fruit concentrates (Garza and Sanchis 1998).

### **1.1.3. Sensory Attributes**

Fresh tart cherry juice concentrate is known for its highly tart flavor, fresh cherry flavor and aroma, and its bright red color. The concentrate's fresh cherry flavor is characterized by the compound benzaldehyde (McGorin 2007). Benzaldehyde has been determined to be one of the most important

compounds in tart cherries due to the aroma and flavor profile it gives the product. Research has shown that benzaldehyde decreases over storage duration, especially at high storage temperatures (Petersen and Poll 1999). The decrease in benzaldehyde is caused by oxidation in the concentrate (Petersen and Poll 1999). In a study of tart cherries it was determined that at high storage temperatures the ethanol and acetic acid concentration increased over time (Petersen and Poll 1999). The development of these compounds can produce off flavors and off aroma within the product. In a shelf life study of tart cherry juice, conducted by (Gonzalez-Mulet 2008), a trained panel concluded that the tart flavor of the product began to diminish over a 24 week storage duration. Over storage as the tart flavors decreased, an increasing sweet flavor became apparent.

#### **1.1.4 Hypothesis**

The independent variables of processing, storage temperature and storage duration have a degradative effect on the dependent variables physico-chemical, microbial, and sensory quality of tart cherry juice concentrate.

### **1.2 Materials and Methods**

#### **1.2.1 Cherry Juice Concentrate Samples**

Montmorency tart cherry concentrate was received in five 5-gallon containers from Shoreline Fruit LLC (Traverse City, Michigan). Shoreline Fruit LLC processed the product (crop year: 2007) to a concentration of 68° Brix. The concentrate was stored in 5-gallon buckets in a Michigan State University

Food Science freezer at -15° C until the concentrate was further processed, packaged and stored.

### **1.2.2 Pasteurization of Concentrate**

Approximately two 5-gallon containers of concentrate were pasteurized at 85°C for 5 seconds. The pasteurization took place in Michigan State University Food Science's pilot plant. The concentrate was pumped through a spiral stainless steel tube that was submerged in water heated in a steam-jacketed kettle.

### **1.2.3 Packaging and Storage of Concentrate**

All samples were stored in clear, square 8, or 16 oz. glass jars. The bottles were acquired from Qorpak (Bridgeville, Pennsylvania). Bottles of concentrate were all labeled indicating if they were unpasteurized or pasteurized. Containers were also labeled with the week number to indicate when the sample would be removed from storage for analysis. All concentrate samples were stored in conditions absent of light. Samples were stored at 4°C in a VWR Scientific Model 2005 Low Temperature Incubator (West Chester, Pennsylvania). Concentrate samples stored at 21°C were stored in a laboratory cupboard with minimal temperature changes and finally samples at 38°C were stored in a Fisher Isotemp® Oven 200 Series Model (Pittsburgh, Pennsylvania). Temperatures were monitored every 2 to 3 days to confirm consistent storage temperatures. After samples were removed from storage they were kept frozen

in an upright Frigidaire freezer (Martinez, Georgia) at approximately -15°C until analysis could be completed. The only exceptions were the samples analyzed for yeast and mold. They were tested immediately after being removed from storage and not frozen.

#### **1.2.4 Shelf Life Schedule**

The shelf life study was conducted over the course of 48 weeks. The samples stored at 4°C and 21°C were tested up to 48 weeks while the samples stored at 38°C were tested only up to 24 weeks. The cherry juice concentrate stored at elevated conditions was no longer stored after 24 weeks because they were no longer of consumable quality. Table 1.1 shows the schedule at which the cherry juice concentrate was analyzed for a particular attribute over the course of the study.

**Table 1.1: Schedule which analysis was performed on cherry juice concentrate**

<b>Week #</b>	<b>TSS</b>	<b>pH</b>	<b>TA</b>	<b>Hunter Color</b>	<b>Turbidity</b>	<b>TPC</b>	<b>Trained Sensory</b>	<b>Consumer Sensory</b>
0	X	X	X	X	X	X	X	
2	X	X	X	X	X			
4	X	X	X	X	X	X	X	
6	X	X	X	X	X			
8	X	X	X	X	X	X	X	
10								
12	X	X	X	X	X	X	X	
14								
16	X	X	X	X	X	X	X	X
18								
20	X	X	X	X	X	X	X	
22								
24	X	X	X	X	X	X	X	X
36	X	X	X	X	X	X	X	X
48	X	X	X	X	X	X	X	X

## **1.2.5 Physico-Chemical Analysis**

### **1.2.5.1 Total Soluble Solids**

Total soluble solids were read in °Brix using a Thermo Fisher Scientific (Waltham, Massachusetts) Abbe-3L refractometer with the sensitivity of 0.1%.

### **1.2.5.2 pH and Titratable Acidity**

The pH and titratable acidity of the cherry juice concentrate was measured according to the guidelines of AOAC Official Method 942.15 (AOAC 2000) using a Corning (Corning, New York) 430 pH meter and a Pinnacle combination electrode (Woburn, Massachusetts). When measuring titratable acidity 100 mL of distilled water was added to 10 grams of cherry juice

concentrate. Samples were neutralized using a 0.1N sodium hydroxide (Mallinckrodt Baker, Phillipsburg, New Jersey) solution to a pH of  $8.1 \pm 0.2$ . Titratable acidity results were expressed in percent malic acid and calculated using equation 1.1.

$$\% \text{ malic acid} = \text{mL } 0.1\text{N NaOH} \times \text{N NaOH} \times 0.067 \text{ meq} \times (100/\text{wt. of sample})$$

**Eq. 1.1**

#### **1.2.5.3 Turbidity**

The turbidity (lack of clarity) of the concentrate was measured using the method of (Krop and Pilnik 1974) and (Ough and others 1975). A sample amount of 10mL was centrifuged at the speed, 11000 rev/minute for 10 minutes. The centrifuge process removed any coarse particles contained within the sample. The absorbance value was read using a Milton Roy (Ivyland, Pennsylvania) Spectronic 21D spectrophotometer at the wavelength of 660nm. This value was then converted to %Transmittance using formula 1.2:

$$\text{Percent Transmittance} = \text{antilog} (2 - \text{absorbance}). \quad \text{Eq. 1.2}$$

The turbidity was measured for the reconstituted juice (14°Brix). This reconstituted juice was analyzed because the high Brix of the concentrate did not allow for measurement on the spectrophotometer.

#### **1.2.5.4 Hunter Color**

Hunter Lab was analyzed by measurement of the L, a, b and  $\Delta E$  values. Analysis was done using a LabScan® XE Spectrophotometer (Reston, Virginia) and EasyMatch® QC software. Color measurements were taken when samples were at room temperature. Using the LabScan glass sample cup

(diameter 1.75 inches) samples were analyzed in 25mL volumes. Data was collected using an Illuminant value of D65 and an Observers value of 10. The spectrophotometer was calibrated using a black tile and a white tile #LX17582 (X=80.37, Y=85.26, Z=89.86). Values a and b are measured in positive and negative values where -a represents green and +a represents red. The -b values represents blue and +b represents yellow. The value "L" is represented by a value between 0 and 100 where the value 0 equals black and 100 equals white. The value  $\Delta E$  is a single value representing total color difference which takes the Hunter L, a, and b values all into account.

#### **1.2.6. Microbial Content**

##### **1.2.6.1 Plate Preparation and Dilution Buffer**

The total plate count for yeast and mold was tested using a Potato Dextrose Agar (Becton, Dickinson and Company, Sparks, Maryland) media. The amount of 39.0 grams of Potato Dextrose Agar (PDA) was added to 1000mL of distilled water. The solution was thoroughly mixed and heated to a boil for 1 minute to completely dissolve all powder. The solution was then autoclaved on the liquid cycle for 15 minutes at 121°C. The agar solution was not acidified due to the high acidity of the cherry juice concentrate. A phosphate buffer, of pH 7.4 was used to dilute the samples prior to plating the samples.

### **1.2.6.2 Sample Preparation**

Three dilutions of the each sample were plated in duplicate. The dilutions included the original concentration ( $10^0$ ) and also  $10^{-1}$  and  $10^{-2}$  of the original concentration. A sample size of 100 $\mu$ L was added to each plate. The plates sat covered for 3 days at room temperature before being analyzed for yeast and mold growth.

### **1.2.7 Sensory Analysis**

#### **1.2.7.1 Environmental Condition**

All sensory panels were performed in the Michigan State Food Science and Human Nutrition sensory evaluation laboratory under controlled sensory conditions. All sensory evaluations were done under full spectrum light. Institutional Review Board approved sensory analysis (IRB Log Number X07-722).

#### **1.2.7.2 Trained Panel**

##### **1.2.7.2.1 Panelist Training**

Panelists ages 18 years and older were recruited by flyers from the Michigan State University campus. Also people who had participated on past panels were contacted for this panel. All panelists were screened where contact information was collected and participants were also asked to complete two triangle tests dealing with the taste and color of cherry juice concentrate. The screening resulted in 15 panelists who were then trained in five, one-hour



training sessions over several weeks. The panelists were taught to rate the cherry juice concentrate and cherry juice using a 15 cm scale on the qualities of color, aroma, off aromas/flavors and overall quality for both the concentrate and the reconstituted juice. Panelists were introduced to reference samples and were also given time to learn, practice, and be tested on. Panelists were also taught all proper sampling methods.

#### **1.2.7.2.2 Sensory Analysis Test Method**

Trained sensory panelists completed descriptive sensory tests rating the concentrate's attributes of color and fresh cherry aroma. The reconstituted juice (14°Brix) was evaluated for color and fresh cherry flavor. A rating was then given for the product's overall quality. Comments on any perceived off-flavors or off-aromas in the juice were also recorded. Both the color of the concentrate and the juice were rated with 15 representing a very good, bright red color and 0 representing a very poor, dull brown color. The concentrate's aroma was evaluated using a score of 15 that represented a good, fresh cherry aroma. Decreasing ratings showed a loss of cherry aroma and development of off-aromas. The juice was evaluated for its flavor using a score of 15 that represented a good, fresh tart cherry flavor. Decreasing flavor ratings indicated a loss of freshness and development of a sweet flavor compared to the desired tart flavor.

#### **1.2.7.2.3 Sample Preparation and Presentation**

Trained panelists were required to read and sign a consent form prior to their participation in the shelf life study. For each training session or sensory panel the panelists were compensated for their time with an ice cream coupon good at the Michigan State University Dairy Store. During each panel, participants received their samples both as a concentrate and as a reconstituted juice. Three mL of the concentrate was presented at room temperature in clear 20 mL scintillation vials from Research Products International Corp (Mount Prospect, Illinois). Alongside the concentrate samples was the reconstituted juice in 25 to 30 mL amounts presented in clear Sweetheart 50 oz. plastic cups (Solo, Highland Park, Illinois). The juice was served at refrigerated temperatures of approximately 4-5°C. Trained panelists were presented with a sample of fresh reconstituted cherry juice made from the concentrate that had not undergone processing or storage to use as a reference. The panelists also received five cherry juice concentrate references that correspond with the quality ratings of 15, 13, 9, 5, and 0 on the 15 cm scale. Panelists were provided distilled water and unsalted saltine crackers and were trained to use them to cleanse their palate prior to tasting and in-between samples. Panelists were given a Styrofoam cup, if they wished to expectorate the samples after tasting.

### **1.2.7.3 Consumer Panel**

#### **1.2.7.3.1 Sensory Analysis Test Method**

A consumer sensory panel of approximately 100 panelists was recruited from faculty, staff and students at Michigan State University. Panelists rated the cherry juice (14°Brix) color, aroma appearance, flavor and overall quality. The sensory panel was conducted using a 9-point hedonic scale where 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely. Consumers were also asked questions about their age, gender and personal juice preferences.

#### **1.2.7.3.2 Sample Preparation and Presentation**

Panelists were required to read and sign a consent form prior to participation and were compensated for their time with an ice cream coupon good at Michigan State University Dairy Store. All samples were served to the participants at refrigerated temperatures at ~4-5°C. Each panelist received between 25 and 30 mL of juice per sample served in a clear Sweetheart 50 oz. plastic cup (Solo, Highland Park, Illinois). Panelists were also provided distilled water and unsalted saltine crackers and encouraged to use them to cleanse their palate between samples. Panelists were allowed to consume all samples however they received a Styrofoam cup if they wished to expectorate the sample.

### **1.2.8 Statistical Analysis**

Physico-chemical attributes were analyzed using linear regression to determine if significant differences occurred over storage duration. To determine if the variables of processing and storage temperature significantly effected physico-chemical data, F tests were used. For the analysis of sensory data, ANOVA analysis was used, using the Turkey HSD test to compare multiple means.

## **1.3 Results and Discussion**

### **1.3.1 Microbial Analysis**

Over the storage period of 48 weeks all counts for yeast and mold resulted in negative results. In addition, no growth was found on the surface of the concentrate prior to plating the samples. These results were expected since tart cherry juice concentrate is a high acid product having a pH lower than 4.6. Having an acidic pH inhibits the growth of mold and yeast in the product.

### **1.3.2 Physico-Chemical**

#### **1.3.2.1 Total Soluble Solids**

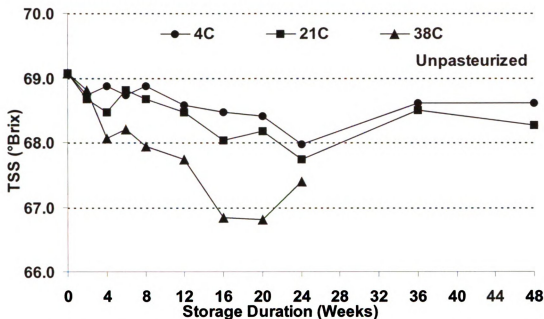
Figures 1.1 and 1.2 display the total soluble solids values for both unpasteurized and pasteurized concentrate at all storage conditions over the duration of storage. Using linear regression, the concentrate stored at 4°C and 21°C showed no significant change over a year of storage for both unpasteurized and pasteurized concentrate (Table 1.2). F tests showed no significant difference between the total soluble solids values among 4°C and

21°C for both unpasteurized ( $p=0.4436$ ) and pasteurized ( $p=0.7632$ ) concentrate. Processing did not affect the results among these storage conditions.

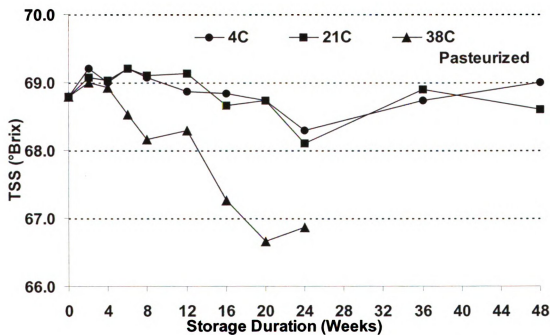
The total soluble solids content for concentrate stored at the elevated 38°C was found to significantly decrease over time (Table 1.2). F tests showed pasteurized concentrate had a significantly higher decreasing slope compared to unpasteurized product ( $p=0.0197$ ). For the unpasteurized concentrate the total soluble solids decreased from its initial value of 69.1 to 67.4°Brix (Figure 1.1), while the pasteurized concentrate decreased from the initial value of 68.8 to 66.9°Brix (Figure 1.2). Cherry juice concentrate is produced to be 68°Brix. If storage at elevated conditions decreases the Brix level below 68° this attribute could limit its shelf life. However, the total soluble solid values do not fall below 68°Brix until week 8 of storage. By this time the trained panelist had already determined the concentrate to be of poor and inconsumable quality (See 1.3.3.2).

**Table 1.2: Linear regression results for total soluble solids (°Brix)**

<b>Unpasteurized</b>	<b>P-value</b>	<b>Slope</b>	<b>Pasteurized</b>	<b>P-value</b>	<b>Slope</b>
<b>4°C</b>	0.1225	No significant difference	<b>4°C</b>	0.2891	No significant difference
<b>21°C</b>	0.0942	No significant difference	<b>21°C</b>	0.1106	No significant difference
<b>38°C</b>	0.0019	-0.0824	<b>38°C</b>	0.0001	-0.1029



**Figure 1.1: Effects of storage time and temperature on the total soluble solids (°Brix) values of unpasteurized cherry juice concentrate**



**Figure 1.2: Effects of storage time and temperature on the total soluble solids (°Brix) values of pasteurized cherry juice concentrate**

### 1.3.2.2 pH

Shown in Figures 1.3 and 1.4 are the unpasteurized and pasteurized pH values for each storage condition over 48 weeks of storage. Using linear regression it was concluded that the pH of the concentrate did not significantly change over the storage period for all storage temperatures, and both processing types (Table 1.3). Using the F test to compare the intercepts of each processing/storage condition it was concluded that pH was not significantly affected by processing or storage temperature (Table 1.3).

The pH for all unpasteurized samples ranged from 3.29 to 3.46; pasteurized samples had a very similar pH range of 3.28 to 3.45. The pH of tart cherry juice concentrate is reported to range from as low as 3.2 to 3.8 (Cherry Marketing Institute 2007a). All pH values from this study fell within this reported pH range.

**Table 1.3: Linear regression results for pH**

Unpasteurized	P-value	Slope	Pasteurized	P-value	Slope
4°C	0.5134	No significant difference	4°C	0.6614	No significant difference
21°C	0.5290	No significant difference	21°C	0.9864	No significant difference
38°C	0.1815	No significant difference	38°C	0.2718	No significant difference

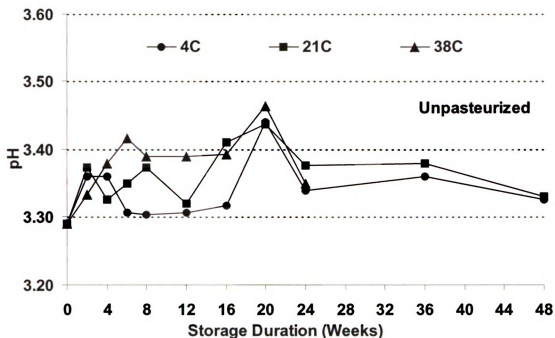


Figure 1.3: Effects of storage time and temperature on the pH values of unpasteurized cherry juice concentrate

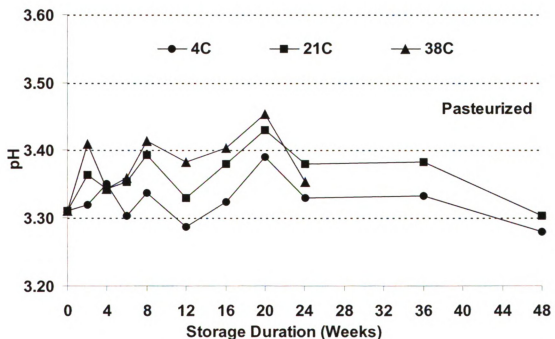


Figure 1.4: Effects of storage time and temperature on the pH values of pasteurized cherry juice concentrate



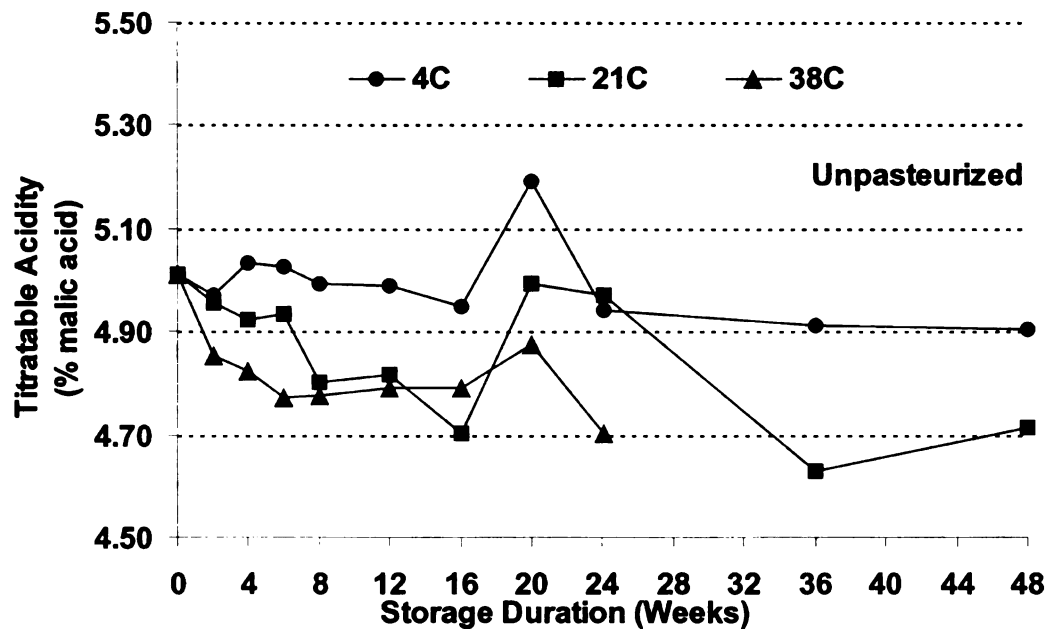
### 1.3.2.3 Titratable Acidity

Figures 1.5 and 1.6 display the titratable acidity values for all storage temperatures and processing types over the duration of storage. Concentrate stored at 4°C had no significant changes over storage (Table 1.4). The F test showed that pasteurization did not have an effect on concentrate at this storage condition ( $p=0.7177$ ).

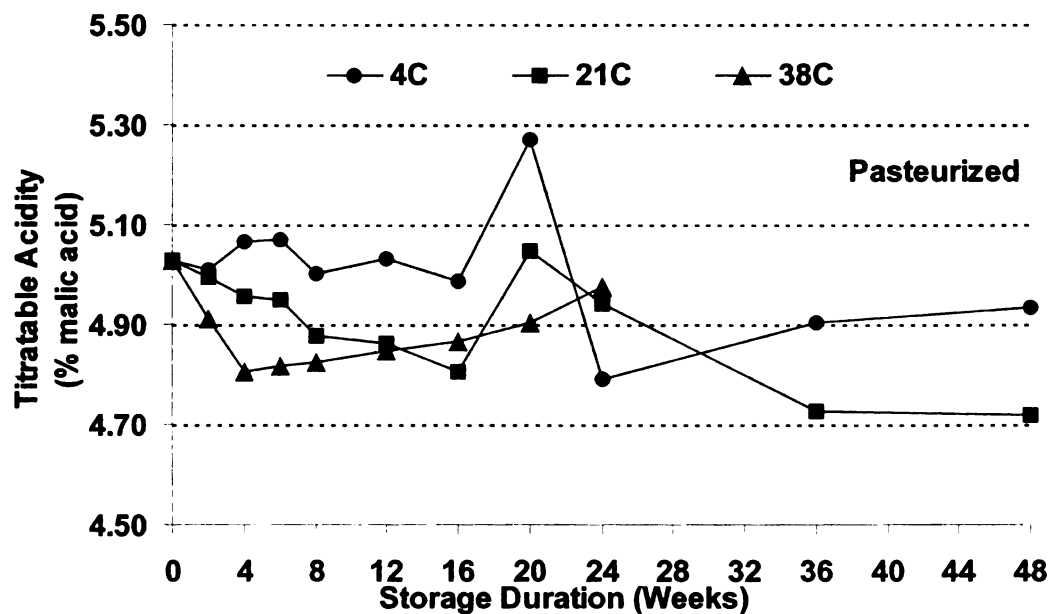
Concentrate held at 21°C had a slight, but significant decrease in titratable acidity over the storage period of 48 weeks (Table 1.4). Analysis of the first 24 weeks of data for 21°C showed no significant difference (unpasteurized  $p=0.6981$ , and pasteurized  $p=0.5599$ ). This indicates that the decrease in titratable acidity occurred after week 24 of storage. No analysis was completed on 38°C concentrate after week 24 due to the low inconsumable quality. Linear regression results showed no significant change in storage temperature 38°C during the 24 weeks, however it is predicted that if analysis continued a significant decrease would have occurred. Though significant decrease occurred in the concentrate's titratable acidity over the duration of 48 weeks, the titratable acidity values for all samples stayed within close range of one another. All samples ranged with 4.70 to 5.27% malic acid.

**Table 1.4: Linear regression results for titratable acidity (% malic acid)**

Unpasteurized	P-value	Slope	Pasteurized	P-value	Slope
4°C	0.2175	No significant difference	4°C	0.2438	No significant difference
21°C	0.0410	-0.0055	21°C	0.0099	-0.0055
38°C	0.1160	No significant difference	38°C	0.8152	No significant difference



**Figure 1.5: Effects of storage time and temperature on the titratable acidity (% malic acid) values of unpasteurized cherry juice concentrate**



**Figure 1.6: Effects of storage time and temperature on the titratable acidity (% malic acid) values of pasteurized cherry juice concentrate**

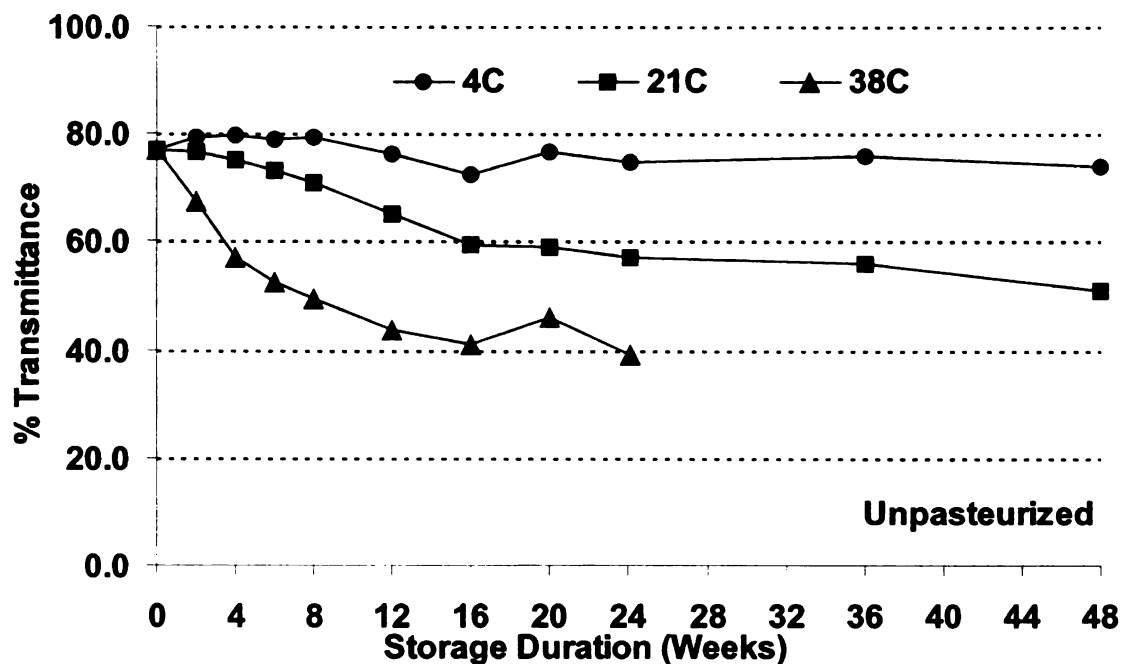
#### 1.3.2.4 Turbidity

Figures 1.7 and 1.8 display the turbidity (% transmittance) values for all storage temperatures and processing types over the duration of storage. A significant decrease over the duration of storage was seen for all cherry juice concentrate samples (Table 1.5).

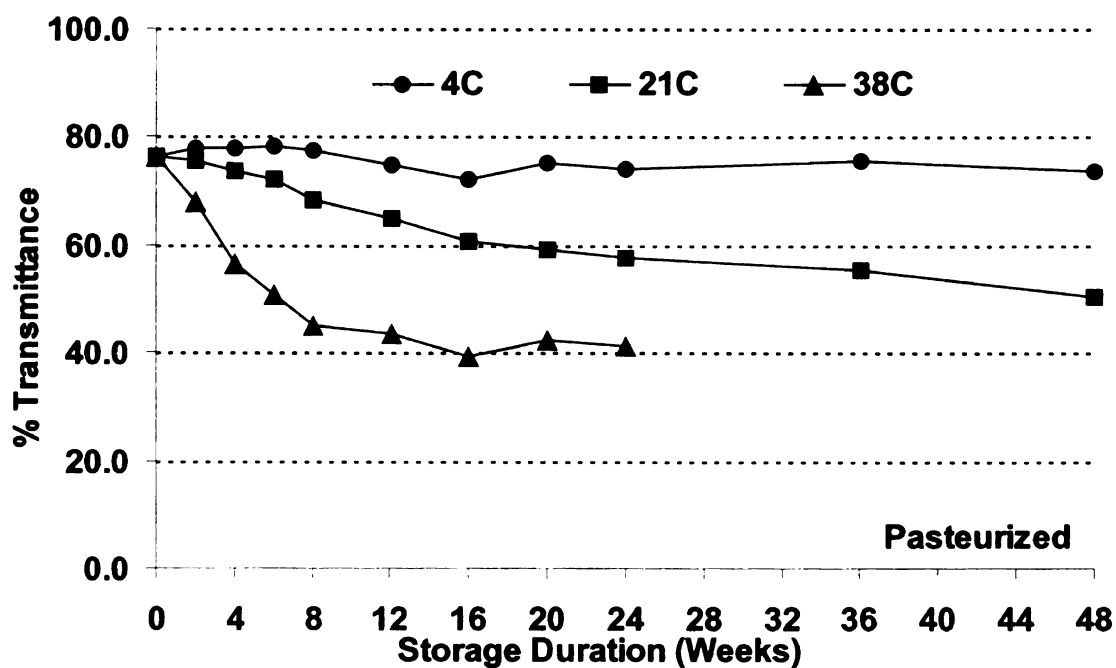
Using the F test, statistical difference was shown among all storage conditions (Table 1.5). Results showed that an increase in storage temperature increased the rate at which the concentrate lost clarity. Concentrate stored at 38°C lost clarity the fastest, losing about half of its percent transmittance over 24 weeks of storage. Concentrate at 4°C lost clarity the slowest, with the percent transmittance decreasing less than 10% transmittance over the year storage period. No statistical differences were seen among unpasteurized and pasteurized concentrate (Table 1.5). Little information is known about the substances that are responsible for the development of haze and turbidity of cherry juice concentrate. However, with research done on apple juice and other beverages the development of the haze may result from proteins, polyphenols, oxidized phenols and insoluble tannins (Meyer and others 2001).

**Table 1.5: Linear regression results for turbidity (% transmittance)**

<b>Unpasteurized</b>	<b>P-value</b>	<b>Slope</b>	<b>Pasteurized</b>	<b>P-value</b>	<b>Slope</b>
<b>4°C</b>	0.0305	-0.1042	<b>4°C</b>	0.0482	-0.0804
<b>21°C</b>	<0.0001	-0.5814	<b>21°C</b>	<0.0001	-0.5564
<b>38°C</b>	0.0030	-1.3102	<b>38°C</b>	0.0063	-1.2892



**Figure 1.7: Effects of storage time and temperature on the turbidity (% transmittance) values of unpasteurized reconstituted cherry juice**



**Figure 1.8: Effects of storage time and temperature on the turbidity (% transmittance) values of pasteurized reconstituted cherry juice**

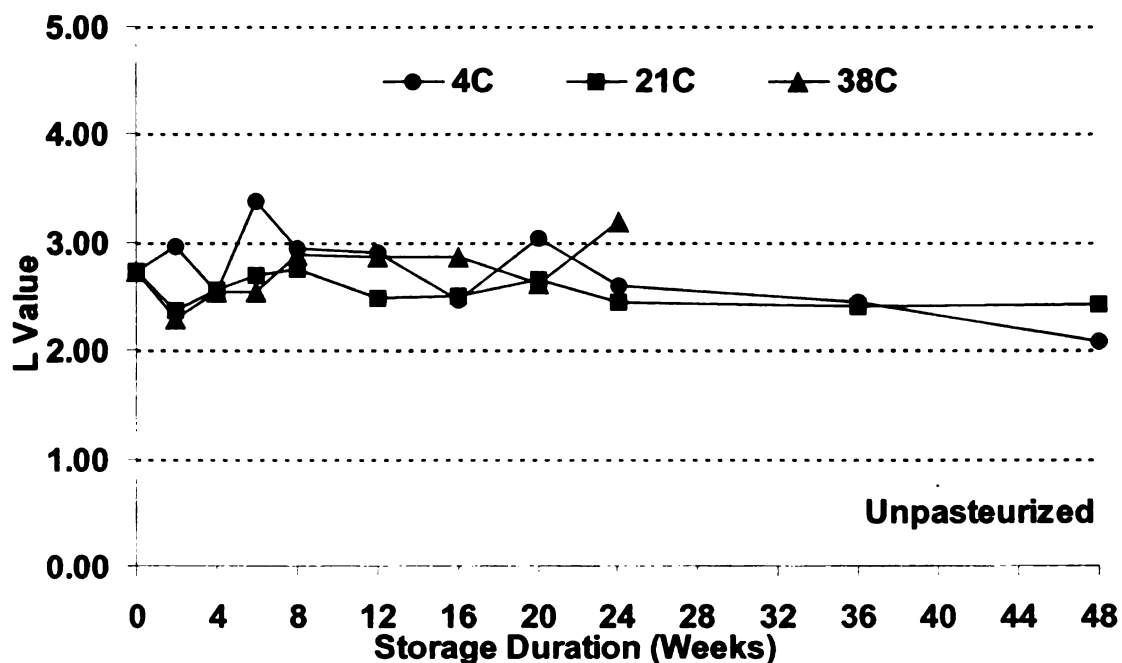
### **1.3.2.5 Hunter Color**

Figures 1.9 and 1.10 display the Hunter color L values for all storage temperatures and processing types over the duration of storage. Using linear regression it was concluded that for the majority of conditions, storage temperature and pasteurization did not have an affect on L values over the duration of storage (Figure 1.6). The only exception was seen for the unpasteurized concentrate stored at 4°C ( $p=0.0234$ ). At week 6 a high peak measurement is seen, which is the likely reason for the small p-value (Figure 1.9).

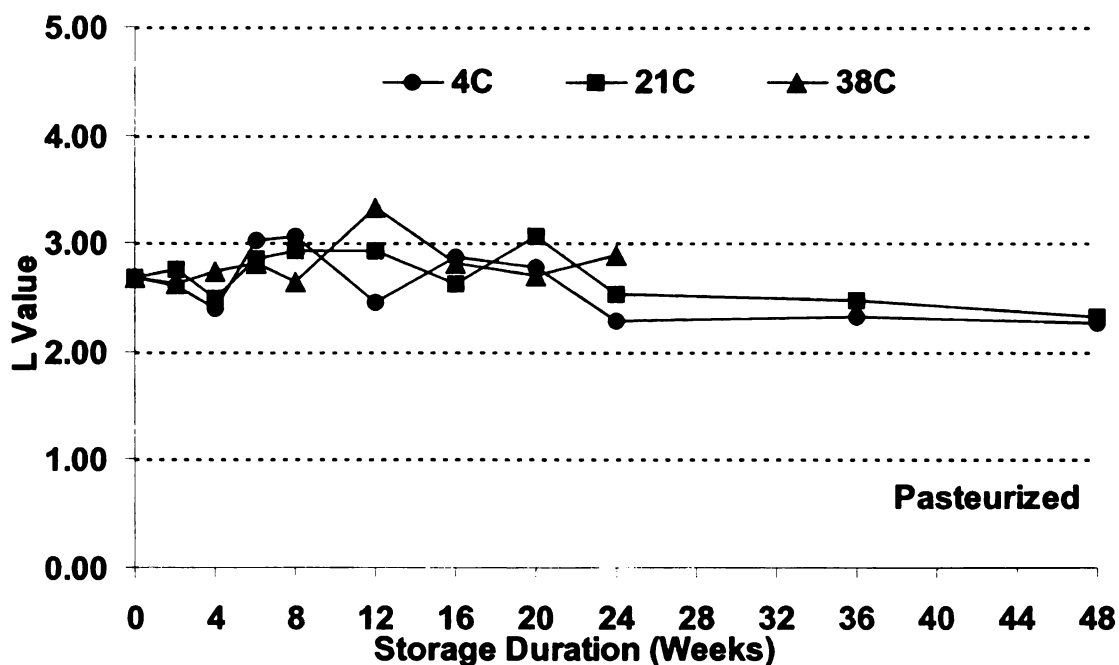
With F test analysis no significant difference was seen between unpasteurized and pasteurized concentrate for all storage conditions (4°C:  $p=0.1147$ , 21°C:  $p=0.0538$ , 38°C:  $p=0.0820$ ). F test showed storage condition, 21°C, to have a significantly higher Hunter L value (or lighter color) compared to 38°C for unpasteurized concentrate ( $p=0.0116$ ). For pasteurized concentrate, the storage condition 4°C had a significantly higher Hunter L value over 38°C ( $p=0.0445$ ). Though these trends are slight, it was confirmed through sensory testing (Section 1.3.3.2) that storage of concentrate at higher storage temperatures yields a dark brown color over storage duration. This dark color that is developed is likely to be correlated with the sample's increased turbidity and decreasing anthocyanins content.

**Table 1.6: Linear regression results for Hunter Color L Value**

Unpasteurized	P-value	Slope	Pasteurized	P-value	Slope
04°C	0.0234	-0.0159	4°C	0.0790	No significant difference
21°C	0.1151	No significant difference	21°C	0.0883	No significant difference
38°C	0.0569	No significant difference	38°C	0.4006	No significant difference



**Figure 1.9: Effects of storage time and temperature on the Hunter color L value of unpasteurized cherry juice concentrate**



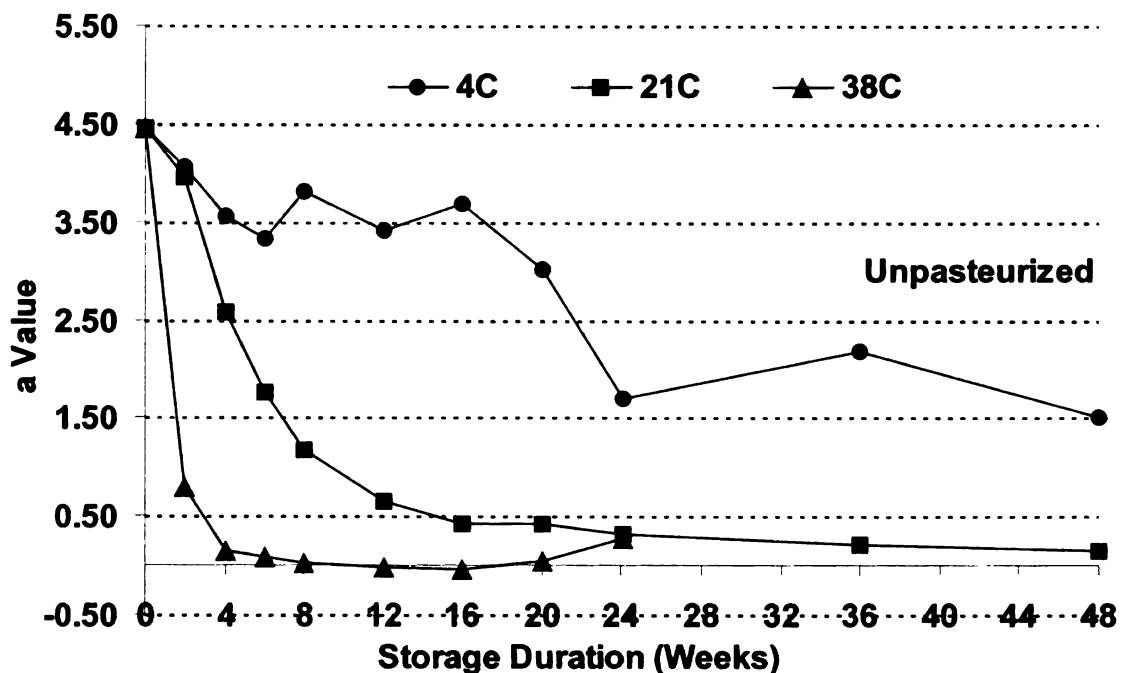
**Figure 1.10: Effects of storage time and temperature on the Hunter color L value of pasteurized cherry juice concentrate**

Figures 1.11 and 1.12 display the Hunter color a values for all storage temperatures and processing types over the duration of storage. The Hunter a value was the most affected Hunter value during the shelf life study. A positive a value represents a red color in the product. Over the course of storage as the concentrate's red color was lost and oxidized, the Hunter a value began to decay. Since the red pigment of cherry juice concentrate is a result of anthocyanin compounds, the decay trends were very comparable to the total anthocyanin decay (Section 2.3.3).

Initial a values were 4.46 for unpasteurized concentrate and 3.95 for pasteurized concentrate. Concentrate stored at 4°C showed a significant decrease over the duration of 48 weeks for both unpasteurized ( $p=0.0002$ ) and pasteurized ( $p=0.0007$ ) concentrate. Among all storage condition refrigerated

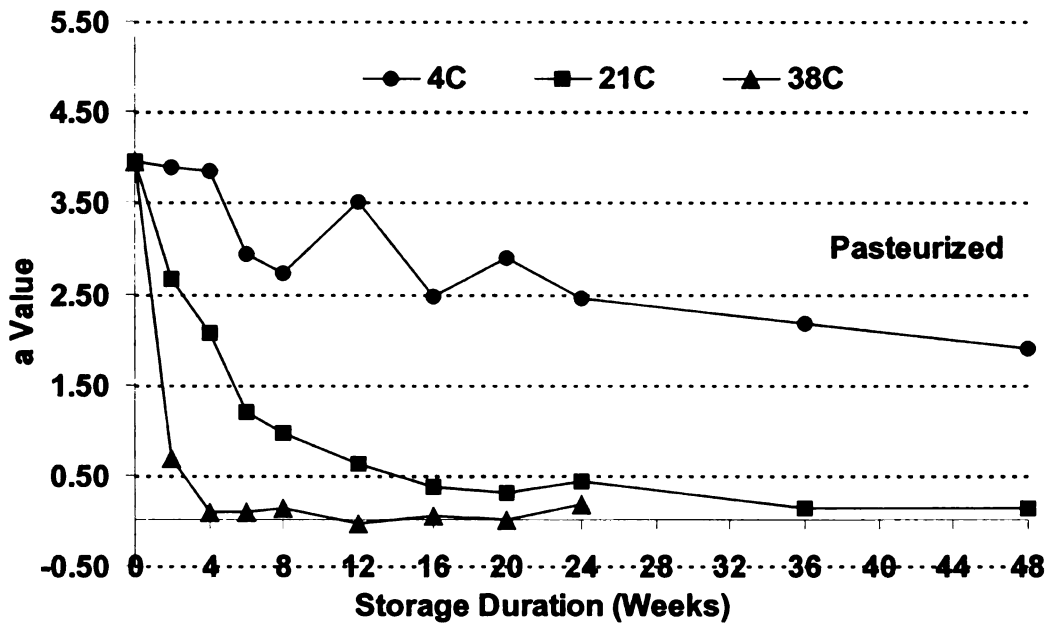
temperatures showed the slowest rate of red color loss. Unpasteurized concentrate show a significantly larger decreasing slope over the 48 weeks of storage ( $p=0.0827$ ) however values were found to be very similar after the storage year (1.51 and 1.89 for unpasteurized and pasteurized concentrate respectively).

The storage temperature of 38°C showed the very steep rate of decay losing 95% of its value within the first 4 weeks of storage. After 4 weeks of storage values were not significantly different throughout the rest of storage for both unpasteurized ( $p=0.5552$ ) and pasteurized ( $p=0.9377$ ) concentrate. Concentrate stored at 21°C showed a slowly significant decreasing exponential decay curve ( $p<0.0001$ ). After 16 weeks of storage the concentrate had lost the majority of its value and no large change occurred throughout the rest of the storage period.



**Figure 1.11: Effects of storage time and temperature on the Hunter color a value of unpasteurized cherry juice concentrate**





**Figure 1.12: Effects of storage time and temperature on the Hunter color a value of pasteurized cherry juice concentrate**

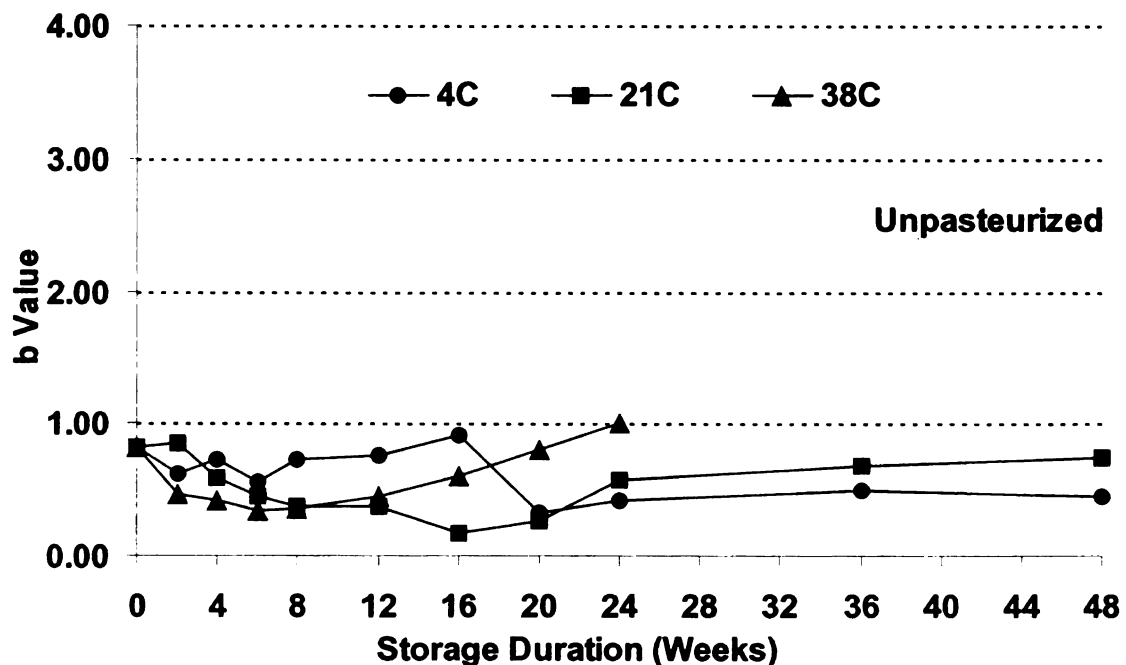
Shown in Figures 1.13 and 1.14 are the Hunter b values over the duration of storage. Linear regression showed that there was no significant change in the Hunter b values over storage duration (Table 1.7). Using the F test it was determined pasteurization does not affect the b value (yellow/blue color) over duration of time by showing no significant difference among the unpasteurized and pasteurized concentrate (Table 1.7).

All Hunter b values were shown to range with the 0 to 1 values. Though all values were found within a small range, F tests showed concentrate stored at 4°C were significantly different than concentrate stored at 38°C. Concentrate at 4°C had a significantly higher Hunter b value for both unpasteurized ( $p=0.0154$ ) and pasteurized ( $p=0.0187$ ) concentrate. Pasteurized concentrate at 4°C also had a significantly higher Hunter b value compared to the storage

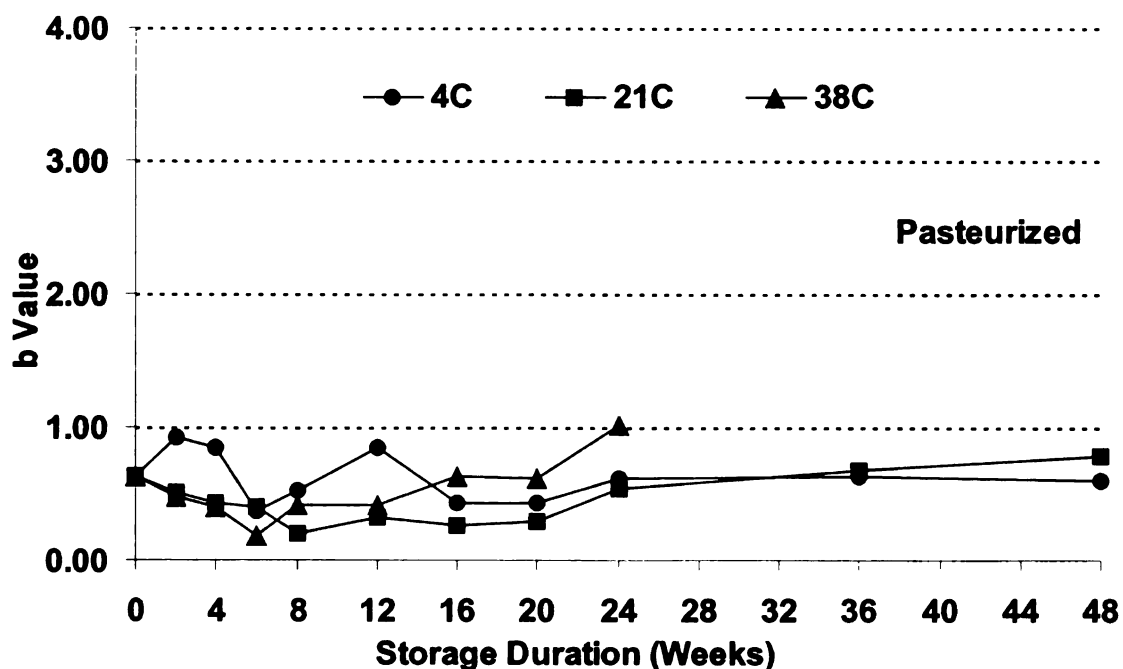
condition of 21°C. Based on results the lower the storage temperature concentrate is stored at the higher the Hunter b value. These higher Hunter b values represent a concentrate with a more yellow color than concentrate stored at elevated conditions.

**Table 1.7: Linear regression results for Hunter Color b Value**

Unpasteurized	P-value	Slope	Pasteurized	P-value	Slope
4°C	0.7019	No significant difference	4°C	0.5211	No significant difference
21°C	0.8675	No significant difference	21°C	0.0932	No significant difference
38°C	0.1059	No significant difference	38°C	0.0635	No significant difference

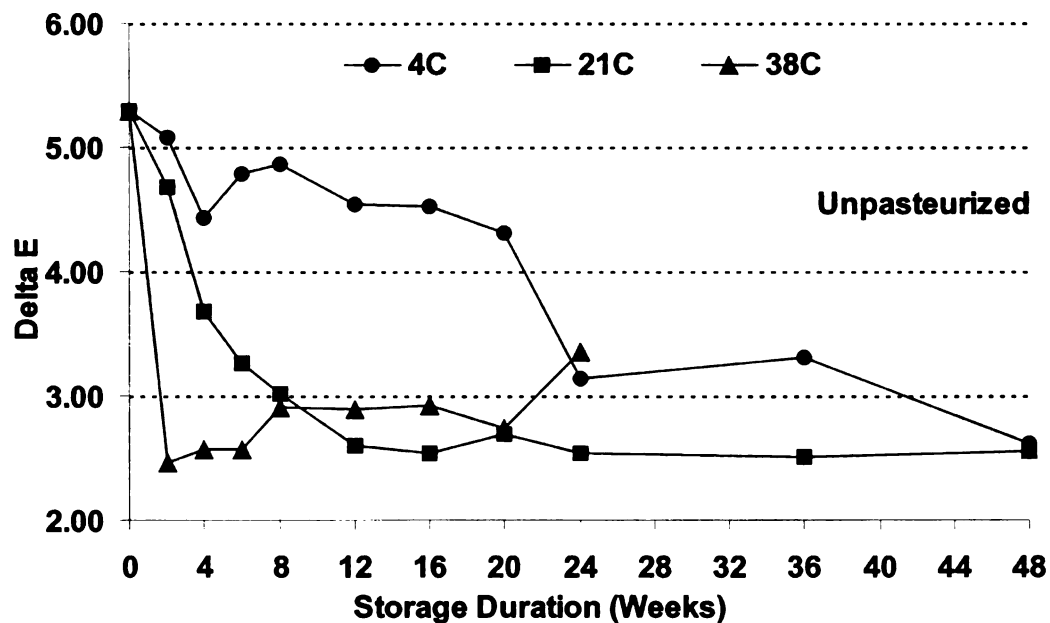


**Figure 1.13: Effects of storage time and temperature on the Hunter color b value of unpasteurized cherry juice concentrate**

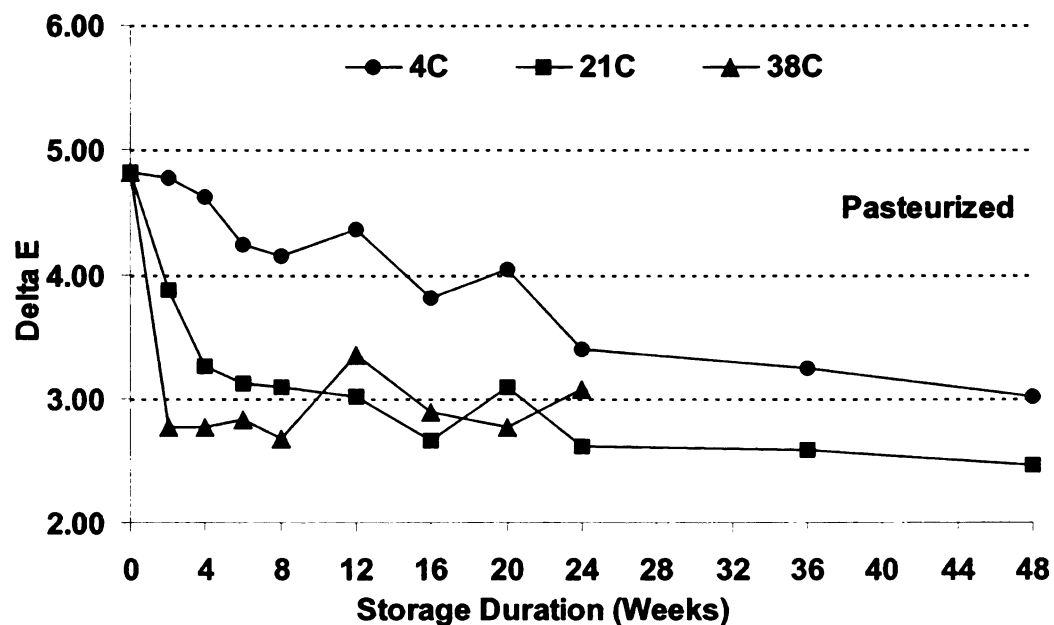


**Figure 1.14: Effects of storage time and temperature on the Hunter color b value of pasteurized cherry juice concentrate**

Shown in Figures 1.15 and 1.16 are the Hunter  $\Delta E$  values over the duration of storage. The value  $\Delta E$  is a single value representing total color difference which takes the Hunter L, a, and b values all into account. Since it was determined that only Hunter a change significantly over time the trend the  $\Delta E$  represent are very similar to the Hunter a values. The concentrate stored at the 4°C showed to have the most stable color over storage duration. Concentrate stored at the elevated 38°C had the most drastic and rapid change in overall color. This color change occurred mostly during the first 2 weeks of analysis.



**Figure 1.15: Effects of storage time and temperature on the Hunter color Delta E value of unpasteurized cherry juice concentrate**



**Figure 1.16: Effects of storage time and temperature on the Hunter color Delta E value of pasteurized cherry juice concentrate**

### **1.3.3 Trained Sensory Panel**

#### **1.3.3.1 Processing and Storage Condition Effect**

Over the duration of the shelf life study the trained sensory panel evaluated the cherry juice concentrates to have slight to no significant difference between the attributes of the unpasteurized and pasteurized samples. However, at the initial analysis (Table 1.8), and week 4 (Table 1.10) and 8 (Table 1.11) panelists found slight significant differences among the samples. These differences were mainly seen in the color attribute. After 8 weeks of storage, no significant difference was seen between unpasteurized and pasteurized concentrate. After 8 weeks, the storage duration had a higher effect on the concentrate than did the processing variable.

Table 1.8 presents the average sensory results and the Tukey's HSD test comparison results for processing of the concentrate. At the initial analysis, the trained panel found that the concentrate's attributes were not significantly different among the unpasteurized and pasteurized samples. The only exception was the fresh cherry flavor of the unpasteurized samples ( $14.50 \pm 0.97$ ), which was found to be significantly higher ( $p=0.0197$ ) than the pasteurized concentrate ( $13.56 \pm 1.55$ ). The average overall quality of the unpasteurized and pasteurized samples was  $14.44 \pm 1.09$  and  $14.19 \pm 1.05$ , respectively ( $p=0.2997$ ).

**Table 1.8: Initial trained panel average sensory ratings for tart cherry concentrate and reconstituted juice**

Attribute	Unpasteurized	Pasteurized	P-value
Concentrate Color	14.75 ± 0.58	14.56 ± 0.89	0.4230
Concentrate Cherry Aroma	14.13 ± 1.71	13.88 ± 1.78	0.3332
Juice Color	14.63 ± 0.89	14.13 ± 1.31	0.1194
Juice Fresh Cherry Flavor	14.50 ± 0.97 <b>A</b>	13.56 ± 1.55 <b>B</b>	0.0197
Overall Quality	14.44 ± 1.09	14.19 ± 1.05	0.2997

n=16, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different

After 2 weeks of storage only the concentrate stored at 38°C was analyzed by panelists. The additional analysis was held due to the predicted and observed high rate of quality decline. Table 1.9 presents the average values and the Tukey's HSD test comparison results for the unpasteurized and pasteurized concentrate stored at 38°C for 2 weeks. All attributes for the unpasteurized and pasteurized samples were not significantly different. The overall quality ratings of the unpasteurized and pasteurized samples were found to be  $9.13 \pm 1.73$  and  $8.47 \pm 2.39$ , respectively. The quality of this concentrate decreased at a very rapid rate over the 2 weeks of storage. The product's initial bright red color had begun to diminish and become darker and duller. Also over these 2 weeks of storage, the juice fresh flavor and concentrate aroma had also decreased.

**Table 1.9: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 2 weeks 4, 21 and 38°C**

Attribute	UP 38°C	P 38°C	P-value
Concentrate Color	9.93 ± 1.44	10.00 ± 1.77	0.8430
Concentrate Cherry Aroma	8.87 ± 2.72	8.67 ± 2.69	0.8142
Juice Color	9.53 ± 1.81	9.53 ± 1.85	1.0000
Juice Fresh Cherry Flavor	8.87 ± 1.60	8.53 ± 2.80	0.6050
Overall Quality	9.13 ± 1.73	8.47 ± 2.39	0.3535

n=15, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P= pasteurized

Table 1.10 presents the average ratings and the Tukey's HSD test comparison results for the processing and storage of the unpasteurized and pasteurized samples at 4 weeks of storage. All p-values were found to be 0.0001 for each attribute. After 4 weeks of storage the sensory ratings for samples stored at 38°C were shown to be significantly lower than those of samples stored at 4°C and 21°C for all attributes analyzed. The concentrate stored at 38° C was evaluated with an average rating of  $5.27 \pm 0.80$  and  $5.67 \pm 1.11$  for the unpasteurized and pasteurized samples. These low sensory ratings represented a concentrate of poor quality with a dull brown color, low cherry aroma and flavor with developing off flavors and aromas. Panelists described off aromas as fermented, and medicine like. They described the samples as having both cooked and prune like flavors.

For concentrate held a 4°C, the overall quality ratings were  $14.13 \pm 1.06$  for unpasteurized, and  $14.47 \pm 0.64$  for pasteurized concentrate while the concentrate stored at 21°C were found to be  $13.60 \pm 1.40$  for unpasteurized and  $12.93 \pm 1.58$  for the pasteurized. The concentrate stored at 4°C and 21°C were not found to be significantly different in the attribute of cherry aroma. For the attributes of color, taste and overall quality it was shown that the pasteurized sample stored at 21°C had a significantly lower rating than the unpasteurized sample (21°C) and the concentrate stored at 4°C.

**Table 1.10: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 4 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.80 ± 0.041AB	14.87 ± 0.35 A	14.53 ± 0.52 AB	14.07 ± 0.59 B	6.00 ± 1.36 C	6.00 ± 1.00 C
<b>Concentrate Cherry Aroma</b>	13.40 ± 1.64 A	13.73 ± 2.12 A	13.60 ± 2.23 A	12.60 ± 2.64 A	5.53 ± 1.77 B	5.47 ± 0.99 B
<b>Juice Color</b>	14.60 ± 0.74 A	14.53 ± 0.64 A	14.20 ± 0.86 A	13.27 ± 1.22 B	4.93 ± 1.16 C	5.60 ± 0.83 C
<b>Juice Fresh Cherry Flavor</b>	14.07 ± 1.03 A	14.40 ± 0.91 A	13.47 ± 1.36 AB	12.80 ± 1.47 B	5.07 ± 1.39 C	5.73 ± 1.75 C
<b>Overall Quality</b>	14.13 ± 1.06 A	14.47 ± 0.64 A	13.60 ± 1.40 AB	12.93 ± 1.58 B	5.27 ± 0.80 C	5.67 ± 1.11 C

n=15, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P= pasteurized

Table 1.11 presents the average scores and the Tukey's HSD test comparison results for processing and storage of the samples after 8 weeks of storage. All p-values for each attribute were 0.0001. The pattern of quality deterioration continued where the samples stored at the elevated temperature (38°C) were significantly lower than samples stored at refrigerated (4°C) and ambient (21°C) temperatures. The cherry concentrate stored at refrigerated and room temperature was found not to be significantly different in the attribute of cherry aroma. This relationship between samples also occurred the previous evaluation (Table 1.10). For the 4°C stored samples, the overall quality ratings were 14.20 ± 1.82 and 13.80 ± 1.47, for unpasteurized and pasteurized samples, respectively. The samples that were stored at 21°C had an overall quality rating of 11.60 for both unpasteurized and pasteurized samples. These ratings represented a good quality sample with slight loss of bright red color, cherry aroma, and fresh cherry flavor. Finally samples at 38°C had an average quality rating of 0.73 ± 1.39 for unpasteurized and 1.67 ± 2.35 for pasteurized.



Concentrate stored at 38°C were not evaluated for its juice's color and flavor because the concentrate was no longer of a consumable quality.

**Table 1.11: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 8 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.60 ± 0.83 A	14.67 ± 0.62 A	12.73 ± 1.03 B	12.60 ± 0.83 B	1.67 ± 1.95 C	1.33 ± 1.68 C
<b>Concentrate Cherry Aroma</b>	13.20 ± 3.73 A	13.53 ± 2.03 A	11.73 ± 2.37 A	12.53 ± 1.41 A	1.47 ± 1.60 B	1.20 ± 1.52 B
<b>Juice Color</b>	14.53 ± 0.74 A	14.27 ± 0.96 AB	12.00 ± 1.51 B	12.00 ± 1.36 B	n/a	n/a
<b>Juice Fresh Cherry Flavor</b>	14.20 ± 1.82 A	13.53 ± 1.64 AB	11.67 ± 1.80 AB	11.40 ± 2.23 B	n/a	n/a
<b>Overall Quality</b>	14.20 ± 1.82 A	13.80 ± 1.47 A	11.60 ± 1.72 B	11.60 ± 1.88 B	0.73 ± 1.39 C	1.67 ± 2.35 C

n=15, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

The average ratings and the Tukey's HSD test comparison results for processing and storage for the concentrate samples after 12 weeks of storage are presented in Table 1.12. The p-values for each attribute were 0.0001. At 12 weeks, the concentrate's attributes at each storage condition were significantly different among one another. Concentration stored at 4°C had the highest ratings while those held at 38°C had the lowest ratings for all attributes evaluated. The average overall quality ratings for unpasteurized and pasteurized concentrate stored at 4°C were 14.33 ± 0.90 and 14.07 ± 1.16, respectively. Concentrate stored at 21°C had ratings that were significantly lower than the concentrate stored under refrigerated conditions. However, the overall quality ratings for the products at 21°C were still were of acceptable quality and very similar to ratings after 8 weeks of storage (11.67 ± 1.92 and 11.27 ± 1.62 for unpasteurized and pasteurized respectively). Unpasteurized

samples stored at 38°C were unacceptable and were given a  $0.80 \pm 2.15$  and  $0.93 \pm 2.63$  rating for unpasteurized and pasteurized, respectively. Concentrate of these low ratings were of very poor and inconsumable quality due to the development brown/oxidized color, poor taste and aroma profile and the development of aged and off-flavors and aromas.

**Table 1.12: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 12 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.73 ± 0.46 A	14.67 ± 0.49 A	12.20 ± 1.61 B	11.53 ± 1.85 B	0.47 ± 0.92 C	0.53 ± 0.99 C
<b>Concentrate Cherry Aroma</b>	13.80 ± 1.94 A	13.53 ± 2.33 A	11.60 ± 2.10 B	11.80 ± 2.76 B	0.67 ± 1.45 C	0.33 ± 0.62 C
<b>Juice Color</b>	14.53 ± 0.52 A	14.33 ± 0.90 A	11.47 ± 1.73 B	11.07 ± 1.67 B	n/a	n/a
<b>Juice Fresh Cherry Flavor</b>	14.33 ± 1.29 A	13.93 ± 1.58 A	11.87 ± 2.07 B	11.20 ± 2.08 B	n/a	n/a
<b>Overall Quality</b>	14.33 ± 0.90 A	14.07 ± 1.16 A	11.67 ± 1.92 B	11.27 ± 1.62 B	0.80 ± 2.15 C	0.93 ± 2.63 C

n=15, 15 cm scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

Table 1.13 displays the trained panel average ratings and the Tukey's HSD test comparison results for processing and storage for the concentrate samples after 16 weeks of storage. The p-values for each attribute were 0.0001. The samples held at 4°C continued to have the highest overall quality ratings for the unpasteurized ( $14.40 \pm 0.91$ ) and pasteurized samples ( $13.47 \pm 1.92$ ). The ratings for the samples held at 21°C continued to decrease with the additional 4 weeks of storage. The average overall quality rating for the unpasteurized 21°C temperature sample was  $9.33 \pm 1.95$  while the pasteurized samples were significantly similar at  $8.87 \pm 2.03$ . These ratings were approximately 35% lower than those of the refrigerated samples stored for 16

weeks. A concentrate with this overall quality rating had lost most of its bright red color and had turned a dull dark red color. Also, the product's cherry aroma became much weaker. Though the product at 21°C ratings reflected a product that was no longer a high quality concentrate, it was not considered poor and inconsumable. A rating of 5.0 or lower was considered the lowest score for consumable product. The quality ratings for the samples held at 38°C were too low to be considered acceptable for consumption (Table 1.13).

**Table 1.13: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 16 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.67 ± 0.62 A	14.67 ± 0.49 A	9.60 ± 1.81 B	9.00 ± 0.76 B	0.27 ± 0.59 C	0.20 ± 0.56 C
<b>Concentrate Cherry Aroma</b>	13.87 ± 1.89 A	14.07 ± 1.44 A	10.00 ± 2.24 B	9.27 ± 1.83 B	0.40 ± 0.63 C	0.73 ± 1.22 C
<b>Juice Color</b>	14.40 ± 1.06 A	13.80 ± 1.15 A	9.33 ± 1.95 B	8.53 ± 1.85 B	n/a	n/a
<b>Juice Fresh Cherry Flavor</b>	14.20 ± 0.94 A	13.47 ± 2.17 A	9.20 ± 2.01 B	8.80 ± 2.27 B	n/a	n/a
<b>Overall Quality</b>	14.40 ± 0.91 A	13.47 ± 1.92 A	9.40 ± 1.68 B	8.87 ± 2.03 B	0.27 ± 0.59 C	0.60 ± 1.30 C

n=15, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

The trained panel average ratings and the Tukey's HSD test comparison results for processing and storage for the concentrate samples for week 20 and week 24 of storage are shown in Tables 1.14 and 1.15. The p-values for each attribute were 0.0001. Trained panels at week 20 and 24 of storage were very similar to one another. Concentrate at 4°C continued to have the highest acceptance ratings, while concentrate stored at 21°C was approximately 45% lower.

**Table 1.14: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 20 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.67 ± 0.49 A	14.40 ± 0.51 A	8.00 ± 1.13 B	8.30 ± 1.15 B	1.07 ± 3.62 C	0.07 ± 0.26 C
<b>Concentrate Cherry Aroma</b>	14.07 ± 1.28 A	13.13 ± 2.00 A	8.47 ± 1.46 B	8.00 ± 2.10 B	1.20 ± 3.61 C	0.40 ± 1.06 C
<b>Juice Color</b>	14.40 ± 0.74 A	14.20 ± 0.78 A	7.87 ± 1.25 B	7.80 ± 1.15 B	n/a	n/a
<b>Juice Fresh Cherry Flavor</b>	14.00 ± 1.41 A	13.67 ± 1.50 A	8.33 ± 1.54 B	7.73 ± 1.83 B	n/a	n/a
<b>Overall Quality</b>	14.07 ± 1.34 A	13.60 ± 1.60 A	8.00 ± 1.25 B	7.67 ± 1.63 B	1.07 ± 3.35 C	0.27 ± 0.80 C

n=15, 15 cm scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

**Table 1.15: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 24 weeks at 4, 21 and 38°C**

Attribute	4°C		21°C		38°C	
	UP	P	UP	P	UP	P
<b>Concentrate Color</b>	14.50 ± 0.65 A	14.50 ± 0.52 A	8.21 ± 0.98 B	7.86 ± 1.17 B	0.07 ± 0.27 C	1.14 ± 4.00 C
<b>Concentrate Cherry Aroma</b>	14.07 ± 1.90 A	13.36 ± 2.71 A	7.29 ± 1.20 B	7.43 ± 1.56 B	0.43 ± 0.94 C	0.21 ± 0.58 C
<b>Juice Color</b>	14.50 ± 0.52 A	14.21 ± 0.80 A	7.71 ± 1.49 B	7.21 ± 1.72 B	n/a	n/a
<b>Juice Fresh Cherry Flavor</b>	14.14 ± 1.10 A	13.64 ± 2.06 A	8.21 ± 1.76 B	7.07 ± 1.82 B	n/a	n/a
<b>Overall Quality</b>	14.36 ± 1.80 A	13.71 ± 2.30 A	7.93 ± 1.33 B	7.50 ± 1.83 B	0.14 ± 0.36 C	0.07 ± 0.27 C

n=14, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

At week 36 and 48 of storage, the concentrate at 38°C was no longer evaluated. All sensory and analytical tests were no longer continued due to the concentrate's poor and unacceptable quality. The trained panel average ratings and the Tukey's HSD test comparison results for processing and storage for the concentrate samples for week 36 and week 48 of storage are shown in Tables 1.16 and 1.17. The p-values for each attribute were 0.0001. At week 36 and

48 the concentrate stored at 21°C had a quality rating 65 to 75% lower than concentrate stored at 4°C.

**Table 1.16: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 36 weeks at 4, and 21°C**

Attribute	4°C		21°C	
	UP	P	UP	P
Concentrate	14.70 ±	14.20 ±	5.40 ±	4.90 ±
Color	0.48 A	0.63 A	1.35 B	1.60 B
Concentrate	13.70 ±	12.80 ±	4.10 ±	3.60 ±
Cherry Aroma	2.06 A	2.97 A	1.73 B	2.37 B
Juice Color	14.40 ±	14.00 ±	4.20 ±	4.10 ±
	0.97 A	1.33 A	1.87 B	2.13 B
Juice Fresh	13.40 ±	13.20 ±	3.70 ±	4.50 ±
Cherry Flavor	2.07 A	2.66 A	2.16 B	1.84 B
Overall Quality	13.80 ±	13.10 ±	3.60 ±	4.50 ±
	1.55 A	2.56 A	1.84 B	2.12 B

n=10, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

**Table 1.17: Trained panel average sensory ratings for tart cherry concentrate and reconstituted juice stored 48 weeks at 4, and 21°C**

Attribute	4°C		21°C	
	UP	P	UP	P
Concentrate	14.45 ±	14.09 ±	4.45 ±	4.55 ±
Color	0.69 A	1.45 A	1.37 B	1.37 B
Concentrate	13.64 ±	13.82 ±	3.64 ±	3.91 ±
Cherry Aroma	1.50 A	1.72 A	2.73 B	2.30 B
Juice Color	13.91 ±	13.64 ±	3.91 ±	3.18 ±
	1.51 A	2.34 A	1.97 B	2.23 B
Juice Fresh	13.82 ±	12.91 ±	3.09 ±	4.18 ±
Cherry Flavor	1.66 A	3.45 A	2.02 B	2.86 B
Overall Quality	13.55 ±	13.36 ±	3.36 ±	4.00 ±
	2.07 A	2.87 A	1.80 B	2.57 B

n=11, 15 cm line scale, Values within each attribute not connected by the same letter are significantly different, UP= unpasteurized, P = pasteurized

### 1.3.3.2 Effect of Storage Duration

Figures 1.17 and 1.18 display the average attribute ratings for the unpasteurized and pasteurized concentrate stored at 4°C over storage duration. Using the Tukey's HSD statistical analysis test, the concentrate stored at 4°C

showed no statistical change in its five rated attributes over 48 weeks of storage. These results were concluded for both unpasteurized and pasteurized concentrate ( $p>0.05$ ). All average attributes ratings ranged within the values, 12 to 15. Showing no statistical difference over 48 weeks of storage demonstrates the benefits of storing tart cherry juice concentrate under refrigerated conditions to best preserve its color, aroma, flavor and overall quality.

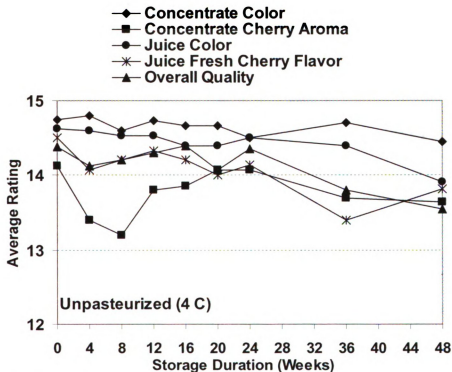
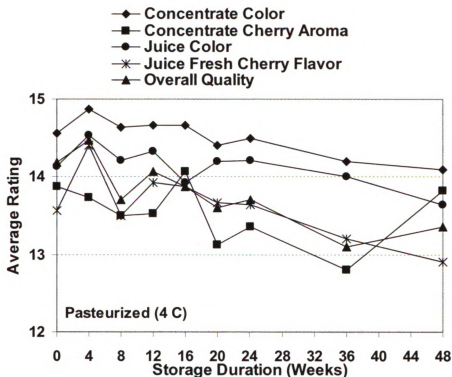


Figure 1.17: Effects of storage duration at 4°C on trained sensory quality for unpasteurized cherry juice concentrate



**Figure 1.18: Effects of storage duration at 4°C on trained sensory quality for pasteurized cherry juice concentrate**

Figures 1.19 and 1.20 present the unpasteurized and pasteurized concentrate's average ratings over the storage period at 21°C. Concentrate stored at 21°C showed a significant decrease in quality over the 48 week storage period ( $p < 0.0001$ ). The largest significant decrease in the concentrate's attributes ratings occurred after weeks 4, 12, and 24. The average concentrate ratings for unpasteurized and pasteurized concentrate are displayed in Table 1.18 and 1.19, along with the Tukey's HSD test comparison over storage duration. After 4 weeks of storage the trained sensory ratings decreased from the 13 to 14 value range to the 10 to 12 value range. Trained panelists commented that at weeks 8 and 12 the concentrate's color was becoming a deeper red and not as bright as it was initially. Though the color

was losing brightness, no browning was detected during these weeks. The concentrate did not have any off-aromas; however, the cherry aroma was beginning to diminish, and had become very light. The reconstituted juice had a good flavor however panelists commented that the product lost some of its tart flavor, and was becoming sweeter in taste. This developing sweet flavor was also seen in a storage study of cherry juice (Gonzalez-Mulet 2008). Even with the change in the concentrate's color, aroma and taste profile, the concentrate at 12 weeks still represented a good quality product.

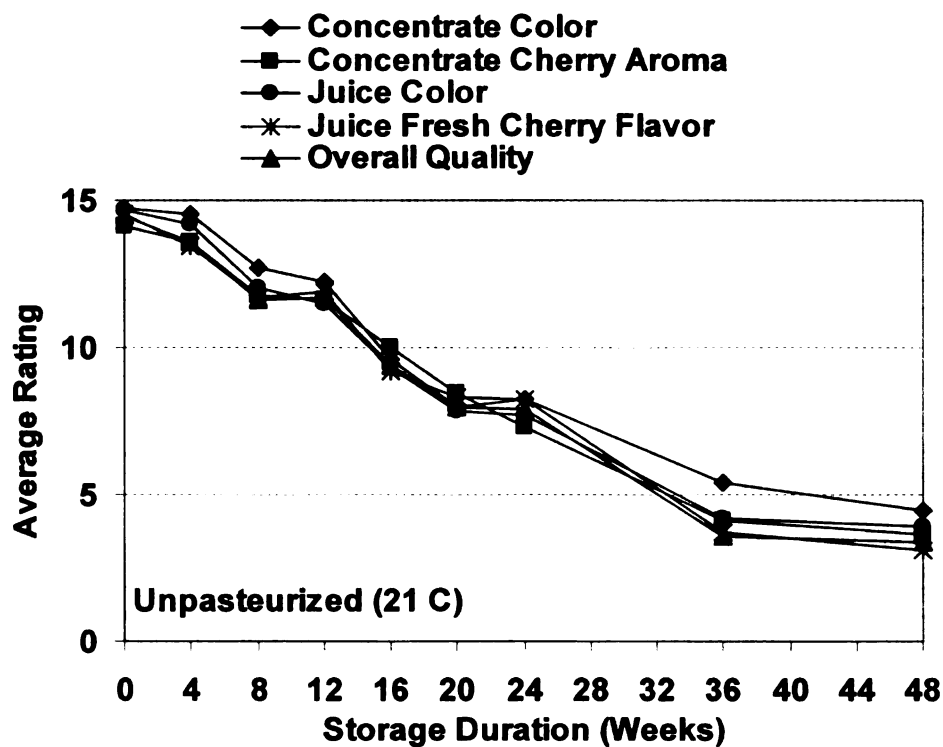
After 12 weeks of storage (Figures 1.19 and 1.20), there was a constant decrease in quality up to week 20. From weeks 20 to 24 there was small significant difference seen in the concentrate's color and aroma, however, the overall quality of the concentrate was constant. Over weeks 18, 20, and 24 of storage the attribute ratings decreased to rating values 7 to 9. Panelists reported that the concentrate now had a color of a weak, less vibrated red, also showing slight brown pigments. Much of the fresh aroma had diminished and the concentrate no longer processed a fresh cherry aroma. Slight off aromas were becoming present including ones of cooked, aged, and prune-like odors. The trained panel believed that for a consumer familiar with tart cherry juice concentrate this product was no longer of quality to be sold.

After week 24 the concentrate's attribute ratings showed a significant decrease at week 26. After week 36, attributes were not significantly different with the additional 12 weeks of storage at week 48. At weeks 36 and 48 attribute values had decreased to a rating within the 3 to 5 value range.

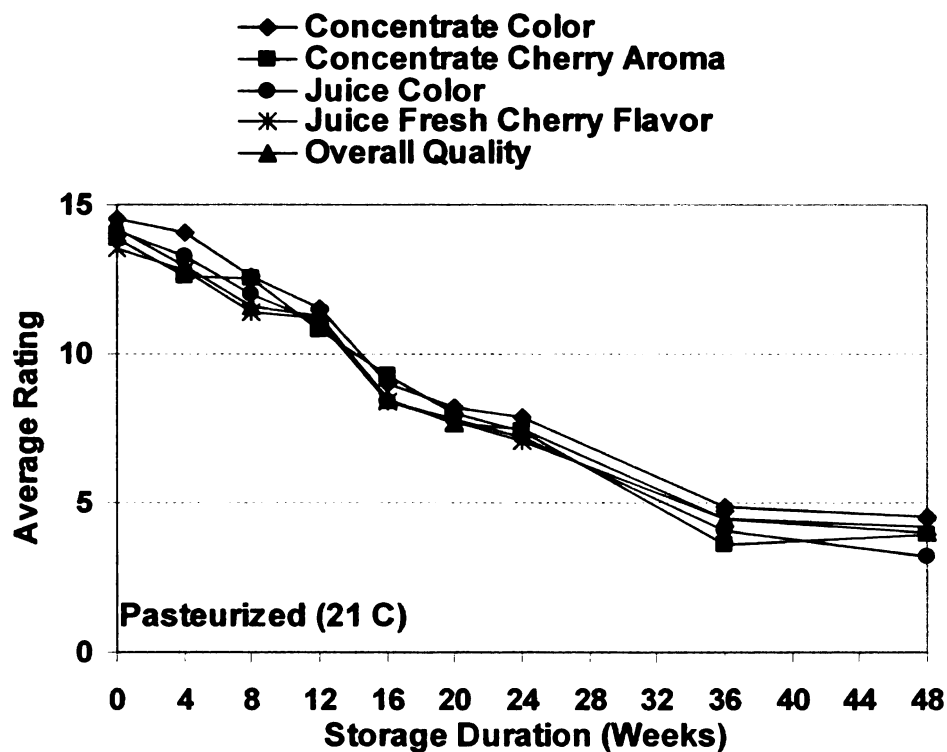


Panelists described these samples with very brown, muddy color which contained no red pigment. The aroma was no longer fresh, and gave off cooked, aged, and medicine-like off-aromas. Panelists reported a majority loss of the juice's tartness. The reconstituted juice was now a very sweet, bland product lacking freshness. A product of these low ratings and poor quality descriptors would definitely not meet quality standards to be of a quality product to be sold to consumers.

A correlation was shown between the trained panel ratings for color results and the Hunter a values. Both the trained panelists and colorimeter measured a constant decrease in red color over storage. However, panelists were not able to view the decreasing color as fast as the instrument. Also, the trained panel saw a decrease in the color attribute throughout the whole 48 weeks while the Hunter a value showed the majority of all red color loss by week 16.



**Figure 1.19: Effects of storage duration at 21°C on trained sensory quality for unpasteurized cherry juice concentrate**



**Figure 1.20: Effects of storage duration at 21°C on trained sensory quality for pasteurized cherry juice concentrate**

**Table 1.18: The average trained panel acceptance ratings and Tukey's HSD test comparison results for storage duration (21°C unpasteurized)**

Week	0	4	8	12	16	20	24	36	48
Concentrate Color	14.75 ± 0.58 A	14.53 ± 0.52 A	12.73 ± 1.03 B	12.20 ± 1.61 B	9.36 ± 1.81 C	8.00 ± 1.13 D	8.21 ± 0.98 CD	5.40 ± 1.35 E	4.46 ± 1.37 E
Concentrate Cherry Aroma	14.13 ± 1.71 A	13.60 ± 2.23 AB	11.73 ± 2.37 BC	11.60 ± 2.10 BC	10.00 ± 2.24 CD	8.47 ± 1.46 DE	7.29 ± 1.20 E	4.10 ± 1.73 F	3.64 ± 2.73 F
Juice Color	14.63 ± 0.89 A	14.2 ± 0.86 A	12.00 ± 1.51 B	11.47 ± 1.73 B	9.33 ± 1.95 C	7.87 ± 1.25 C	7.71 ± 1.49 C	4.20 ± 1.87 D	3.91 ± 1.97 D
Juice Fresh Cherry Flavor	14.50 ± 0.97 A	13.47 ± 1.36 AB	11.67 ± 1.80 B	11.87 ± 2.07 B	9.20 ± 2.01 C	8.33 ± 1.54 C	8.21 ± 1.76 C	3.70 ± 2.16 D	3.09 ± 2.02 D
Overall Quality	14.44 ± 1.09 A	13.60 ± 1.40 A	11.60 ± 1.72 B	11.67 ± 1.92 B	9.40 ± 1.68 C	8.00 ± 1.25 C	7.93 ± 1.33 C	3.60 ± 1.84 D	3.36 ± 1.80 D

15-point cm scale, \*Values not connected by the same letter within each attribute are significantly different

**Table 1.19: The average trained panel acceptance ratings and Tukey's HSD test comparison results for storage duration (21°C pasteurized)**

Week	0	4	8	12	16	20	24	36	48
Concentrate Color	14.56 ± 0.98 A	14.07 ± 0.59 A	12.60 ± 0.83 B	11.53 ± 1.85 B	9.00 ± 0.76 C	8.20 ± 1.15 C	7.86 ± 1.17 C	4.90 ± 1.60 D	4.55 ± 1.37 D
Concentrate Cherry Aroma	13.88 ± 1.78 A	12.60 ± 2.64 AB	12.53 ± 1.41 AB	10.80 ± 2.76 BC	9.27 ± 1.83 CD	8.00 ± 2.10 D	7.43 ± 1.56 D	3.60 ± 2.37 E	3.91 ± 2.30 E
Juice Color	14.13 ± 1.31 A	13.27 ± 1.22 AB	12.00 ± 1.36 BC	11.07 ± 1.67 C	8.40 ± 1.85 D	7.80 ± 1.15 D	7.21 ± 1.72 D	4.10 ± 2.13 E	3.18 ± 2.23 E
Juice Fresh Cherry Flavor	13.56 ± 1.55 A	12.80 ± 1.47 AB	11.40 ± 2.23 AB	11.20 ± 2.08 B	8.40 ± 2.20 C	7.73 ± 1.83 C	7.07 ± 1.82 C	4.50 ± 1.84 D	4.18 ± 2.86 D
Overall Quality	14.19 ± 1.05 A	12.93 ± 1.58 AB	11.60 ± 1.88 B	11.27 ± 1.62 B	8.47 ± 2.03 C	7.67 ± 1.63 C	7.50 ± 1.83 C	4.50 ± 2.12 D	4.00 ± 2.57 D

15-point cm scale, \*Values not connected by the same letter within each attribute are significantly different

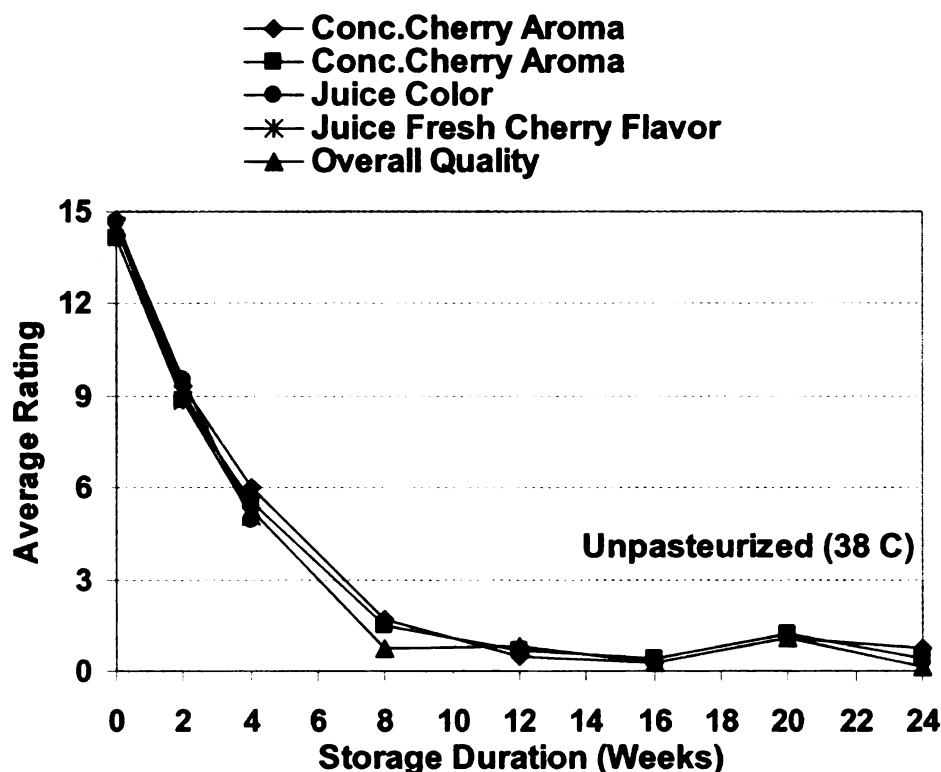
Figures 1.21 and 1.22 present the unpasteurized and pasteurized concentrate's average ratings over the 24 week storage duration at the storage condition of 38°C. Using the Tukey's HSD test for statistical analysis, the samples stored at 38°C showed a significant decrease in quality over the year storage period ( $p < 0.0001$ ). Average sensory ratings for all concentrate are displayed in Table 1.20 and 1.21, along with the Tukey's HSD test comparison over storage duration. The concentrate's quality attributes significantly decreased at 2 weeks, and week 4 and 8. After week 8 of storage the concentrate's attributes were no longer significantly different. There was no significant decrease after week 8 because the concentrate had then already lost all quality and no longer able to decrease in acceptance ratings.

At week 2 of storage the concentrate's ratings had decreased to an 8 to 10 range. Panelists commented that these samples were on the verge of no longer being acceptable for sale. At week 2 the color of the concentrate had lost a majority of its bright red pigment, while brown pigments began to develop. The concentrate had developed a slightly aged, cooked aroma, and became much sweeter in taste. The overall freshness of the sample had slightly decreased; however the cherry flavor was still present.

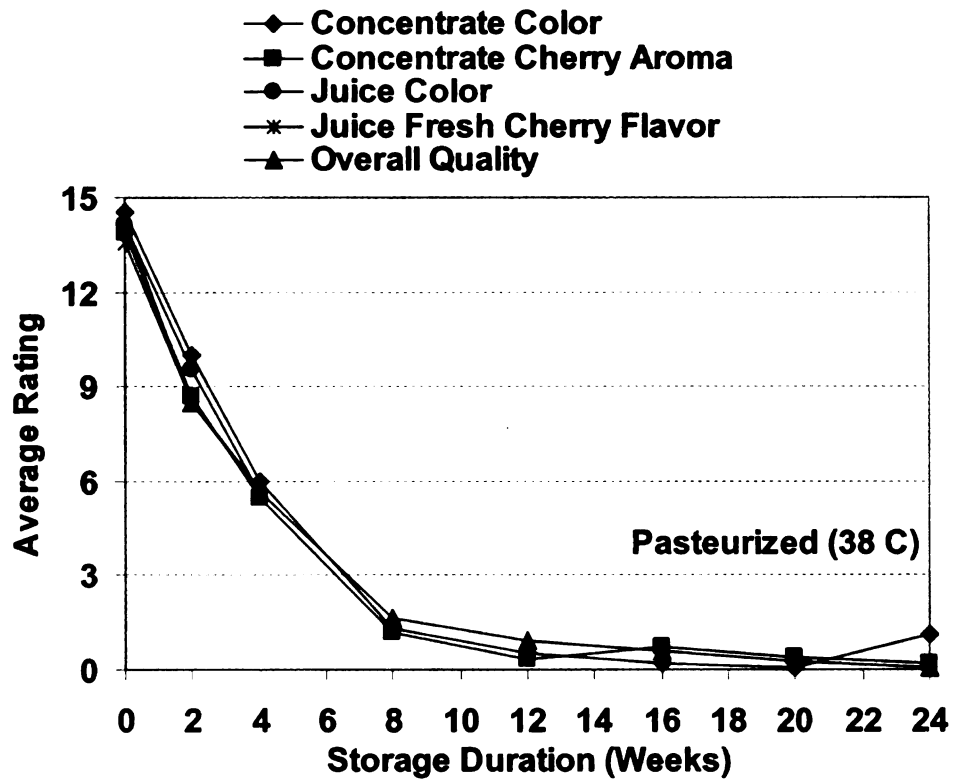
At week 4 the quality of the concentrate decreased to the acceptance range 5 to 6. These ratings were given for the concentrate's poor brown color and for having only small amount of red pigments. Trained panelists commented that the aroma of the concentrate was very poor, and the fresh cherry aroma had completely diminished. Off aromas and flavors were

beginning to become present giving the concentrate a fermented, aged aroma, and an aged, cooked taste. After 4 weeks of storage the reconstituted juice had lost the majority of its tartness and was very sweet, with only a slight cherry flavor.

From week 8 throughout the rest of the storage period the reconstituted juice was no longer evaluated due to its very low quality. At week 8 through 24 the concentrate's color had become a dark, dull muddy brown. The concentrate's aroma lacked freshness, cherry aroma, and contained off-aromas including those fermented, cooked, and medicine-like aromas.



**Figure 1.21: Effects of storage duration at 38°C on trained sensory quality for unpasteurized cherry juice concentrate**



**Figure 1.22: Effects of storage duration at 38°C on trained sensory quality for pasteurized cherry juice concentrate**

**Table 1.20: The average trained panel acceptance ratings and Tukey's HSD test comparison results for storage duration (38°C unpasteurized)**

Week	0	2	4	8	12	16	20	24
Concentrate Color	14.75 ± 0.58 A	9.33 ± 1.44 B	6.00 ± 1.36 C	1.67 ± 1.95 D	0.47 ± 0.92 D	0.27 ± 0.59 D	1.07 ± 3.62 D	0.71 ± 0.27 D
Concentrate Cherry Aroma	14.13 ± 1.71 A	8.87 ± 2.72 B	5.53 ± 1.77 C	1.47 ± 1.60 D	0.67 ± 1.45 D	0.40 ± 0.63 D	1.20 ± 3.61 D	0.43 ± 0.94 D
Juice Color	14.63 ± 0.89 A	9.53 ± 1.81 B	4.93 ± 1.16 C	n/a	n/a	n/a	n/a	n/a
Juice Fresh Cherry Flavor	14.50 ± 0.97 A	8.87 ± 1.60 B	5.07 ± 1.39 C	n/a	n/a	n/a	n/a	n/a
Overall Quality	14.44 ± 1.09 A	9.13 ± 1.73 B	5.27 ± 0.80 C	0.73 ± 1.39 D	0.80 ± 2.15 D	0.27 ± 0.59 D	1.07 ± 3.35 D	0.14 ± 0.36 D

15-point cm scale, \*Values not connected by the same letter are significantly different

**Table 1.21: The average trained panel acceptance ratings and Tukey's HSD test comparison results for storage duration (38°C pasteurized)**

Week	0	2	4	8	12	16	20	24
Concentrate Color	14.56 ± 0.98 A	10.00 ± 1.77 B	6.00 ± 1.00 C	1.33 ± 1.68 D	0.53 ± 0.99 D	0.20 ± 0.56 D	0.07 ± 0.26 D	1.14 ± 4.00 D
Concentrate Cherry Aroma	13.88 ± 1.78 A	8.67 ± 2.69 B	5.47 ± 0.99 C	1.20 ± 1.52 D	0.33 ± 0.62 D	0.73 ± 1.22 D	0.40 ± 1.06 D	0.21 ± 0.58 D
Juice Color	14.13 ± 1.31 A	9.53 ± 1.85 B	5.60 ± 0.83 C	n/a	n/a	n/a	n/a	n/a
Juice Fresh Cherry Flavor	13.56 ± 1.55 A	8.53 ± 2.80 B	5.73 ± 1.75 C	n/a	n/a	n/a	n/a	n/a
Overall Quality	14.19 ± 1.05 A	8.47 ± 2.39 B	5.67 ± 1.11 C	1.67 ± 2.35 D	0.93 ± 2.63 D	0.60 ± 1.30 D	0.27 ± 0.80 D	0.07 ± 0.27 D

15-point cm scale, \*Values not connected by the same letter are significantly different

### **1.3.4 Consumer Sensory Panel**

#### **1.3.4.1 Processing and Storage Condition Effect**

After 16 weeks of storage, an acceptance consumer panel was conducted to evaluate both the unpasteurized and pasteurized concentrate stored at 4 and 21°C. The samples stored at 38°C were not evaluated because the trained panel determined that they were not fit for consumption after 4 weeks of storage. Average acceptance ratings of each attribute rated are displayed in Table 1.22. Also shown is the Tukey HSD statistical analysis on each rated attribute. Consumers found that the color among the reconstituted juice stored at 4 and 21°C were significantly different. Concentrate stored at 21°C had significantly poorer acceptance ratings than samples held at 4°C. Concentrate stored at 4°C had average color ratings of  $7.58 \pm 1.10$  and  $7.32 \pm 1.16$  for unpasteurized and pasteurized concentrate respectively. These ratings represent a color that is moderately liked by the consumer. Concentrate stored at 21°C had average color ratings of  $5.89 \pm 1.77$  and  $5.89 \pm 1.78$  for the unpasteurized and pasteurized concentrate respectively. Ratings represented a color of a product that was neither liked or disliked by the consumer. The aroma and flavor among the four samples were found to have no significant difference in acceptance. Similar to color, the overall acceptability of the juice was significantly different among the concentrate stored at 4°C and 21°C. Concentrate stored at 4°C were found to have a greater degree of acceptability in overall acceptance.



**Table 1.22: Average consumer acceptance ratings and Tukey's HSD test comparison at week 16 of storage (unpasteurized and pasteurized)**

Attribute	4°C		21°C		P-value
	UP	P	UP	P	
<b>Color</b>	7.58 ± 1.10 <b>A</b>	7.32 ± 1.16 <b>A</b>	5.87 ± 1.77 <b>B</b>	5.89 ± 1.78 <b>B</b>	0.0001
<b>Aroma</b>	6.51 ± 1.49	6.46 ± 1.33	6.43 ± 1.61	6.54 ± 1.49	0.9019
<b>Flavor</b>	6.87 ± 1.70	6.78 ± 1.61	6.50 ± 1.66	6.57 ± 1.63	0.2518
<b>Overall Acceptability</b>	6.90 ± 1.58 <b>A</b>	6.88 ± 1.33 <b>A</b>	6.42 ± 1.67 <b>B</b>	6.51 ± 1.59 <b>B</b>	0.0153

n=91, 9 point hedonic scale, Values within each attribute connected by different letters are significantly different, UP= unpasteurized, P = pasteurized

The consumer panel average acceptance ratings and Tukey HSD statistical analysis for storage week 24 are shown in Table 1.23. The ratings for aroma and flavor attributes were found to be significantly the same for all samples evaluated by consumers. The concentrate stored at 4°C had significantly higher color ratings and overall acceptability over concentrate stored at 21°C. The overall acceptability for concentrate stored at 4°C was 6.90 ± 1.46 and 6.89 ± 1.25 while concentrate stored at 21°C had an acceptance rating of 6.24 ± 1.62 and 6.13 ± 1.73. Though both storage temperatures had significantly different overall acceptability ratings all concentrate's ratings fell within the "like slightly" definition.

**Table 1.23: Average consumer acceptance ratings and Tukey's HSD test comparison at week 24 of storage (unpasteurized and pasteurized)**

Attribute	4°C		21°C		P-value
	UP	P	UP	P	
<b>Color</b>	7.67 ± 1.07 <b>A</b>	7.55 ± 0.94 <b>A</b>	4.96 ± 1.91 <b>B</b>	5.10 ± 2.02 <b>B</b>	0.0001
<b>Aroma</b>	6.56 ± 1.26	6.55 ± 1.31	6.35 ± 1.46	6.72 ± 1.37	0.1826
<b>Flavor</b>	6.71 ± 1.81	6.86 ± 1.42	6.49 ± 1.69	6.38 ± 1.90	0.1067
<b>Overall Acceptability</b>	6.90 ± 1.46 <b>A</b>	6.89 ± 1.25 <b>A</b>	6.24 ± 1.62 <b>B</b>	6.13 ± 1.73 <b>B</b>	0.0001

n=100, 9 point hedonic scale, Values not connected by the same letter are significantly different, UP=unpasteurized, P=pasteurized

In Table 1.24 are the consumer panel average acceptance ratings and Tukey HSD statistical analysis for storage week 36. Unlike the consumer panels at week 16 and 24, week 36 is the first consumer panel that participants significantly saw a difference in flavor between the two storage conditions. Consumers saw no significant difference among the unpasteurized and pasteurized concentrate, but they did see a significant difference between color, flavor and overall acceptability. The concentrate's color played a large factor in the consumer's perception of overall quality. Concentrate stored at 4°C received an average hedonic rating of “like moderately” while concentrate at 21°C received an average “dislike slightly” ratings. Concentrate stored at refrigerated conditions received higher overall acceptability with acceptance ratings of  $6.88 \pm 1.46$  and  $6.95 \pm 1.48$ , while concentrate stored at ambient temperatures received ratings of  $5.52 \pm 1.72$  and  $5.40 \pm 1.84$ .

**Table 1.24: Average consumer acceptance ratings and Tukey's HSD test comparison at week 36 of storage (unpasteurized and pasteurized)**

Attribute	4°C		21°C		P-value
	UP	P	UP	P	
<b>Color</b>	$7.60 \pm 1.35$ A	$7.67 \pm 1.07$ A	$4.22 \pm 1.86$ B	$4.17 \pm 1.97$ B	0.0001
<b>Aroma</b>	$6.32 \pm 1.41$	$6.48 \pm 1.32$	$6.16 \pm 1.55$	$6.18 \pm 1.63$	0.2374
<b>Flavor</b>	$6.76 \pm 1.58$ A	$6.70 \pm 1.58$ A	$6.09 \pm 1.79$ B	$5.84 \pm 2.00$ B	0.0001
<b>Overall Acceptability</b>	$6.88 \pm 1.46$ A	$6.95 \pm 1.48$ A	$5.52 \pm 1.72$ B	$5.40 \pm 1.84$ B	0.0001

n=101, 9 point hedonic scale, Values not connected by the same letter are significantly different, UP=unpasteurized, P=pasteurized

For the final consumer panel, panelists evaluated the unpasteurized concentrate which was held at 4°C for 48 weeks and “fresh” unpasteurized concentrate which was held at frozen temperatures since production. These

products were chosen for the 24 week consumer panel because the concentrate stored at 21°C at 48 weeks received very low ratings by the trained panel. These low ratings represented a product, which was poor quality and would not be sold to consumers. Pasteurized concentrate was not analyzed because it was already determined that the average consumer does not see a significant difference among these samples.

Table 1.25 displays the consumer panel's average acceptance ratings and Tukey HSD statistical analysis for storage week 48. The consumer panel showed that after 48 weeks, concentrate stored at refrigerated conditions had a significantly lower acceptance rating for color than fresh concentrate. However, both concentrates were shown to not be significantly different in the attributes of aroma and flavor and the overall acceptability of the products.

**Table 1.25: Average consumer acceptance ratings and Tukey's HSD test comparison at week 48 of storage (Fresh vs. Stored)**

Attribute	Fresh (UP)	4°C (UP)	P- value
Color	7.55 ± 1.14 A	6.75 ± 1.43 B	0.0001
Aroma	6.57 ± 1.39	6.41 ± 1.38	0.3021
Flavor	6.55 ± 1.70	6.92 ± 1.43	0.0735
Overall Acceptability	6.73 ± 1.52	6.88 ± 1.36	0.4106

n=96, 9 point hedonic scale, Values not connected by the same letter are significantly different, UP=unpasteurized, P=pasteurized

From analyzing the effects of pasteurization and storage condition on the consumer's acceptance of cherry juice concentrate it can be concluded that consumers do not detect a significant difference between unpasteurized and pasteurized concentrate. Consumers saw no significant difference in the aroma profile among the 4°C and 21°C storage conditions. This was also true for flavor, however at week 36 a taste difference between 4°C and 21°C became present. The attribute that was most influential on the overall acceptance of the

concentrate was the color attribute. In all consumer panels concentrate stored at 4°C had the higher acceptance ratings over concentrate stored at ambient temperatures.

During each consumer panel, panelists were also asked marketing questions to better understand their likeness of the product and purchasing habits. Of the consumers who participated in the panels approximately 75 to 80% of them were consumers of juice made from concentrate. Panelists were asked if they were likely to purchase their favorite juice sample. On a 5-point scale ranging from “definitely not likely” to “definitely likely” only a small percentage of ranging from 7 to 17% answered that they would “probably” or “definitely” purchase the product. 22 to 33% of the panelists were unsure of their purchasing decisions. A number of the panelists commented that the product was too tart or sour for their taste, however since tart cherry juice concentrate is known for being a tart product, it can be assumed that a number of consumers are not familiar with the product. Even with the low number of consumers reporting that they would purchase their favorite sample, a quite high percentage (91%) said that they would more willing to purchase the product for its health benefiting attributes.

#### **1.3.4.2 Storage Duration Effect**

When analyzing consumer sensory data over storage duration (week 16, 24, and 36) using Tukey HSD statistical analysis test it was determined that consumers saw no statistical difference in color, aroma, flavor and overall quality for concentrate stored at 4°C.

Tukey's HSD p-values are shown in Table 1.26 for each sample evaluated. For concentrate stored at 21°C consumers saw a significant decrease in color acceptance at weeks 24 and 36. For the aroma and flavor attributes of concentrate stored at 21°C consumer panels saw no statistical difference for the unpasteurized concentrate. For pasteurized concentrate there was a statistical difference between weeks 24 and 36 for aroma and a statistical difference between week 16 and 36 for flavor. For the overall quality of the samples stored at 21°C, consumers did not see a statistical decrease until week 36.

**Table 1.26: Tukey's HSD p-values for consumer panel evaluation over storage duration of 36 weeks**

<b>Attribute</b>	<b>4°C Unpasteurized</b>	<b>4°C Pasteurized</b>	<b>21°C Unpasteurized</b>	<b>21°C Pasteurized</b>
<b>Color</b>	0.8563	0.0798	<0.0001	<0.0001
<b>Aroma</b>	0.4226	0.8715	0.4437	0.0347
<b>Flavor</b>	0.8133	0.7701	0.1601	0.0194
<b>Overall Quality</b>	0.9948	0.9226	0.0005	<0.0001

#### **1.4 Conclusion**

A significant decrease in total soluble solids was only seen at elevated conditions. Processing, storage temperature and storage duration had no statistical affect on the pH of the product. Titratable acidity was only affected by storage conditions 21°C or higher and after storage duration of 24 weeks. The clarity of the product was greatly impacted by both storage temperature and duration, showing the most rapid rate of decay at high storage temperatures. Hunter color a values were shown to have the largest significant effect caused

by storage duration and storage temperature of all color measurements. All storage temperatures showed a significant decrease in a values over time, with higher temperature showing the largest rate of degradation.

The trained panel found a slight significant difference between the unpasteurized and pasteurized concentrate during the early stages of storage. The untrained panel of consumers did not see significant effect of pasteurization. Due to the minimal effect pasteurization has on sensory acceptance it is likely that any of sensory quality loss caused by heat occurs during concentration process.

Trained panelists were able to distinguish significant flavor and aroma difference among samples earlier than consumer panelists. Consumers only saw a significant difference in the samples' color, which affected the overall acceptance of the juice at weeks 16 and 24. It was only at week 36 that consumers saw a significantly higher flavor acceptance of 4°C over 21°C.

The manner at which the storage duration affected the sensory quality of the concentrate depended highly on the concentrate's storage temperature. It is shown that storing concentrate at refrigerated (4°C) conditions for 48 weeks does not significantly affect the sensory properties and only minimally affects the physico-chemical properties of the product.

Trained and consumer panels were able to see a steady decreasing trend in the sensory attributes of concentrate stored at 21°C. Consumers were not able to pick up on flavor and aroma differences until week 36; however, the trained panel that was familiar with the product saw a decrease in aroma and

flavor quality after only 4 weeks. Consumer evaluation concluded that the color of the product highly affects the overall acceptance. Though concentrate stored at ambient temperatures (21°C) are shown to be accepted equally in flavor and aroma as refrigerated (4°C) concentrate until week 36, the color is the first quality attribute to noticeably decrease, limiting the shelf life of the concentrate at ambient storage condition. Based on the trained and consumer panel results it is not recommended to store concentrate at ambient temperature longer than 12 weeks.

High storage temperatures greatly reduced the quality of cherry juice concentrate in a short period of time. Based on data collected it not recommended exposing concentrate to elevated temperatures as its sensory attributes, especially color, are greatly affected. If the product is exposed to elevated conditions, maximum exposure should not be greater than 2 weeks

## **2. EFFECTS OF PROCESSING, STORAGE TEMPERATURE AND STORAGE DURATION ON ANTIOXIDANT PROPERTIES OF MONTMORENCY CHERRY JUICE CONCENTRATE**

### **2.1 Literature Review**

#### **2.1.1 Antioxidants**

##### **2.1.1.1 Background of Antioxidants**

Research has shown that tart Montmorency cherries contain a high number of compounds that convey health benefits. These compounds, including anthocyanins and phenolics, act as antioxidants within the body. An antioxidant is “a substance that opposes oxidation or inhibits reaction promoted by oxygen or peroxides” (Huang 2005). Preventing oxidation within the body is very important because oxidative damage, caused by free radicals, can cause harm to the body in a number of ways.

There are a number of types of free radicals including reactive nitrogen species (RNS), reactive chlorine species (RCS), reactive oxygen species (ROS) and reactive bromine species (RCS) (Halliwell and Gutteridge 2007). These reactive species, or free radicals, are formed in a variety of ways within the body and its surrounding environment. Free radicals can result from smog, ozone, drugs, chemicals, radiation and physiological processes (Gropper and others 2005). A free radical is defined as one or more unpaired electrons, which are in an orbit and can cause oxidation of DNA, proteins, lipids, and uric acid (Halliwell and Gutteridge 2007). The natural antioxidants found in tart cherries quench free radicals within the body to form a stable compound. Antioxidants form this stable compound through the donation of a hydrogen



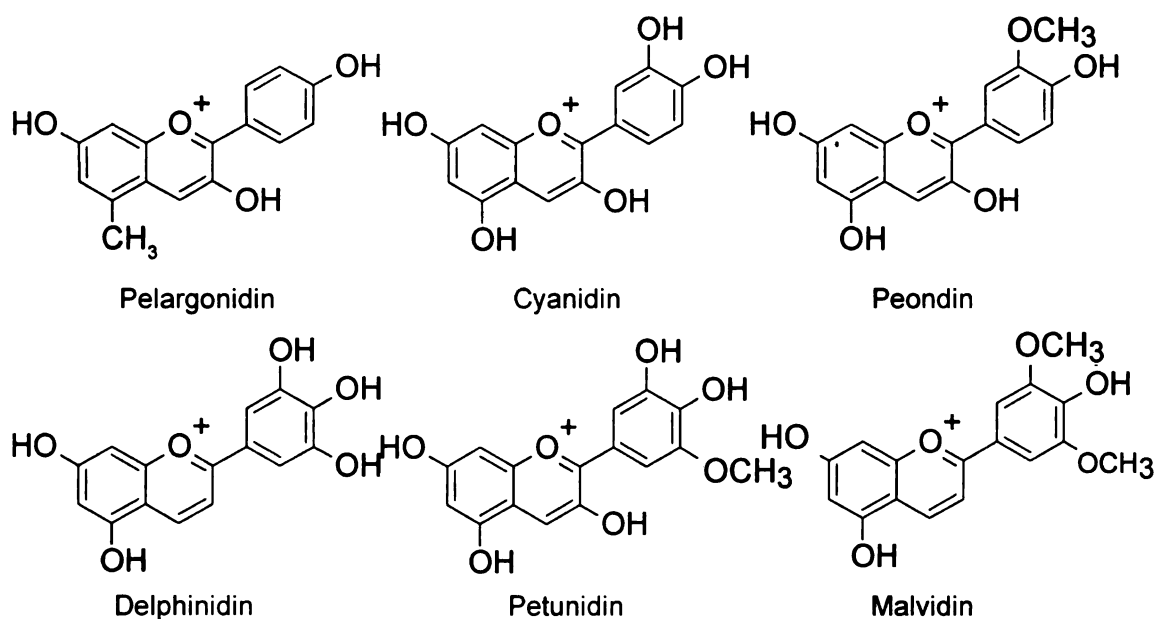
atom to the reactive free radical. Eating foods that contain high levels of antioxidants reduces the overall level of free radicals in the body, which prevent oxidative stress and oxidative damage from occurring in the body.

There are a number of commonly used assays used to measure total antioxidant content of foodstuffs such as Total Radical-Trapping Antioxidant Parameter (TRAP), Trolox Equivalent Antioxidant Capacity (TEAC), and Ferric Reducing/Antioxidant Power (FRAP) (Mermelstein 2008). However, the oxygen radical absorbance capacity (ORAC) assay was chosen for the shelf life study to measure the total antioxidant capacity.

Cherry juice concentrate has one of the highest ORAC values among other fruit and fruit products. The high levels are partly due the highly concentrated product. In a Cherry Nutritional Report (Reiter) tart cherry concentrate was found to yield an ORAC value of 128 (micromole TE/g). Though tart cherry juice concentrate is always known for its high ORAC values, these levels can vary from season to season.

#### **2.1.1.2. Anthocyanins**

Anthocyanins are the pigment that gives an orange, red, blue or purple color to a number of plants, flowers and fruits in nature. For tart cherries, in particular, anthocyanins give the fruit their deep red color. Anthocyanins are one of the major group of pigments found in nature and belong to the secondary metabolite group of flavonoids (Blando et al. 2004). In Figure 2.1 are the chemical structures of the six most common anthocyanins found in nature, including pelargonidin, cyanidin, peonidin delphinidin, petunidin and malvidin.



**Figure 2.1: Six common anthocyanins found in plants**

Anthocyanin structures are unique from one another by the number of hydroxyl groups within the molecule, the degree of methylation of the hydroxyl groups, the nature, number and position of sugars attached to the molecule and the nature and number of aliphatic or aromatic acids attached to the sugars within the molecule (Mazza and Miniati 1993). Tart cherries are known to contain a variety of anthocyanins including cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside, cyanidin 3-arabinosylrutinoside, pelargonidin 3-glucoside and peonidin 3-rutinoside (Kim and others 2005).

The stability of the anthocyanin structure can be affected in various ways. Anthocyanins are most stable in an acidic solutions and lose their pigment, due to structural changes as the pH becomes more alkaline (Mazza and Miniati 1993). Other affecting factors include light, oxygen, acetaldehyde,

ascorbic acids, sugars, and others (Mazza and Miniati 1993). It has also been shown that the by-products of degradation products, such as ascorbic acid can accelerate the anthocyanin degradation (Mazza and Miniati 1993) and (Alighourchi and Barzegar 2009). As temperature storage or processing temperature rises the degradation rate of the anthocyanins increase (Ma 2007). Berry fruit products and beverages from these fruits can provide the consumer 10's to 100's of milligrams of anthocyanins in a single serving (McGhie and Walton 2007). It is common for fruit and vegetable juices to contain an total anthocyanin content to from 50 to 500 mg/liter (Giusti and Wrolstad 2005).

#### **2.1.1.3 Phenolics**

Phenolics are a diverse group of aromatic compounds with at least one hydroxyl group (Kim and others 2005). They are known for being beneficial to one's health due to their various biological activities (Kim and others 2005). Foodstuffs and plants contain a large array of phenolic derivatives including simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans and lingins (Shahidi and Naczsk 2004).

Tart Montmorency cherries have one of the highest total phenolic contents of all tart cherry varieties (Kim and others 2005). It is reported fresh Montmorency cherries contain 4.07 mg GAE/g of fresh weight (Chaovanalkit and Wrolstad 2004) and Montmorency cherry juice concentrate contain  $4013 \pm 578$   $\mu$ g GAE/g dry weight (Kirakosyan and others 2009). However the most comparable total phenolic value is found in a study on dark fruit juices. Total phenolic content for a sour cherry nectar juice (including sour cherry and apple

juice) showed a total phenolic content ranging from approximately 1043 to 1604 mg GAE/L (Piljac-Zegarac and others 2009).

#### **2.1.1.4 Health Benefits**

The antioxidant-acting compounds, anthocyanins and phenolics, have a wide range of potential benefits for the body. These compounds have been shown to inhibit the growth of cancer cells and protect against other chronic diseases such as heart disease (Kim and others 2005; Piccolella and others 2008). Phenolic compounds in particular have been shown to have the potential to decrease the risk of certain types of Alzheimer's disease (Kim and others 2005) and to defend against infection (Piccolella and others 2008). In mice studies it has been shown that anthocyanins from tart cherries inhibited the growth of a developing tumor (Blando and others 2004). The anthocyanins found in tart cherries have the potential to slow cardiovascular disease (Piccolella and others 2008; Zafra-Stone and others 2007) and retard the aging process (Piccolella and others 2008) and to improve neuronal and cognitive brain functions (Zafra-Stone and others 2007). Anthocyanins and phenolics have the ability to reduce oxidative stress and inflammation in the body (Kirakosyan and others 2009; Kim and others 2005).

#### **2.1.2 Analysis of Antioxidant Capacity**

##### **2.1.2.1 Oxygen Radical Absorbance Capacity**

The oxygen radical absorbance capacity (ORAC) allows for the measurement of all antioxidant activity within a sample. The measurement

includes anthocyanins, and phenolic compounds along with all other compounds with antioxidant potential. The ORAC assay has come to be a very common and widely used method. The ORAC method has been used in many research areas including nutraceutical, pharmaceutical and food industries (Huang and others 2002).

The assay quantitatively determines antioxidant activity by measuring the ability of the antioxidants contained in the samples to inhibit peroxy radical induced oxidation. The antioxidants inhibit the oxidation by quenching free radicals by the donation of hydrogen atoms. This type of reaction is classified as a hydrogen atom transfer (HAT). The ORAC method uses a fluorescent probe that when oxidized by peroxy radicals, yields an unfluorescent product. The oxidation reaction is initiated by a 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) solution.

The ORAC assay has been credited and developed by (Ghiselli and others 1995), (Glazer 1990) and (Cao and others 1993). Originally the reaction was performed using B-phycoerythrin (B-PE), a fluorescent protein that is isolated from *Porphyridium cruentum*. Eventually the method was changed to use fluorescein (FL; 3',6'-dihydroxy- spiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one), a synthetic, non-protein probe. The change was done because fluorescein overcomes some of the disadvantages that B-PE has. Fluorescein is photostable unlike B-PE, which loses roughly 50% of its fluorescence after 35 minutes of exposure to excitation light (Huang and others 2002). Also B-PE

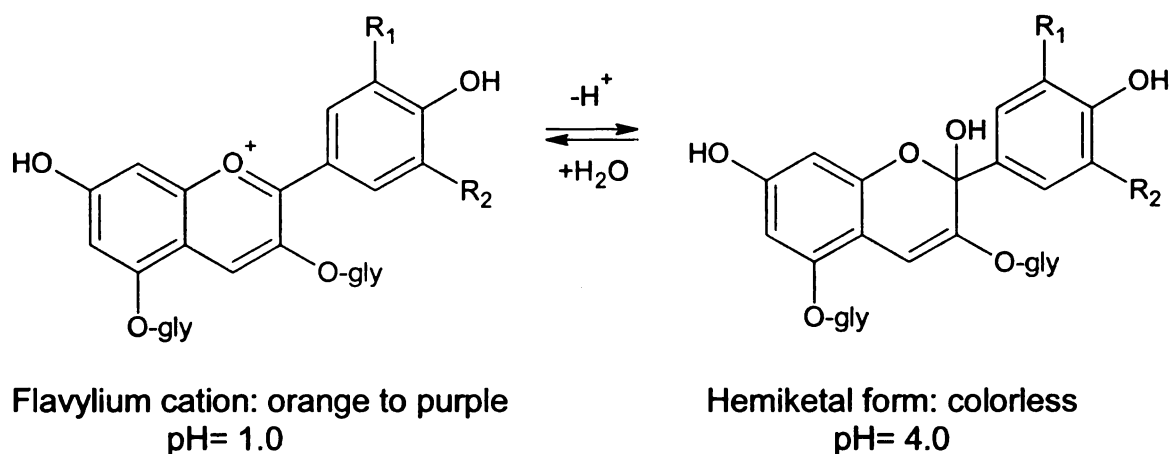
has shown to have lot-to-lot variability, which affects the overall assay results (Huang and others 2005).

During the ORAC procedure, loss of fluorescence is measured over time and decay curves for both sample and blank sample are built. With the decay curves the net area under the curve (AUC) is calculated using the trapezoidal rule and the equation  $\text{Net AUC} = \text{AUC Sample} - \text{AUC Blank}$ . A standard curve is built using the antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble vitamin E analog (Prior and others 2005). Like with the sample and blanks, decay curves for five Trolox concentrations are made. The net area under the curve is found and the Trolox concentrations are plotted against the AUC to build the standard curve. The net AUC for the samples is compared against that of the standard curve to obtain a value expressed in micromoles of Trolox equivalents per gram or liter of sample ( $\mu\text{mol TE/g}$  or  $\mu\text{mol TE/L}$ ).

#### **2.1.2.2 Total Anthocyanins - pH Differential Method**

The pH differential method quantitatively measures total monomeric anthocyanins within a variety of food products. This assay was originally designed by Sondheimer and Kertesz in 1948 to determine the anthocyanin content in strawberry jams. Later on in 1968 Fuleki and Francis adjusted the pH buffers from 2.0 and 3.4 to 1.0 and 4.5 so the assay could be applied to cranberries and also a wider variety of food products (Giusti and Wrolstad 2005).

The pH differential method is a rapid and easy assay that works effectively even when in the presence of a number of obstructive compounds. Among the few materials that do cause interference within this method are the red pigments FD&C Red No. 40, FD&C Red No. 3, cochineal and beet powder (Giusti and Wrolstad 2005). Using optical spectroscopy the assay measures the structural transformation of the anthocyanin compounds dependent upon different pH environments. Anthocyanin pigments can range in colors from red, blue and purple. At a pH lower than 2 the anthocyanins form flavylium cations which produce a red or yellow color. If the pH of the system is raised to a pH between 4 and 6 the anthocyanin structure changes to a colorless hemiketal form due to the hydration of the flavylium cation (Mazza and Miniati 1993). Figure 2.2 shows the structural change between the colored structure flavylium cation and the colorless hemiketal form that takes place during this assay.



**Figure 2.2: Structural changes of an anthocyanin at pH 1.0 and pH 4.0**

The pH differential method measures the absorbance of both the flavylium cation (pH 1.0) and hemiketal (pH 4.5) anthocyanin structures. The absorbance is measured at  $\lambda_{\text{vis-max}}$  (nm) and at 700 nm. No absorbance occurs at the wavelength of 700 nm however the measurement is taken to correct any absorbance readings that occur due to haze or sediment within the sample. The  $\lambda_{\text{vis-max}}$  (nm) is determined based off the anthocyanin compound being measured within the sample. For cherry juice concentrate the anthocyanin cyanidin-3-glucoside (520 nm) is chosen as it is a common anthocyanin found within tart cherries (Blando and others 2004).

#### **2.1.2.3 Total Phenolics - Folin-Ciocalteu Assay**

Folin and Ciocalteu first developed the Folin-Ciocalteu method in 1927 for the analysis of tyrosine and tryptophan in proteins. (Folin and Ciocateu 1927) Singleton and Rossi first used the Folin-Ciocalteu method in a food application for wine analysis in 1965 (Waterhouse 2005). Since then it has been adopted as the official procedure for determining total phenolic content in wine and tea. The procedure is used for a number of food products and is one of the most popular procedures for total phenolic analysis in foods due to being fast and simple. It is also commonly used because results can be compared directly to other results reported in literature (Bravo and Mateos 2008). The assay uses gallic acid as a standard and reports all results in gallic acid equivalents. There are a number of standards that can be used however, gallic



acid is used most commonly because it is inexpensive in its pure form and it is stable in its dry form (Waterhouse 2005).

The chemistry of the procedure involves the oxidation of the phenolate ion within the foodstuff and the reduction of the phosphotungstic-phosphomolybdic, also called the Folin-Ciocalteu reagent (Bravo and Mateos 2008). The metal oxide reduction produces a blue phosphotungstic-phosphomolybdic complex. This blue complex can be measured spectroscopically because it exhibits a broad light absorption at a maximum of 765 nm. The intensity of light the sample absorbs at 765 nm is proportional to the total phenolic concentration of the sample (Waterhouse 2005).

### **2.1.3 Hypothesis**

The independent variables of processing, storage temperature and storage duration have a degradative effect on the dependent variables physico-chemical, microbial, and sensory quality of tart cherry juice concentrate.

## **2.2 Materials and Methods**

### **2.2.1 Cherry Juice Concentrate Samples**

Montmorency tart cherry concentrate was received in five 5-gallon containers from Shoreline Fruit LLC (Traverse City, Michigan). Shoreline Fruit LLC processed the product (crop year: 2007) to a concentration of 68° Brix. The concentrate was stored in the 5-gallon buckets in a Michigan State University Food Science freezer at approximately -15°C until the concentrate was further processed, packaged and stored.

### **2.2.2 Pasteurization of Concentrate**

Pasteurization took place in the Michigan State University Food Science's pilot plant. The concentrate was pumped through a spiral stainless steel tube that was submerged in water heated in a steam-jacketed kettle. Approximately two 5-gallon containers of concentrate were pasteurized at 85°C for 5 seconds. Three pasteurized samples were collected and stored to represent different times during the pasteurization of the concentrate (beginning of the run, middle, and end of the run). These pasteurized samples were represented as pasteurized batch 1= beginning of run, pasteurized batch 2= middle of run and pasteurized batch 3= end of run.

### **2.2.3 Packaging and Storage of Concentrate**

All samples were stored in clear, square 1 oz. glass jars. The bottles were acquired from Qorpak (Bridgeville, Pennsylvania). Bottles of concentrate were all labeled indicating if they were unpasteurized or pasteurized. Containers were also labeled with the week number to indicate when the sample would be removed from storage for analysis. All concentrate samples were stored in conditions absent of light. Samples were stored at 4°C in a VWR Scientific Model 2005 Low Temperature Incubator (West Chester, Pennsylvania). Concentrate samples stored at 21°C were stored in a laboratory cupboard with minimal temperature changes and finally samples at 38°C were stored in a Fisher Isotemp® Oven 200 Series Model (Pittsburgh, Pennsylvania). Temperatures were monitored every 2 to 3 days to confirm consistent storage

temperatures. After samples were removed from storage they were kept frozen in an upright Frigidaire freezer (Martinez, Georgia) at approximately -15°C until analysis could be completed.

#### **2.2.4 Shelf Life Schedule**

The shelf life was conducted over the course of one year. The samples stored at 4°C and 21°C were tested up to the one year while the samples stored at 38°C were only tested up to 24 weeks. The cherry juice concentrate stored at elevated conditions was no longer stored after 24 weeks because it was no longer of consumable quality. Total antioxidants, total anthocyanins, and total phenolics were measured every 6 weeks for 24 weeks. They were analyzed again at week 36 and week 48.

#### **2.2.5 Oxygen Radical Absorbance Capacity**

##### **2.2.5.1 Reagent and Sample Preparation**

The oxygen radical absorbance capacity (ORAC<sub>FL</sub>) assay was based off of (Huang and others 2005). The ORAC<sub>FL</sub> assay used the chemical solutions; sodium phosphate buffer pH 7.4 (75mM), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) stock solution (2.0mM), fluorescein sodium salt stock solution ( $4 \times 10^{-3}$  mM) and also an 153 mM 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) solution. The sodium phosphate buffer solution was prepared by adding 11.741 grams of dibasic

sodium phosphate (Sigma-Aldrich, St. Louis, Missouri) and 4.306 grams of monobasic monohydrate sodium phosphate (Mallinckrodt Baker Phillipsburg, New Jersey) to 1000 mL of distilled water and adjusting the pH to 7.4 with 1M sodium hydroxide (Mallinckrodt Baker Phillipsburg, New Jersey) and 1M hydrochloric acid (Columbus Chemical Industries, Columbus, Wisconsin). The 2.0 mM trolox solution was prepared by adding 0.25 grams of trolox (Sigma-Aldrich, St. Louis, Missouri) to 500 mL of 75 mM sodium phosphate (buffer pH 7.4). The trolox stock solution was stored in the refrigerator between uses and also wrapped in foil to prevent light from reaching the solution.

A fluorescein stock solution at a concentration of  $4 \times 10^{-3}$  mM was prepared by dissolving 0.1 grams of fluorescein sodium salt (Sigma-Aldrich, St. Louis, Missouri) in 50 mL of sodium phosphate buffer (75 mM, pH 7.4). This created a  $5.3153 \times 10^{-3}$  M solution. Finally 0.752 mL of the  $5.3153 \times 10^{-3}$  M fluorescein solution was added to a 1 L volumetric flask and the volume as brought to 1 L with sodium phosphate buffer (75 mM, pH 7.4). The stock solution was stored at refrigerated conditions, wrapped in aluminum foil for up to three months. During each ORAC analysis a working solution was prepared by diluting the fluorescein stock solution (1:1000) in sodium phosphate buffer (75 mM, pH 7.4). A fresh 153 mM 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) solution was made for each ORAC analysis. This solution was prepared by adding 0.414 grams of AAPH to 10 mL of sodium phosphate buffer (75 mM, pH 7.4).

Trolox samples were prepared in five concentrations to build a standard curve. The concentrations (6.25, 12.5, 25, 50, 100  $\mu$ L) were prepared using sodium phosphate buffer (75 mM, pH 7.4). All cherry juice concentrate samples were diluted using sodium phosphate buffer (75 mM, pH 7.4) with a dilution factor of 11. This sample was then diluted into four more dilutions adding 100, 75, 50, and 25  $\mu$ L of the sample to 10 mL of sodium phosphate buffer (75 mM, pH 7.4).

#### **2.2.5.2 Experimental Procedure**

The ORAC procedure was performed using an FLx800 Multi-Detection Microplate Reader (Biotek Instruments, Winooski, Vermont) and the Biotek software Gen 5. The microplate reader was set at the detection parameters of 528 nm, 20 nm bandpass, emission filter and 485 nm, 20 nm bandpass excitation filter. Black polystyrene 96-well, round bottom microplates (Corning, Corning, New York) were used. The exterior wells of the plated were left empty, while the interior wells were used for the analysis. To all wells that would be used for analysis, 150  $\mu$ L of the diluted Fluorescein solution ( $4 \times 10^{-6}$  mM) was added. Twenty-five  $\mu$ L of sodium phosphate buffer (75 mM, pH 7.4) was added to the wells that would represent a blank sample. The remaining wells contained 25  $\mu$ L of the diluted Trolox samples for the standard curve and 25  $\mu$ L of the diluted cherry juice concentrate samples. The microplate was then incubated for 30 minutes at 37°C in the Biotek reader. After the incubation period 25  $\mu$ L of the 153 mM AAPH solution was added to all experimental wells.

After the addition of the AAPH solution the Biotek microplate reader measured the relative fluorescence intensity every two minutes for up to five hours.

Decay curves for the blank, standards and samples were built by plotting the fluorescence intensity versus the time. The normalized area under the curve (AUC) for each decay curve was calculated using the trapezoidal rule (Eq. 2.1).

$$AUC = \left( \frac{R_1}{2} + R_2 + R_3 + \dots + R_{n-1} + \frac{R_n}{2} \right) \frac{\Delta t}{R_1} \quad \text{Eq. 2.1}$$

Where  $R_1$  represents the fluorescence reading at the initial time of the reaction,  $R_n$  represents the final fluorescence reading and  $\Delta t$  is the change in time between readings equaling two minutes. The blank and the sample's AUC was then used to calculate the Net AUC where  $\text{Net AUC} = \text{AUC}_{\text{Sample}} - \text{AUC}_{\text{Blank}}$ . A standard curve was built using the AUC of the standards versus the concentration of the standards. The sample's Net AUC was then compared to the standard curve in order to determine the sample's  $\text{ORAC}_{\text{FL}}$  of micromole of Trolox equivalent per gram of liter of sample ( $\mu\text{M TE/g}$  or  $\mu\text{M TE/L}$ ).

## **2.2.6 Total Anthocyanins- pH Differential Assay**

### **2.2.6.1 Reagent Preparation**

The total anthocyanins- pH differential assay was followed according to them method of Giusti and Wrolstad (2005). A 0.025 M potassium chloride buffer pH 1.0 and 0.4 M sodium acetate buffer pH 4.5 solutions were prepared for the total anthocyanin assay. To prepare the potassium chloride buffer, 1.86

grams of KCl (Mallinckrodt Baker Phillipsburg, New Jersey) was mixed with 980 mL of distilled water. The pH was adjusted to 1.0 using concentrate hydrochloric acid (Columbus Chemical Industries, Columbus, Wisconsin). The solution was transferred to a 1 L volumetric glass and the volume was brought up to 1 L. The sodium acetate buffer was prepared by mixing 54.43 grams of  $\text{CH}_3\text{CO}_2\text{Na} \cdot 3 \text{H}_2\text{O}$  (Mallinckrodt Baker Phillipsburg, New Jersey) with ~960 mL of distilled water. The pH was adjusted to 4.5 using concentrate hydrochloric acid. The solution was transferred to a 1 L volumetric flask and the volume was brought up to 1 L.

#### **2.2.6.2 Experimental Procedure**

The appropriate dilution factor was determined to be 40. This value was found by diluting the cherry juice concentrate sample with the potassium chloride buffer until it was confirmed that the absorbance reading was within linear range of the spectrophotometer at the wavelength  $\lambda_{\text{vis-max}}$  (510 nm). It was advised that the sample volume not exceed the total sample volume by 20% so as not to exceed the buffer's capacity.

Two solutions were prepared for each sample, one with potassium chloride buffer, pH 1.0 and the other with the sodium acetate buffer pH 4.5. The dilutions were allowed to equilibrate for 15 minutes prior to absorbance reading. Distilled water was used as a blank to calibrate the spectrophotometer. The absorbance value for each sample was read using a Milton Roy Spectronic 21D spectrophotometer (Ivyland, Pennsylvania) at both

the  $\lambda_{\text{vis-max}}$  (510 nm) and 700 nm. The absorbance (A) of the samples were calculated using equation 2.2.

$$A = (A_{\lambda \text{ vis-max}} - A_{700})_{\text{pH 1.0}} - (A_{\lambda \text{ vis-max}} - A_{700})_{\text{pH 4.5}} \quad \text{Eq. 2.2}$$

Using the absorbance value the monomeric anthocyanin pigment (mg/L) was calculated using equation 2.3:

$$\text{Monomeric anthocyanin pigment (mg/liter)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times 1)$$

### Eq. 2.3

In equation 2.3 the MW is the molecular weight of Cyanidin-3-glucoside (449.2 g/mole), DF is the dilution factor (40) and  $\epsilon$  is the molar absorptivity of Cyanidin-3-glucoside (26,900).

## 2.2.7 Total Phenolics- Folin-Ciocalteu Assay

### 2.2.7.1 Reagent and Sample Preparation

The total phenolics- Folin-Ciocalteu's assay was performed according to the Waterhouse's method (Waterhouse 2005). A gallic acid stock solution and sodium carbonate solution was prepared for the total phenolic assay. To prepare the gallic acid solution 0.5 grams of gallic acid (Sigma-Aldrich, St. Louis, Missouri) was added to 10 mL of ethanol (EMD Chemicals, Gibbstown, New Jersey) and then diluted to 100 mL of distilled water. The gallic acid stock solution was stored in an amber glass container at refrigerated temperatures for up to 2 weeks. Five gallic acid standard dilutions were prepared using the premade gallic acid stock solution and distilled water. The dilutions included the concentrations of 50, 100, 200, 300, and 400 mg/L.



The saturated sodium carbonate solution was prepared by dissolving 200 grams of anhydrous sodium carbonate (Mallinckrodt Baker, Phillipsburg, New Jersey) into 800mL of water. The solution was brought to a boil and then allowed to cool. After cooling a few crystals of sodium carbonate was added to the solution and then it was allowed to sit for 24 hours at room temperature. The solution was filtered through Whatman No. 1 paper (Whatman Inc., Clifton, New Jersey). The volume was brought up to 1 L with distilled water and was stored at room temperature.

Cherry juice concentrate samples were prepared by diluting the sample with distilled water with the dilution factor of 41.

#### **2.2.7.2 Experimental Procedure**

For each five gallic acid concentrates and diluted cherry juice concentrate samples the amount of 0.5 mL was added to separate glass test tubes. For a blank sample 0.5 mL of distilled water was added to an empty test tube. The amount of 7.5 mL of distilled water was added to all tests tubes (blank, standards, samples) followed by 0.5 mL of the Folin- Ciocalteu reagent (Sigma-Aldrich, St. Louis, Missouri). All test tubes were mixed using a Fisher Scientific vortex mixer (Pittsburg, Pennsylvania). The test tubes were allowed to incubate at room temperature for one to eight minutes. Following by the incubation 1.5 mL of the saturated sodium carbonate solution was added to each test tube. All samples were mixed using the vortex mixer for the second time. The test tubes were covered and then allowed to incubate for two hours at room temperature. After the incubation period the samples' absorbance was

read at a wavelength of 765nm using a Spectronic 21D Milton Roy spectrophotometer (Ivyland, Pennsylvania). The blank sample prepared was used to calibrate the spectrophotometer prior to reading the standard curve and cherry juice concentrate samples.

The absorbencies of the five gallic acid concentrations were used to create a standard curve of absorbance values versus the gallic acid concentrations. Using the absorbance readings of the cherry juice concentrate samples and the standard curve, total phenolic values were calculated and expressed in gallic acid equivalents (GAE). The values were also corrected using the sample's dilution factor.

### **2.2.8 Statistical Analysis**

Antioxidant properties were analyzed using a three-way factorial design using the GLIMMIX procedure in the statistical software SAS 9.1 (Raleigh, North Carolina).

## **2.3 Results and Discussion**

### **2.3.1 Oxygen Radical Absorbance Capacity**

#### **2.3.2.1 Interaction Effect**

Using a three-way factorial design it was concluded that the interactions among the variables of processing, storage temperature and storage duration did not significantly affect the concentrate's ORAC values ( $p>0.05$ ). Analysis of the effect of each individual variable showed that only the storage duration had

a significant affect ( $p < 0.0001$ ). P-values for all variables and variable interactions are presented in Table 2.1.

**Table 2.1: Summary of p-values of variables and interactions for ORAC values (micromole TE/g tart cherry juice concentrate)**

<b>Variable and Interactions</b>	<b>p-value</b>
Pasteurization	0.2034
Storage Temperature	0.0627
Storage Duration	<0.0001
Storage Duration*Storage Temperature	0.5006
Storage Duration*Pasteurization	0.1870
Storage Temperature*Pasteurization	0.8368
Storage Duration *Storage Temperature*Pasteurization	0.9659

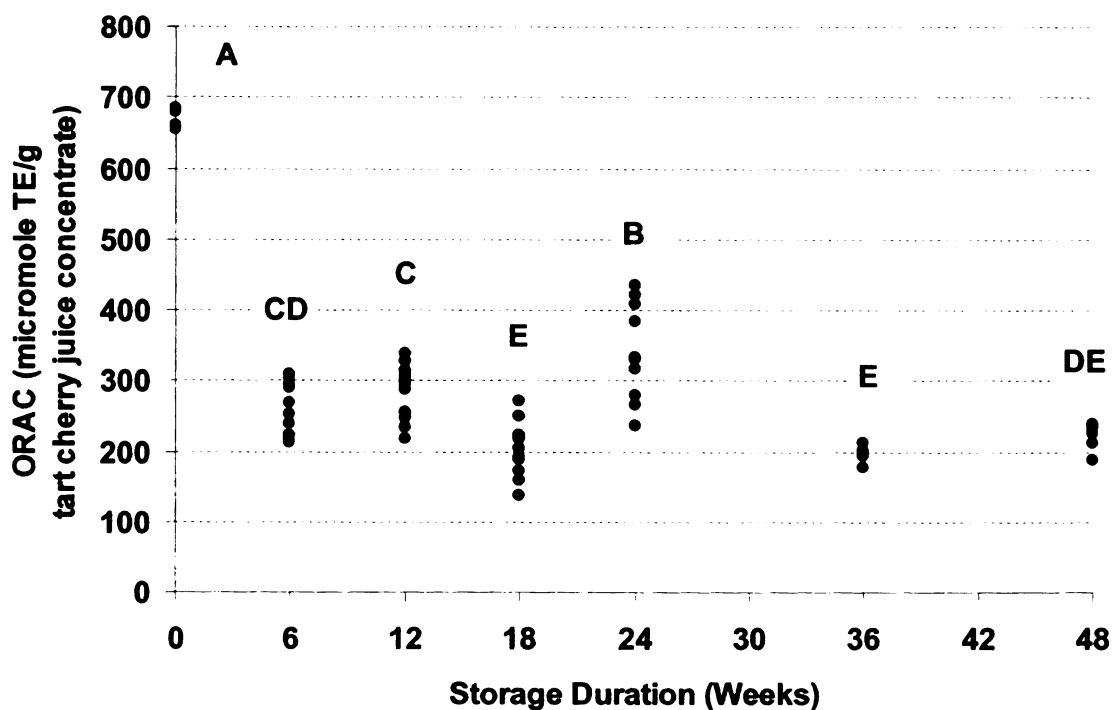
### **2.3.2.2 Storage Duration Effect**

Since it was determined that storage duration was the only variable significantly affecting the ORAC value, the main effects of storage duration were analyzed, while excluding the effects of the processing (pasteurization) and storage temperature variables. This analysis was completed using the Tukey Honest Significant Difference test. In Table 2.2 are the results of the Tukey HSD test showing the average ORAC values at each 6 week analysis. Each week includes all storage condition data. Figure 2.3 displays the same results in a graph format. Graphs for each individual processing type and storage temperature are found in Appendix 2.

**Table 2.2: The average ORAC values (micromole TE/g tart cherry juice concentrate) and Tukey's HSD test comparison results for storage duration**

Storage Duration (Weeks)	ORAC Values
0	670 ± 102 A
6	260 ± 50 CD
12	285 ± 107 C
18	203 ± 50 E
24	340 ± 114 B
36	198 ± 34 E
48	216 ± 35 DE

**\*Values not connected by the same letter are significantly different**



**Figure 2.3: Effects of storage duration on the average of all storage temperature and processing types ORAC values (micromole TE/g tart cherry juice concentrate) for cherry juice concentrate**

**\*Values not connected by the same letter are significantly different**

Analysis of the ORAC content over the one year storage period showed that the ORAC value at the initial day 0 measurement was statistically greatest. Average ORAC values at the initial analysis were  $670 \pm 102$  micromole TE/g tart cherry juice concentrate. At week 6 the ORAC value significantly decreased to between approximately 40% of its initial ORAC content. It is likely that the reason for the large ORAC value decay within the first 6 weeks was due to the loss of the concentrate's most abundant antioxidant. Research is not available on what antioxidant is the most abundant in tart cherries, however it is known that they contain a variety of anthocyanins, phenolics and high levels of other antioxidant compounds such as the melatonin (Burkhardt and others 2001). After week 6, the concentrate's ORAC values significantly increased and decreased throughout the rest of the year storage period. Though significant changes were occurring, average ORAC values stayed within the range of  $198 \pm 34$  to  $340 \pm 114$  micromole TE/g tart cherry juice concentrate. It is likely that biological variation within the cherry juice concentrate samples was the cause for some of the ORAC value fluctuation.

In a Cherry Nutritional Report (Reiter; Cherry Marketing Institute 2007b) tart cherry concentrate was found to yield an ORAC value of 128 (micromole TE/g). The fresh tart cherry concentrate in this study yielded an ORAC value 5 times that amount. After storage of the concentrate, the ORAC value yielded approximately twice the amount as reported in the Cherry Nutritional Report. It must be noted that little information is known about the cherry juice concentrate reported in the Cherry Nutritional Report, including the age and the storage

conditions of the concentrate. The total antioxidant capacity of tart cherry juice concentrate is also dependent on the growing season.

## **2.3.2 Total Anthocyanins**

### **2.3.2.1 Interaction Effects**

With the analysis of a three-way factorial design it was concluded all variables and the interactions of these variables had a significant affect on the total anthocyanin content of the tart cherry juice concentrate ( $p < 0.05$ ). The p-values of each individual variable and the variable interactions are presented in Table 2.3.

**Table 2.3: Summary of p-values of variables and interactions for total anthocyanin values (mg cyn-3-glu/liter tart cherry juice concentrate)**

<b>Variable and Interactions</b>	<b>p-value</b>
Pasteurization	<0.0001
Storage Temperature	<0.0001
Storage Duration	<0.0001
Storage Duration*Storage Temperature	<0.0001
Storage Duration*Pasteurization	<0.0001
Storage Temperature*Pasteurization	<0.0001
Storage Duration *Storage Temperature*Pasteurization	0.0362

### **2.3.2.2 Pasteurization Effect**

The effects of each interaction were shown through the simple effects comparison of the interaction, storage duration\*storage temperature\*processing least squares means sliced by that particular interaction. The effects of processing with the interaction of storage duration\*storage temperature showed the majority of significant difference among processing types occurring during

the initial analysis at day 0. No significant difference occurred among the three pasteurization batches ( $p>0.05$ ) at the initial analysis. However, significantly higher total anthocyanin values were seen for the unpasteurized concentrate ( $p<0.05$ ), with an average total anthocyanin value of  $538.37 \pm 28.44$  mg cyn-3-glu/liter tart cherry juice concentrate.

For the storage condition  $38^{\circ}\text{C}$  no significant difference was seen among processing types during the 24 weeks of storage ( $p>0.05$ ). This was also seen for the storage condition  $21^{\circ}\text{C}$ . However, there was one exception, which occurred early in the study at week 6. At this condition a significant difference was observed among the concentrate pasteurized at the beginning of processing and at the end of processing ( $p=0.0390$ ).

The remaining effects of processing occurred throughout the duration of the storage study within the storage temperature,  $4^{\circ}\text{C}$ . Significant differences occurred at weeks 6, 18, and 48 of analysis. At week 6, the unpasteurized concentrate had a significantly higher total anthocyanin value over pasteurized batch 1 ( $p=0.0006$ ). At week 18 of storage unpasteurized concentrate had a significantly higher value over all three pasteurized batches ( $p<0.05$ ). Finally, at week 48, unpasteurized concentrate had a significantly higher total anthocyanin content over pasteurized batch 1 ( $p=0.0178$ ), while pasteurized batch 1 being significantly large compared to batch 3 ( $p<0.0001$ ).

Based on analysis of total anthocyanin content it can be concluded that any minimal temperature fluctuations that occur during the pasteurization process have minimal to no affect on the total anthocyanin content. Results

show that the interactions of storage temperature and storage duration have a greater effect on total anthocyanin content than the variable of processing. However, for concentrate stored at 4°C the interaction effect of storage temperature and storage duration are minimal, giving the variable of processing a greater effect on total anthocyanin content.

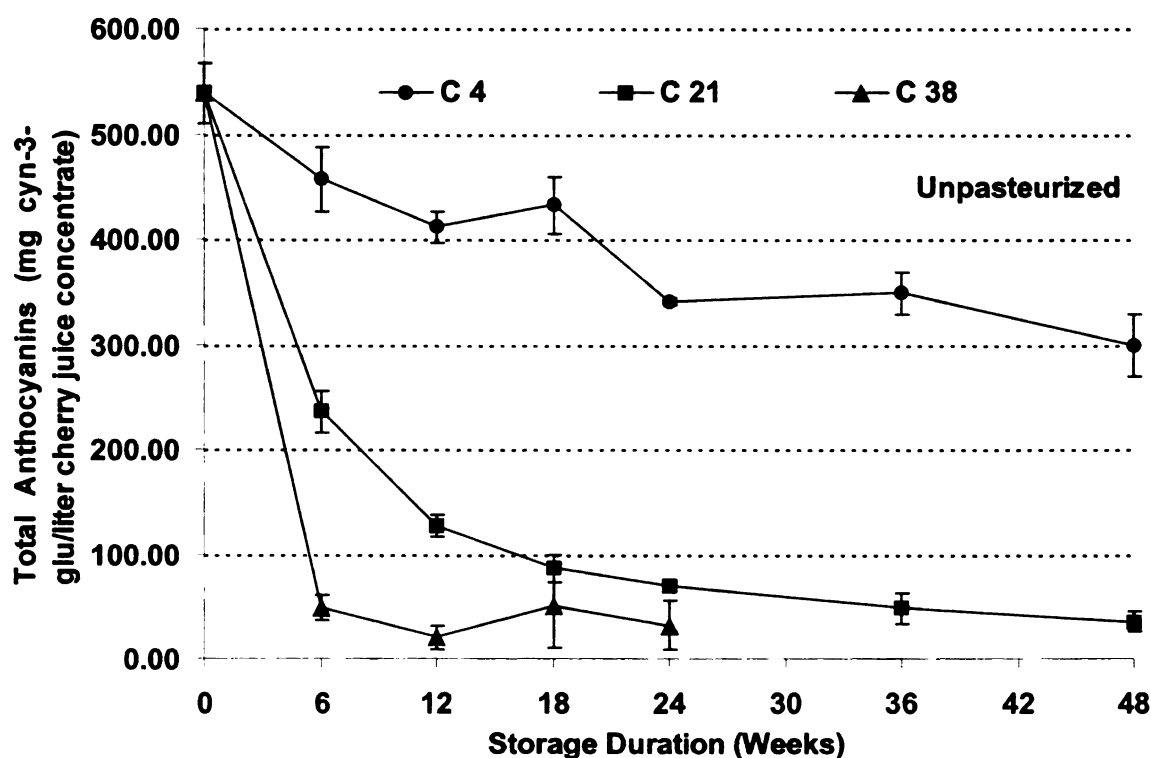
### **2.3.2.3 Storage Temperature Effect**

The effects of storage temperature with the interaction of storage duration\*processing showed a high significant difference among the three storage conditions ( $p < 0.05$ ). At each 6 week analysis, the concentrate stored at the 4°C contained the highest total anthocyanin content. The concentrate stored at the 21°C yielded the second highest, with the concentrate stored at 38°C yielding the lowest total anthocyanin values. The only exceptions seen were during week 18 and 24 of analysis. At week 18, pasteurized batch 2 showed no significant difference among the 21 and 38°C storage conditions ( $p = 1.000$ ). At week 24 of analysis pasteurized batch 1 and 2 also showed no significant difference among storage conditions 21 and 38°C ( $p > 0.05$ ). The cause for these non significant values was due to the very low total anthocyanin content seen for both storage temperature 21 and 38°C after week 12 of storage (Figures 2.4, 2.5, 2.6 and 2.7).

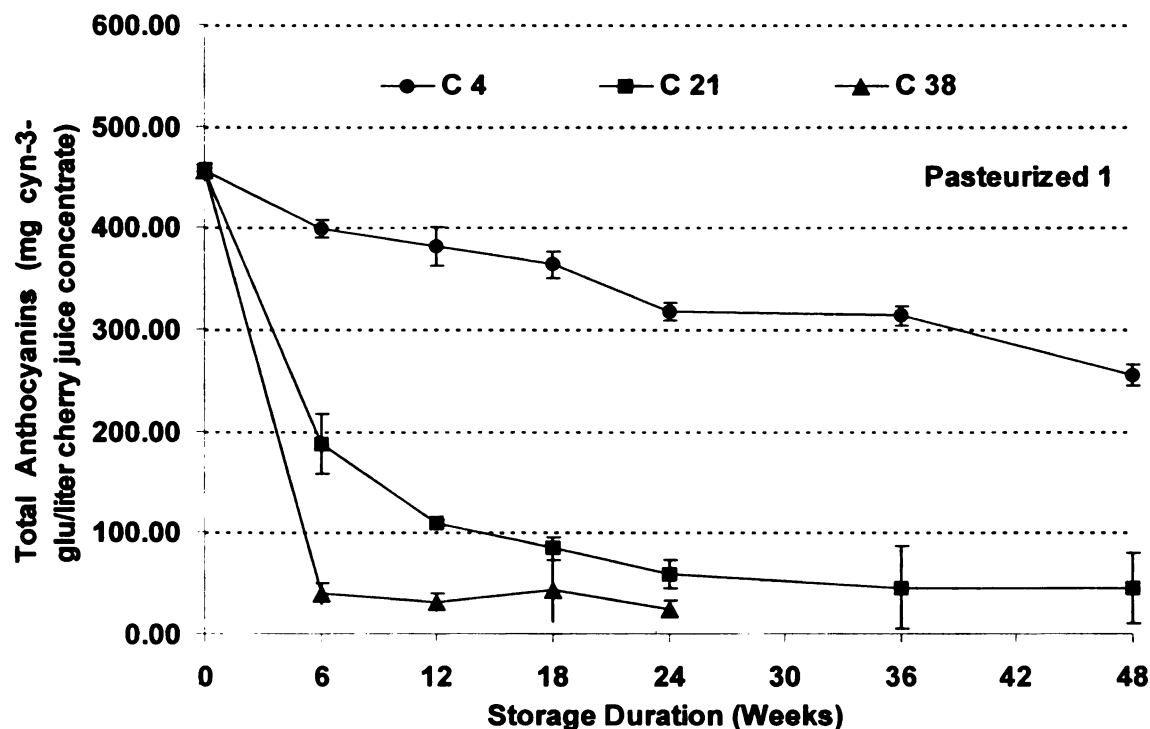
The trend seen in this anthocyanin analysis is similar to those seen in a number of other analyses of other fruit products. In a study with reconstituted pomegranate juice, a fruit that also contains high levels of anthocyanins, the juice was stored at storage temperatures of 4, 20, and 38°C for 220 days. The



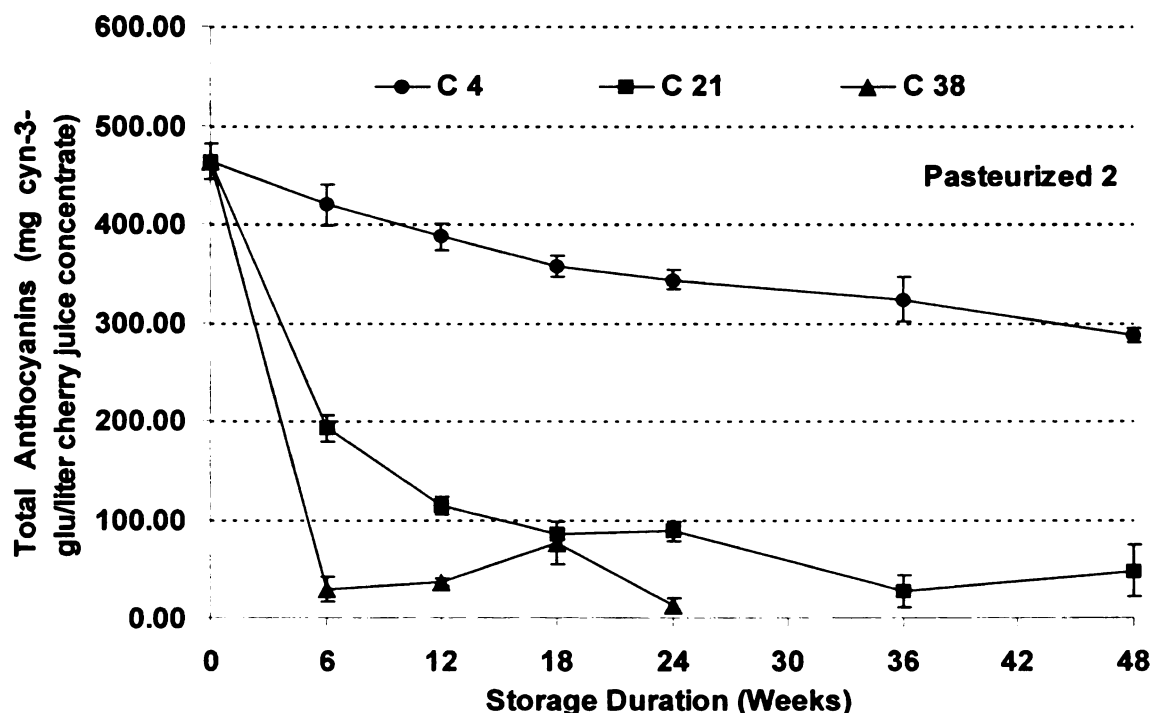
anthocyanin degradation was found to be very similar to the results found within the cherry concentrate. The study found that the pomegranate juice stored at 4°C had the smallest anthocyanin degradation over time while the juice stored at 38°C had the highest degradation on anthocyanins (Alighourchi and Barzegar 2009). This trend was also seen in a study looking at the total anthocyanin content of raspberry pulp. Over the storage of 55 days at the storage temperatures of 4, 20, and 37°C there was a significant decrease in all storage conditions, however the higher storage temperature greatly increased the rate of total anthocyanin decay (Ochoa and others 1999).



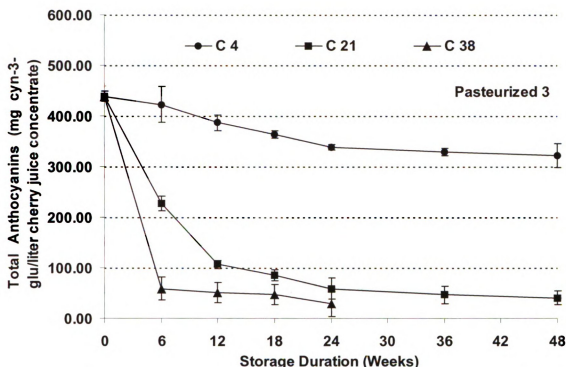
**Figure 2.4: Effects of storage time and temperature on the average total anthocyanin values (mg cyn-3-glu/liter tart cherry juice concentrate) for unpasteurized cherry juice concentrate**



**Figure 2.5: Effects of storage time and temperature on the average total anthocyanin values (mg cyn-3-glu/liter tart cherry juice concentrate) for pasteurized (batch 1) cherry juice concentrate**



**Figure 2.6: Effects of storage time and temperature on the average total anthocyanin values (mg cyn-3-glu/liter tart cherry juice concentrate) for pasteurized (batch 2) cherry juice concentrate**



**Figure 2.7: Effects of storage time and temperature on the average total anthocyanin values (mg cyn-3-glu/liter tart cherry juice concentrate) for pasteurized (batch 3) cherry juice concentrate**

#### 2.3.2.4 Storage Duration Effect

The effects of storage over a 1 year duration with the interaction of storage temperature\*processing showed a significant decrease over the year of storage for all simple effect levels. The concentrate at the storage condition 38°C had the largest decay rate of anthocyanins while the storage condition, 4°C had the smallest.

The concentrate stored at the 4°C showed a slow rate of decay becoming significantly smaller at most 6-week intervals. Significant decay in total anthocyanins were seen as early as week 6 of storage for the unpasteurized and pasteurized concentrate ( $p < 0.05$ ) and as late as week 12 for

pasteurized batches 2, and 3 ( $p < 0.05$ ). Initial total anthocyanin values ranged from  $238.85 \pm 9.63$  to  $538.37 \pm 28.44$  mg cyn-3-glu/liter tart cherry juice concentrate. Sample held at the  $4^{\circ}\text{C}$  lost 27 to 45% of their overall total anthocyanin content over the year of storage, resulting in final values ranging from  $254.71 \pm 10.65$  to  $321.95 \pm 24.24$  mg cyn-3-glu/liter tart cherry juice concentrate.

Concentrate stored at  $21^{\circ}\text{C}$  had a moderately steep total anthocyanin decay. At weeks 6 and 12 a significant loss in total anthocyanins occurred for each processing type ( $p < 0.05$ ). At week 6 the unpasteurized and pasteurized batches had already lost 48 to 59% of its overall anthocyanin content, and by week 12, the samples had lost 75 to 76% of their overall anthocyanin content. After week 12 the decay of the anthocyanin began to decrease to a slow rate. Significant loss was seen between weeks 12 and 36 ( $p < 0.05$ ), however anthocyanin content was not significantly different between analysis at week 36 and 48 ( $p > 0.05$ ). By week 36 the concentrate had lost the majority of its anthocyanins, losing over 89% of the concentrate's initial value. The final total anthocyanin content after a year of storage ranged from  $34.96 \pm 9.73$  to  $47.42 \pm 26.12$  mg cyn-3-glu/liter tart cherry juice concentrate.

Concentrate stored at the elevated temperature of  $38^{\circ}\text{C}$  lost the majority of its anthocyanin value after only the first 6 weeks of storage. After week 6 until the end of storage no significant change occurred in total anthocyanin content ( $p > 0.05$ ). In the storage study of raspberry pulp the majority of the total anthocyanin lost occurred around day 40 (approximately 6 weeks) for the

sample stored at 37°C (Ochoa et al. 1999). From week 12 to week 24 the anthocyanin content stayed significantly the same due to losing most of the value within the first 6 weeks of storage. At week 6 the concentrates had already lost from 87 to 94% of its original anthocyanin content. At week 24 the values had decreased to a measurement of  $11.80 \pm 7.14$  to  $31.84 \pm 23.86$  mg cyn-3-glu/liter tart cherry juice concentrate.

### 2.3.3 Total Phenolics

#### 2.3.3.1 Interaction Effects

Three-way factorial design results showed that all variables and the interactions of these variables had a significant effect on the total phenolic content of the tart cherry juice concentrate ( $p < 0.05$ ). The p-values of each individual variable and the variable interactions are presented in Table 2.4.

**Table 2.4: Summary of p-values of variables and interactions for total phenolic (grams GAE/liter tart cherry juice concentrate)**

<b>Variable and Interactions</b>	<b>p-value</b>
Pasteurization	<0.0004
Storage Temperature	<0.0001
Storage Duration	<0.0001
Storage Duration*Storage Temperature	<0.0001
Storage Duration*Pasteurization	<0.0001
Storage Temperature*Pasteurization	<0.0001
Storage Duration *Storage Temperature*Pasteurization	<0.0001

### **2.3.3.2 Pasteurization Effect**

The effects that each interaction had on the total phenolic content were shown through the simple effects comparison of the interaction, storage duration\*storage temperature\*processing least squares means spliced by that particular interaction. The effects of processing with the interaction of storage duration\*storage temperature did not show a consistently clear trends throughout the study. Statistical differences among processing types occurred in samples at week 0, 6, 12, 18, 24 and 48 within all storage temperature conditions. At the initial analysis at day 0 the unpasteurized concentrate had a significantly higher total phenolic content over the three pasteurized batches ( $p<0.05$ ). This same occurrence appeared again at week 24/storage condition 4°C ( $p<0.05$ ). However, at week 12/storage condition 38°C the unpasteurized concentrate had a significantly lower total phenolic content compared to the pasteurized batches, with pasteurized batch 1 having the significantly highest values ( $p<0.05$ ). Unpasteurized concentrate once again had a significantly lower total phenolic content at week 18/storage condition 38°C and week 24/storage condition 38°C.

A storage durations of 6, 12, 18, 24, and 48 weeks significant difference was seen among the three pasteurized batches ( $p<0.05$ ), however no pasteurized batches was shown to consistently have the significantly higher or lower totally phenolic content among the rest of the batches.

Though a number of statistical differences were seen among processing types throughout the storage study, no results occur consistently to draw a conclusion from the data.

### 2.3.3.3 Storage Condition Effect

For the unpasteurized concentrate (see Table 2.5) there were no clear trends or storage conditions, which consistently showed the highest or lowest total phenolic content over the year of storage. For the pasteurized concentrate (Table 2.6, 2.7 and 2.8) during the first 12 weeks of storage no clear trends were present in the relationship among the three storage conditions. At week 18 and forward a trend became clear. In most cases the concentrate stored at 21°C had a significantly higher total phenolic content over 4°C ( $p < 0.05$ ). Also the concentrate stored at 38°C had a significantly higher total phenolic content over the 4 and 21°C ( $p < 0.05$ ).

**Table 2.5: The average total phenolic (g GAE/liter liter tart cherry juice concentrate) results for storage conditions (unpasteurized)**

<b>Storage Period (Weeks)</b>	<b>4°C</b>	<b>21°C</b>	<b>38°C</b>
<b>6</b>	<b>11.45 ± 0.24</b>	10.86 ± 0.15	11.08 ± 0.11
<b>12</b>	10.59 ± 0.18	10.40 ± 0.15	<b>11.44 ± 0.19</b>
<b>18</b>	12.03 ± 0.11	<b>13.00 ± 0.23</b>	12.45 ± 0.13
<b>24</b>	10.97 ± 0.18	10.94 ± 0.05	<b>13.63 ± 0.71</b>
<b>36</b>	11.10 ± 0.17	<b>11.40 ± 0.12</b>	n/a
<b>48</b>	11.20 ± 0.05	<b>11.52 ± 0.13</b>	n/a

\*Highest Total Phenolic content is bolded

**Table 2.6: The average total phenolic (g GAE/liter liter tart cherry juice concentrate) results for storage conditions (pasteurized batch 1)**

<b>Storage Period (Weeks)</b>	<b>4°C</b>	<b>21°C</b>	<b>38°C</b>
<b>6</b>	11.02 ± 0.18	10.99 ± 0.32	<b>11.42 ± 0.0</b>
<b>12</b>	10.42 ± 0.21	10.97 ± 0.21	<b>12.54 ± 0.10</b>
<b>18</b>	12.43 ± 0.21	<b>13.67 ± 0.22</b>	13.61 ± 0.30
<b>24</b>	10.34 ± 0.14	10.94 ± 0.04	<b>14.58 ± 0.32</b>
<b>36</b>	11.40 ± 0.13	<b>11.66 ± 0.07</b>	n/a
<b>48</b>	11.15 ± 0.18	<b>11.93 ± 0.1</b>	n/a

\*Highest Total Phenolic content is bolded

**Table 2.7: The average total phenolic (g GAE/liter liter tart cherry juice concentrate) results for storage conditions (pasteurized batch 2)**

<b>Storage Period (Weeks)</b>	<b>4°C</b>	<b>21°C</b>	<b>38°C</b>
<b>6</b>	11.05 ± 0.15	10.99 ± 0.11	<b>11.16 ± 0.24</b>
<b>12</b>	10.22 ± 0.09	10.20 ± 0.22	<b>12.82 ± 0.45</b>
<b>18</b>	12.43 ± 0.23	12.88 ± 0.05	<b>13.32 ± 0.18</b>
<b>24</b>	10.83 ± 0.13	11.38 ± 0.11	<b>14.61 ± 0.10</b>
<b>36</b>	11.19 ± 0.11	<b>11.59 ± 0.15</b>	n/a
<b>48</b>	11.46 ± 0.05	<b>11.79 ± 0.10</b>	n/a

\*Highest Total Phenolic content is bolded

**Table 2.8: The average total phenolic (g GAE/liter liter tart cherry juice concentrate) results for storage conditions (pasteurized batch 3)**

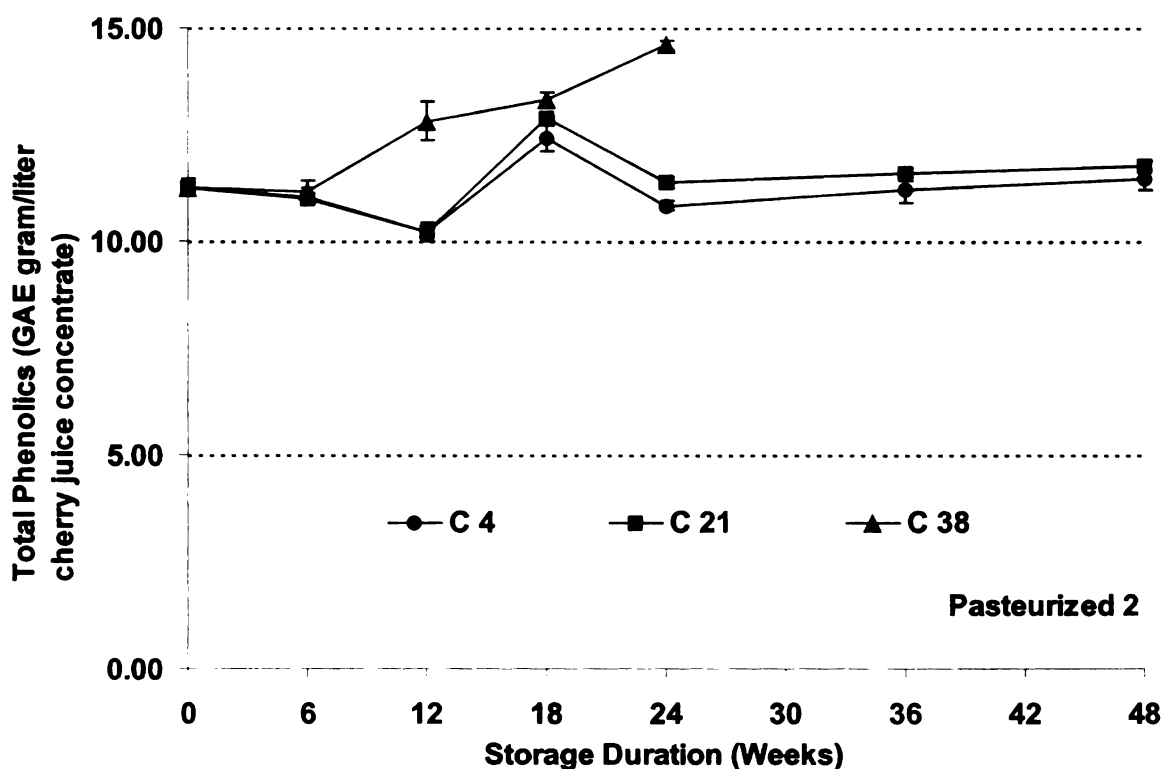
<b>Storage Period (Weeks)</b>	<b>4°C</b>	<b>21°C</b>	<b>38°C</b>
<b>6</b>	<b>11.25 ± 0.20</b>	10.58 ± 0.20	10.95 ± 0.33
<b>12</b>	10.45 ± 0.07	10.36 ± 0.15	<b>12.22 ± 0.31</b>
<b>18</b>	12.26 ± 0.30	13.43 ± 0.31	<b>13.73 ± 0.22</b>
<b>24</b>	10.46 ± 0.11	11.45 ± 0.31	<b>14.07 ± 0.26</b>
<b>36</b>	11.35 ± 0.30	<b>11.61 ± 0.19</b>	n/a
<b>48</b>	10.93 ± 0.24	<b>11.74 ± 0.12</b>	n/a

\*Highest Total Phenolic content is bolded



### 2.3.3.4 Storage Duration Effect

Figure 2.8 presents the average total phenolic values (GAE gram/liter tart cherry juice concentrate) for pasteurized batch 2 of cherry juice concentrate. Figures for all other storage conditions are found in Appendix 1.



**Figure 2.8: Effects of storage time and temperature on the average total phenolic values (g GAE/liter liter tart cherry juice concentrate) for pasteurized (batch 2) cherry juice concentrate**

For the analysis of total phenolics there was no constant significant increase or decrease of values for the storage conditions of 4°C and 21°C. In both these storage conditions the total phenolic value decreased up to week 12, increased at week 18 and then decreased again until week 36 of storage. No significant difference was seen between week 36 and week 48 ( $p>0.05$ ). In all

processing conditions a spike of values can be seen at week 18 (Figure 2.13). This spike could be the cause of instrumental or human error. However it is also noted that the total phenolic values through the year of storage have a small distribution of values ranging from approximately 10 to 14 GAE gram/liter tart cherry juice concentrate. Due to this small range any slight peak is considerably more noticeable.

At the elevated storage of 38°C the total phenolic content steadily increased over the 24 weeks of storage. At week 24 the total phenolic value was significantly the highest of all weeks of analysis. However, it is not likely that the phenolic content of this product will increase with time. Because of this it can be concluded that over the storage period there was the development of interfering compounds. These compounds reacted within the total phenolic method, and caused an overestimation of the measurement.

Through the analysis of these results it can be concluded that the total phenolic content of cherry juice concentrate goes through a number of significant fluctuations over storage duration. Though these fluctuations are significant it is observed that all values are within a fairly small range. This conclusion was also made in a study done on the fluctuations of total phenolic content of dark fruit juices in refrigerated conditions. The study had a much shorter storage period of 29 days; however, it was concluded that even with significant increasing and decreasing fluctuations in the total phenolic content values remain stable during storage (Piljac-Zegarac and others 2009). Due to

interference caused by developing compounds in concentrate stored at 38°C no definite conclusion can be made for high temperature storage.

## **2.4 Conclusion**

Based on the results of this research it can be concluded that pasteurization will not greatly affect the health-benefiting properties of the tart cherry juice concentrate. However, a significant difference was seen among unpasteurized and pasteurized concentrate during the early stages of storage, at low storage conditions for total anthocyanins. Storage temperature of concentrate can have harmful effects on the total anthocyanin content; however, storage temperature has minimal effect on total phenolic content and total antioxidant capacity. Concentrate stored at 4°C yields higher and most stable anthocyanin content over a year of storage; however, total antioxidant decay is shown to be unavoidable for concentrate stored over 6 weeks at any storage temperature 4°C and higher. Concentrate stored at 21°C was shown to lose the majority of its total anthocyanin content by week 12 while concentrate stored at 38°C lost the majority of its total anthocyanin content by week 6. Though fluctuations were seen in the total phenolic content over the year of storage, the storage duration had only minimal affect on its content.

## **3. OVERALL CONCLUSIONS AND FUTURE RECOMMENDATIONS**

Through the analysis of physico-chemical, sensory quality and antioxidant content it was determined that the pasteurization process has little to no effect

during storage duration at all storage conditions. Refrigerated storage conditions had the best and most stable quality concentrate over the one year storage period. However, concentrate stored over time at any storage condition, experienced significant decline total antioxidants by week 6. If concentrate were to be marketed as having high antioxidant properties the shelf life of this product would be significantly shorter. Ambient storage temperature was possible for short-term storage; however, continual storage at 21°C would greatly affect sensory quality and anthocyanin content. Taking into account both these properties concentrate should not be stored at ambient temperatures longer than 12 weeks. Elevated storage temperatures had harmful and rapid effects on the concentrate's sensory quality and antioxidant properties, reducing the shelf life to no more than 2 weeks.

Future recommendations for continued research of this topic include:

1. A repeated study on the analysis of different crop seasons to show repeatability of attribute results.
2. A more in-depth, detailed sensory analysis of the first two weeks of storage for the elevated (38°C) storage condition.
3. Further ORAC analysis determining the changes in total antioxidant capacity during the first six weeks of storage.
4. Investigation into packaging alternatives to better protect the antioxidant properties of the product.

## APPENDICES

### APPENDIX 1 RAW PHYSCIO-CHEMICAL DATA

**Table A.1.1 Raw data for total soluble solids (°Brix)**

	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	69.1	69.1	69.1	68.8	68.8	68.8
<b>2</b>	68.7	68.7	68.8	69.2	69.1	69.0
<b>4</b>	68.9	68.5	68.1	69.0	69.0	68.9
<b>6</b>	68.7	68.8	68.2	69.2	69.2	68.5
<b>8</b>	68.9	68.7	67.9	69.1	69.1	68.2
<b>12</b>	68.6	68.5	67.7	68.9	69.1	68.3
<b>16</b>	68.5	68.0	66.8	68.8	68.7	67.3
<b>20</b>	68.4	68.2	66.8	68.7	68.7	66.7
<b>24</b>	68.0	67.7	67.4	68.3	68.1	66.9
<b>36</b>	68.6	68.2	n/a	68.7	68.9	n/a
<b>48</b>	68.6	68.3	n/a	69.0	68.6	n/a

**Table A.1.2 Raw data for pH**

	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	3.29	3.29	3.29	3.31	3.31	3.31
<b>2</b>	3.36	3.37	3.33	3.32	3.36	3.41
<b>4</b>	3.36	3.33	3.38	3.35	3.34	3.34
<b>6</b>	3.31	3.35	3.42	3.30	3.35	3.36
<b>8</b>	3.30	3.37	3.39	3.34	3.39	3.41
<b>12</b>	3.31	3.32	3.39	3.29	3.33	3.38
<b>16</b>	3.32	3.41	3.39	3.32	3.38	3.40
<b>20</b>	3.44	3.44	3.46	3.39	3.43	3.45
<b>24</b>	3.34	3.38	3.35	3.33	3.38	3.35
<b>36</b>	3.36	3.38	n/a	3.33	3.38	n/a
<b>48</b>	3.33	3.33	n/a	3.28	3.30	n/a

**Table A.1.3 Raw data for titratable acidity (% malic acid)**

	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	5.01	5.01	5.01	5.03	5.03	5.03
<b>2</b>	4.97	4.96	4.85	5.01	4.99	4.91
<b>4</b>	5.03	4.92	4.82	5.07	4.96	4.81
<b>6</b>	5.03	4.93	4.77	5.07	4.95	4.82
<b>8</b>	4.99	4.80	4.78	5.00	4.88	4.83
<b>12</b>	4.99	4.82	4.79	5.03	4.86	4.85
<b>16</b>	4.95	4.70	4.79	4.99	4.81	4.87
<b>20</b>	5.19	4.99	4.88	5.27	5.05	4.90
<b>24</b>	4.94	4.97	4.70	4.79	4.94	4.98
<b>36</b>	4.91	4.63	n/a	4.90	4.73	n/a
<b>48</b>	4.91	4.71	n/a	4.94	4.72	n/a

**Table A.1.4 Raw data for turbidity (% transmittance)**

	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	76.9	76.9	76.9	76.3	76.3	76.3
<b>2</b>	79.4	76.7	67.5	78.0	75.4	67.9
<b>4</b>	79.6	75.1	57.1	77.9	73.8	56.5
<b>6</b>	79.0	73.2	52.4	78.1	72.3	50.6
<b>8</b>	79.2	70.8	49.4	77.6	68.2	44.9
<b>12</b>	76.2	65.3	43.7	74.7	64.8	43.4
<b>16</b>	72.3	59.5	40.8	72.1	60.9	39.1
<b>20</b>	76.7	59.1	45.9	75.1	59.3	42.4
<b>24</b>	74.8	57.1	38.9	74.2	57.8	41.3
<b>36</b>	75.8	55.9	n/a	75.4	55.3	n/a
<b>48</b>	74.0	50.9	n/a	73.8	50.5	n/a

**Table A.1.5 Raw data for Hunter Color L value**

	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	2.73	2.73	2.73	2.68	2.68	2.68
<b>2</b>	2.96	2.37	2.28	2.60	2.76	2.63
<b>4</b>	2.54	2.55	2.54	2.39	2.48	2.73
<b>6</b>	3.38	2.70	2.55	3.02	2.85	2.82
<b>8</b>	2.93	2.75	2.89	3.06	2.93	2.64
<b>12</b>	2.91	2.49	2.85	2.45	2.92	3.33
<b>16</b>	2.46	2.49	2.86	2.88	2.62	2.82
<b>20</b>	3.03	2.65	2.61	2.78	3.06	2.70
<b>24</b>	2.60	2.45	3.19	2.28	2.52	2.89
<b>36</b>	2.44	2.40	n/a	2.31	2.48	n/a
<b>48</b>	2.08	2.43	n/a	2.26	2.32	n/a

**Table A.1.6 Raw data for Hunter Color a value**

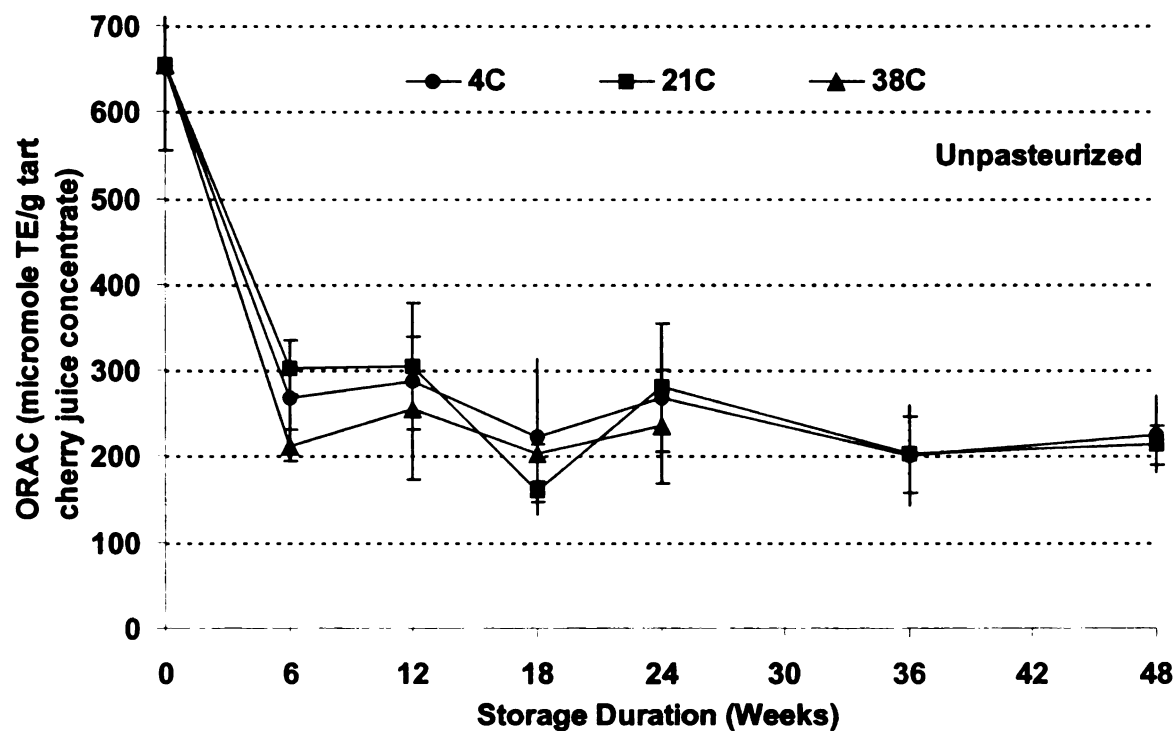
	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	4.46	4.46	4.46	3.95	3.95	3.95
<b>2</b>	4.07	3.95	0.80	3.89	2.67	0.69
<b>4</b>	3.55	2.57	0.15	3.85	2.06	0.10
<b>6</b>	3.33	1.76	0.09	2.94	1.19	0.09
<b>8</b>	3.82	1.18	0.02	2.73	0.97	0.13
<b>12</b>	3.41	0.64	-0.02	3.50	0.61	-0.02
<b>16</b>	3.68	0.43	-0.03	2.46	0.36	0.04
<b>20</b>	3.01	0.41	0.04	2.89	0.31	0.01
<b>24</b>	1.70	0.32	0.28	2.45	0.44	0.19
<b>36</b>	2.18	0.22	n/a	2.18	0.13	n/a
<b>48</b>	1.51	0.15	n/a	1.89	0.13	n/a

**Table A.1.7 Raw data for Hunter Color b value**

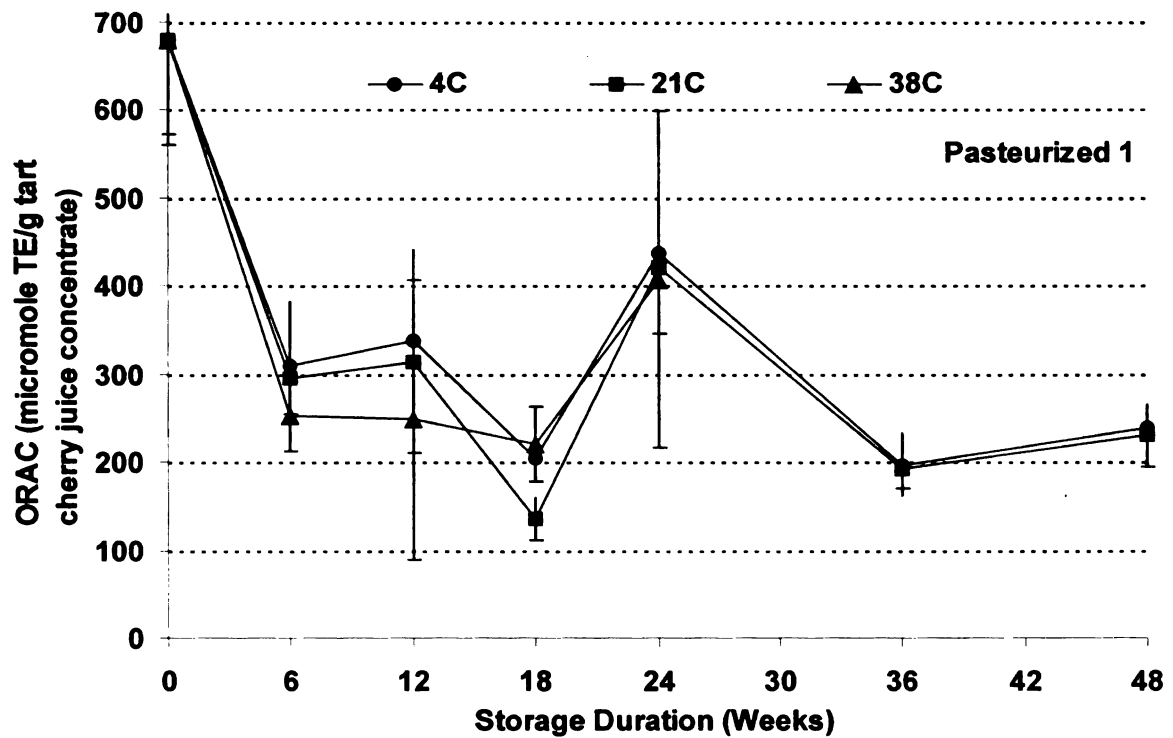
	Unpasteurized			Pasteurized		
	4°C	21°C	38°C	4°C	21°C	38°C
<b>0</b>	0.81	0.81	0.81	0.63	0.63	0.63
<b>2</b>	0.62	0.85	0.47	0.92	0.50	0.48
<b>4</b>	0.73	0.59	0.41	0.85	0.44	0.39
<b>6</b>	0.55	0.45	0.34	0.38	0.40	0.19
<b>8</b>	0.72	0.37	0.35	0.53	0.20	0.42
<b>12</b>	0.76	0.38	0.45	0.84	0.32	0.41
<b>16</b>	0.92	0.16	0.61	0.43	0.26	0.62
<b>20</b>	0.33	0.26	0.81	0.42	0.30	0.62
<b>24</b>	0.42	0.57	1.01	0.61	0.54	1.02
<b>36</b>	0.50	0.68	n/a	0.64	0.68	n/a
<b>48</b>	0.45	0.74	n/a	0.60	0.79	n/a



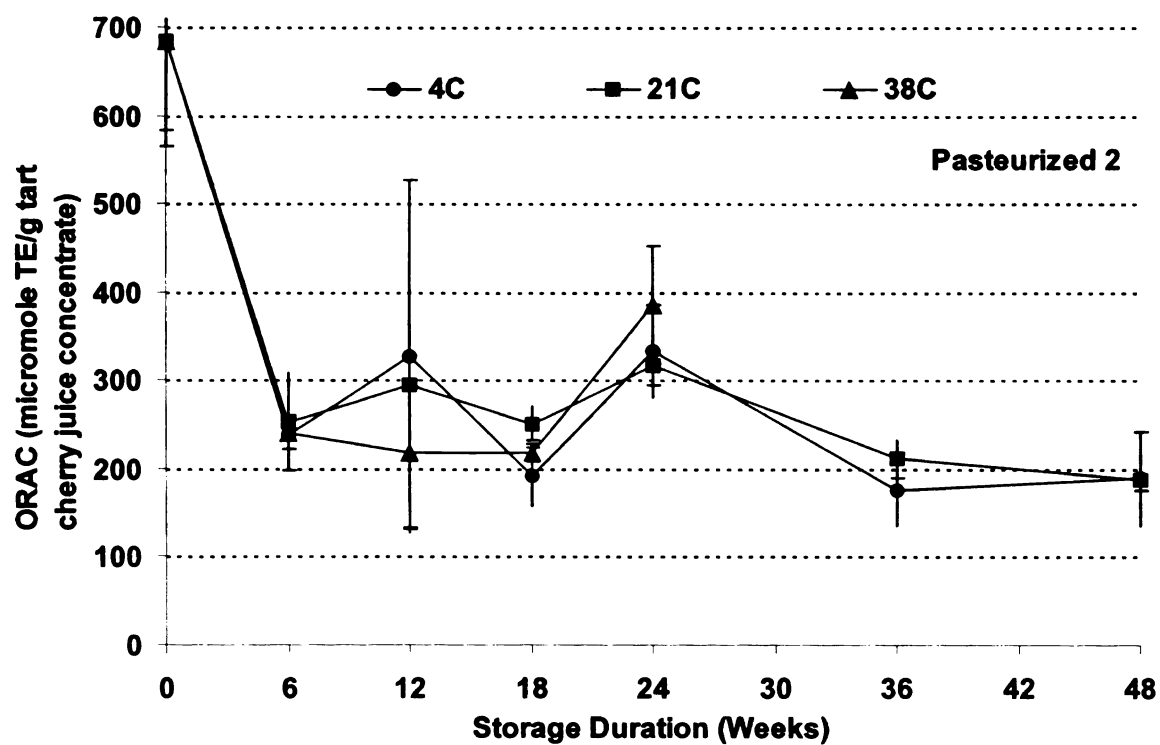
## APPENDIX 2 RAW ANTIOXIDANT DATA



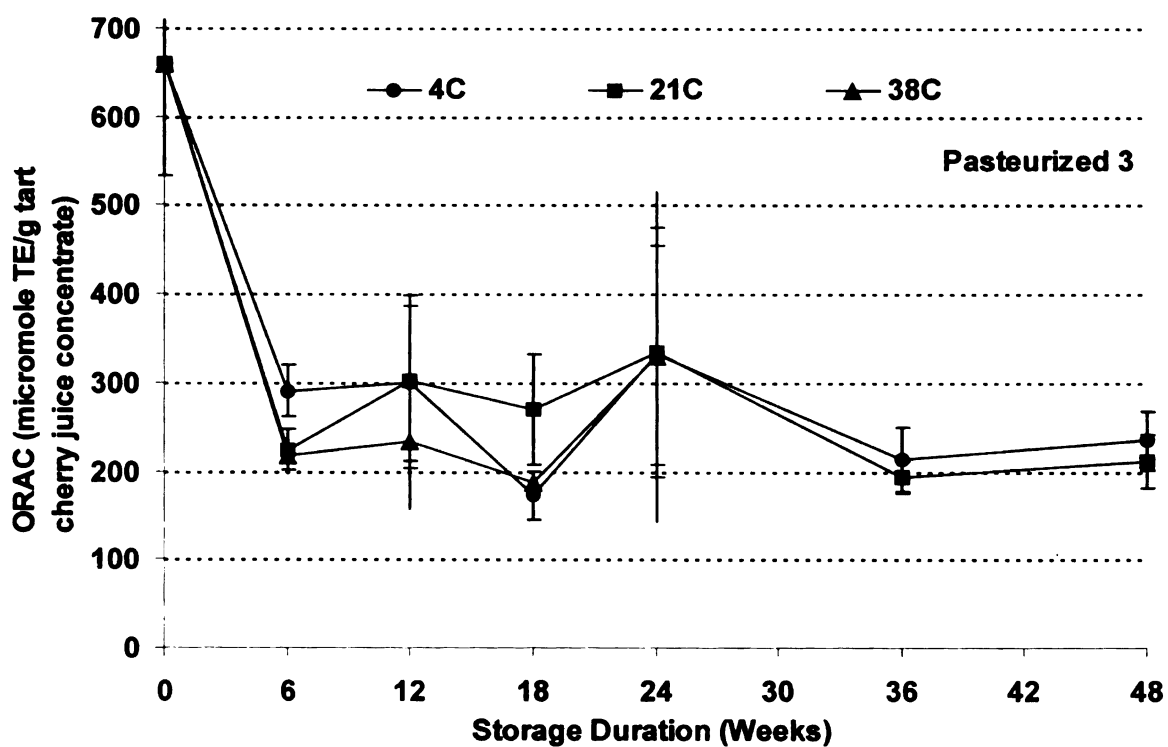
**Figure A.2.1: Effects of storage time and temperature on the average ORAC values (micromole TE/g tart cherry juice concentrate) for unpasteurized cherry juice concentrate**



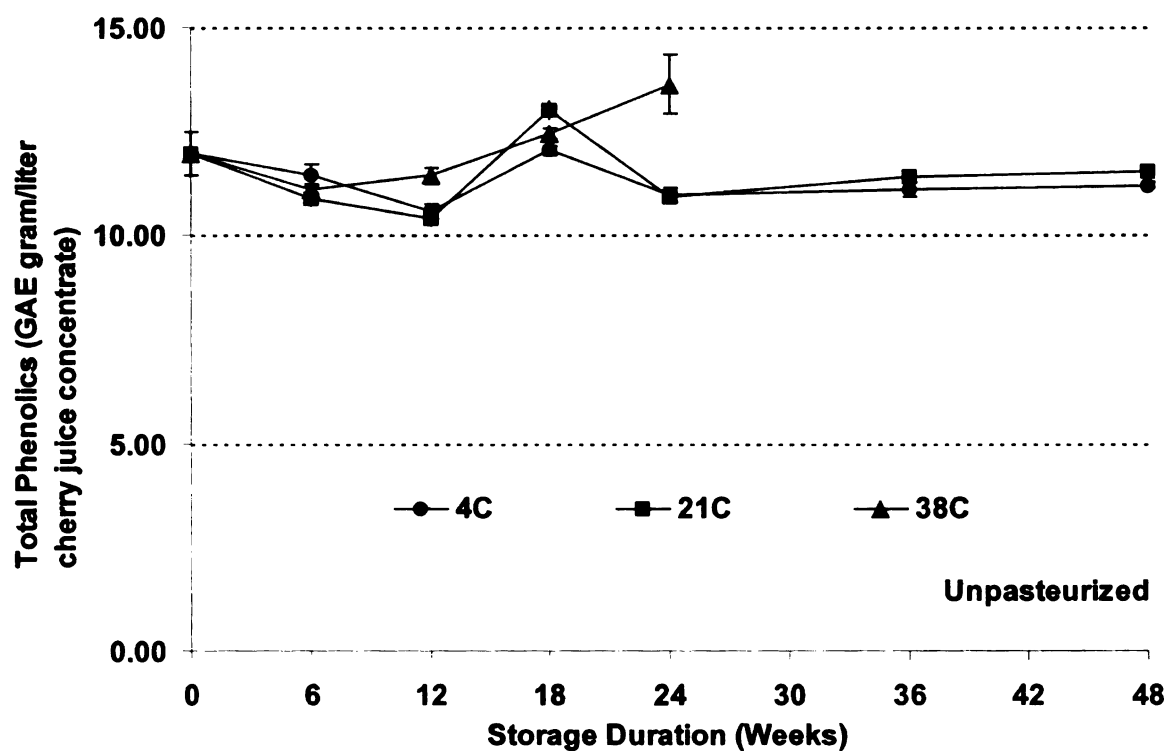
**Figure A.2.2: Effects of storage time and temperature on the average ORAC values (micromole TE/g tart cherry juice concentrate) for pasteurized (batch 1) cherry juice concentrate**



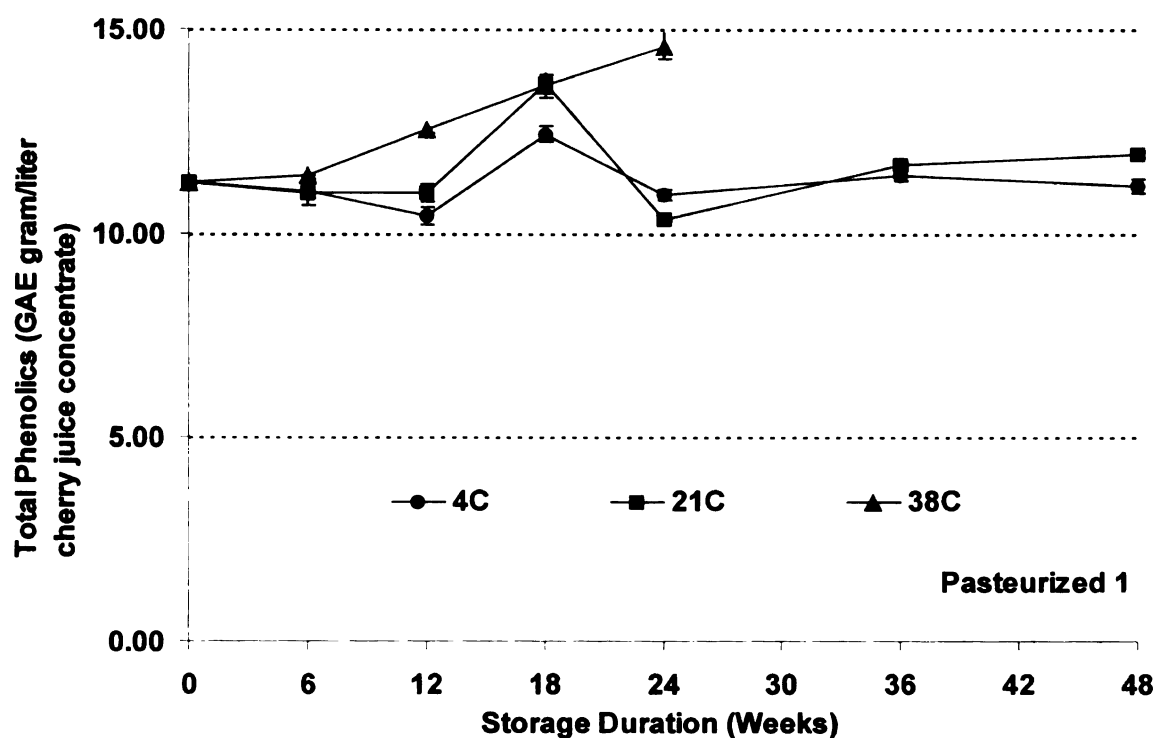
**Figure A.2.3: Effects of storage time and temperature on the average ORAC values (micromole TE/g tart cherry juice concentrate) for pasteurized (batch 2) cherry juice concentrate**



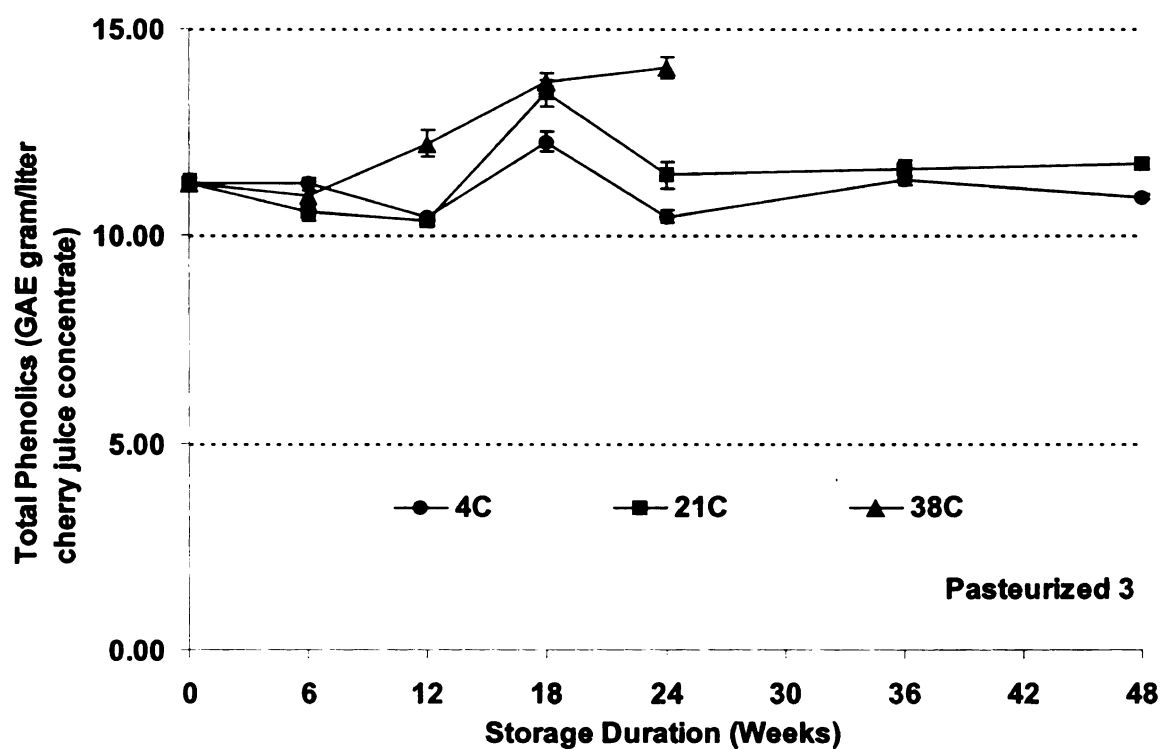
**Figure A.2.4: Effects of storage time and temperature on the average ORAC values (micromole TE/g tart cherry juice concentrate) of pasteurized (batch 3) cherry juice concentrate**



**Figure A.2.5: Effects of storage time and temperature on the average total phenolic values (g GAE/liter liter tart cherry juice concentrate) for unpasteurized cherry juice concentrate**



**Figure A.2.6: Effects of storage time and temperature on the average total anthocyanin values (g GAE/liter liter tart cherry juice concentrate) for pasteurized (batch 1) cherry juice concentrate**



**Figure A.2.7: Effects of storage time and temperature on the average total anthocyanin values (g GAE/liter liter tart cherry juice concentrate) for pasteurized (batch 3) cherry juice concentrate**





**Table A.2.1 Raw data for Oxygen Radical Absorbance Capacity (ORAC<sub>FL</sub>) for tart cherry juice concentrate**

Storage Period (Weeks)	Storage Temperature (°C)	Processing	Total Antioxidants (micromole TE/g tart cherry juice concentrate)	Average	Standard Deviation
0	n/a	Unpasteurized	569 571 717 764	655	100
0	n/a	Pasteurized (1)	613 615 651 836	679	106
0	n/a	Pasteurized (2)	583 653 643 857	684	119
0	n/a	Pasteurized (3)	582 662 558 837	660	126
6	4	Unpasteurized	224 224 293 332	268	53
6	4	Pasteurized (1)	226 290 315 403	309	73
6	4	Pasteurized (2)	212 260 227 253	238	22
6	4	Pasteurized (3)	261 272 310 319	290	29
6	21	Unpasteurized	273 312 284 344	303	32
6	21	Pasteurized (1)	292 351 281 255	295	41

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
6	21	Pasteurized (2)	198 226 262 327	253	56
6	21	Pasteurized (3)	191 230 227 246	224	23
6	38	Unpasteurized	185 223 214 228	212	19
6	38	Pasteurized (1)	193 207 290 318	252	61
6	38	Pasteurized (2)	202 238 227 295	240	39
6	38	Pasteurized (3)	201 235 201 235	218	20
12	4	Unpasteurized	207 266 322 352	287	64
12	4	Pasteurized (1)	225 285 381 460	338	104
12	4	Pasteurized (2)	198 243 242 624	327	199
12	4	Pasteurized (3)	224 243 308 419	299	88

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
12	21	Unpasteurized	216 276 343 385	305	74
12	21	Pasteurized (1)	211 261 335 449	314	104
12	21	Pasteurized (2)	163 254 230 534	295	164
12	21	Pasteurized (3)	228 246 290 441	301	97
12	38	Unpasteurized	181 210 259 370	255	83
12	38	Pasteurized (1)	191 208 230 365	248	79
12	38	Pasteurized (2)	151 168 99 449	217	158
12	38	Pasteurized (3)	194 204 186 348	233	77
18	4	Unpasteurized	183 236 128 342	222	91
18	4	Pasteurized (1)	184 201 213 222	205	16

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
18	4	Pasteurized (2)	155 210 227 173	191	33
18	4	Pasteurized (3)	192 177 186 132	172	27
18	21	Unpasteurized	155 164 173 144	159	12
18	21	Pasteurized (1)	151 154 138 102	136	24
18	21	Pasteurized (2)	221 256 263 263	251	20
18	21	Pasteurized (3)	206 271 247 354	270	62
18	38	Unpasteurized	191 213 202 210	204	10
18	38	Pasteurized (1)	192 175 236 276	220	46
18	38	Pasteurized (2)	172 210 213 273	217	42
18	38	Pasteurized (3)	182 196 183 193	188	7

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
24	4	Unpasteurized	211 261 279 316	267	43
24	4	Pasteurized (1)	338 400 441 567	436	97
24	4	Pasteurized (2)	269 349 320 393	333	52
24	4	Pasteurized (3)	217 262 347 496	331	123
24	21	Unpasteurized	193 255 308 365	280	74
24	21	Pasteurized (1)	369 384 400 533	421	76
24	21	Pasteurized (2)	284 323 338 325	317	23
24	21	Pasteurized (3)	197 239 401 499	334	141
24	38	Unpasteurized	167 206 245 322	235	66
24	38	Pasteurized (1)	312 383 392 542	407	97

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
24	38	Pasteurized (2)	230 249 418 640	385	191
24	38	Pasteurized (3)	194 193 342 588	329	186
36	4	Unpasteurized	135 171 235 262	201	58
36	4	Pasteurized (1)	162 176 210 240	197	35
36	4	Pasteurized (2)	136 170 164 233	176	41
36	4	Pasteurized (3)	176 216 261 201	333	52
36	21	Unpasteurized	151 178 232 248	213	36
36	21	Pasteurized (1)	167 199 219 182	202	45
36	21	Pasteurized (2)	188 198 225 231	192	22
36	21	Pasteurized (3)	167 202 209 192	211	21

**Table A.2.1 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Antioxidants (micromole TE/g tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
48	4	Unpasteurized	173 213 236 281	193	18
48	4	Pasteurized (1)	218 223 253 260	226	45
48	4	Pasteurized (2)	191 176 132 259	238	21
48	4	Pasteurized (3)	200 258 267 214	189	53
48	21	Unpasteurized	179 225 231 218	235	33
48	21	Pasteurized (1)	189 219 239 273	213	23
48	21	Pasteurized (2)	179 203 176 189	230	36
48	21	Pasteurized (3)	175 198 240 232	187	12

**Table A.2.2 Raw data for total anthocyanins for tart cherry juice concentrate**

Storage Period (Weeks)	Storage Temperature (°C)	Processing	Total Anthocyanins (mg cyn-3-glu/liter tart cherry juice concentrate)	Average	Standard Deviation
0	n/a	Unpasteurized	569.77 531.02 514.33	538.37	28.44
0	n/a	Pasteurized (1)	463.56 448.87 454.88	455.77	7.39
0	n/a	Pasteurized (2)	476.92 468.90 443.52	463.12	17.44
0	n/a	Pasteurized (3)	428.16 441.52 446.86	438.85	9.63
6	4	Unpasteurized	422.82 464.23 482.93	456.66	30.76
6	4	Pasteurized (1)	394.09 393.43 408.79	398.77	8.68
6	4	Pasteurized (2)	405.45 409.46 442.19	419.03	20.15
6	4	Pasteurized (3)	391.42 414.13 460.89	422.15	35.42
6	21	Unpasteurized	197.05 204.39 235.79	212.41	20.58
6	21	Pasteurized (1)	217.09 158.31 185.02	186.80	29.43
6	21	Pasteurized (2)	176.34 199.05 201.05	192.15	13.73
6	21	Pasteurized (3)	211.74 227.10 241.13	226.66	14.70
6	38	Unpasteurized	42.08 40.75 63.46	48.76	12.74
6	38	Pasteurized (1)	29.39 33.40 48.09	36.96	9.85



**Table A.2.2 Continued**

Storage Period (Weeks)	Storage Temperature (°C)	Processing	Total Anthocyanins (mg cyn-3-glu/liter tart cherry juice concentrate)	Average	Standard Deviation
6	38	Pasteurized (2)	14.70 39.41 32.73	28.94	12.78
6	38	Pasteurized (3)	36.07 58.78 80.82	58.56	22.38
12	4	Unpasteurized	428.16 406.78 400.11	411.68	14.65
12	4	Pasteurized (1)	361.36 384.07 398.10	381.18	18.54
12	4	Pasteurized (2)	377.39 379.40 402.78	386.52	14.11
12	4	Pasteurized (3)	401.44 386.75 371.38	386.52	15.03
12	21	Unpasteurized	138.93 120.23 122.24	127.13	10.27
12	21	Pasteurized (1)	112.22 104.87 109.54	108.88	3.72
12	21	Pasteurized (2)	114.89 122.24 104.20	113.78	9.07
12	21	Pasteurized (3)	99.53 104.87 116.22	106.87	8.53
12	38	Unpasteurized	30.73 22.04 7.35	20.04	11.82
12	38	Pasteurized (1)	40.75 24.05 30.06	31.62	8.46
12	38	Pasteurized (2)	32.06 39.41 37.41	36.29	3.80
12	38	Pasteurized (3)	70.80 49.43 32.06	50.76	19.41

**Table A.2.2 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Anthocyanins (mg cyn-3-glu/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
18	4	Unpasteurized	405.45 458.22 433.50	432.39	26.40
18	4	Pasteurized (1)	350.01 366.04 375.39	363.81	12.84
18	4	Pasteurized (2)	344.66 366.71 360.03	357.13	11.30
18	4	Pasteurized (3)	356.02 363.37 370.05	363.15	7.02
18	21	Unpasteurized	93.51 71.47 94.18	86.39	12.92
18	21	Pasteurized (1)	105.54 82.83 84.18	94.18	11.36
18	21	Pasteurized (2)	86.17 78.15 88.17	84.16	5.30
18	21	Pasteurized (3)	78.82 80.82 97.52	85.72	10.27
18	38	Unpasteurized	96.19 24.71 30.06	50.32	39.81
18	38	Pasteurized (1)	78.15 28.05 22.71	42.97	30.58
18	38	Pasteurized (2)	100.19 66.80 60.12	75.70	21.47
18	38	Pasteurized (3)	25.38 53.44 63.46	47.42	19.74
24	4	Unpasteurized	344.00 335.98 342.66	340.88	4.29
24	4	Pasteurized (1)	324.63 317.95 307.26	316.61	8.76

**Table A.2.2 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Anthocyanins (mg cyn-3-glu/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
24	4	Pasteurized (2)	350.68 331.31 346.67	342.88	10.23
24	4	Pasteurized (3)	343.33 338.65 331.97	337.99	5.71
24	21	Unpasteurized	74.14 72.14 63.46	69.91	5.68
24	21	Pasteurized (1)	50.76 71.14 52.10	59.00	13.13
24	21	Pasteurized (2)	76.15 96.19 90.17	87.50	10.28
24	21	Pasteurized (3)	51.43 42.75 82.83	59.00	21.08
24	38	Unpasteurized	41.41 49.43 4.68	31.84	23.86
24	38	Pasteurized (1)	30.39 29.39 15.36	25.05	8.40
24	38	Pasteurized (2)	8.02 20.04 7.35	11.80	7.14
24	38	Pasteurized (3)	0.67 46.09 40.08	28.94	24.67
36	4	Unpasteurized	360.70 325.96 360.70	349.12	20.05
36	4	Pasteurized (1)	323.96 304.59 310.60	313.05	9.91
36	4	Pasteurized (2)	310.60 310.60 348.67	323.29	21.98
36	4	Pasteurized (3)	335.31 321.29 331.31	329.30	7.23

**Table A.2.2 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Anthocyanins (mg cyn-3-glu/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
36	21	Unpasteurized	65.46 38.74 40.08	48.09	15.05
36	21	Pasteurized (1)	42.08 50.10 45.42	45.87	4.03
36	21	Pasteurized (2)	40.75 9.35 32.06	27.39	16.21
36	21	Pasteurized (3)	58.11 54.10 27.39	46.53	16.70
48	4	Unpasteurized	267.18 301.92 325.96	298.35	29.55
48	4	Pasteurized (1)	259.83 261.84 242.47	254.71	10.65
48	4	Pasteurized (2)	287.22 279.21 293.23	286.55	7.04
48	4	Pasteurized (3)	349.34 303.25 313.27	321.95	24.24
48	21	Unpasteurized	42.75 38.07 24.05	34.96	9.73
48	21	Pasteurized (1)	38.07 14.03 83.49	45.20	35.28
48	21	Pasteurized (2)	22.71 44.75 74.81	47.42	26.15
48	21	Pasteurized (3)	36.07 55.44 30.73	40.75	13.00

**Table A.2.3 Raw data for total phenolics for tart cherry juice concentrate**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Phenolics (GAE g/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
0	n/a	Unpasteurized	11.22 11.36 12.30	11.96	0.52
0	n/a	Pasteurized (1)	11.19 11.36 11.15	11.23	0.11
0	n/a	Pasteurized (2)	11.15 11.36 11.28	11.26	0.11
0	n/a	Pasteurized (3)	11.06 11.45 11.28	11.26	0.19
6	4	Unpasteurized	11.22 11.70 11.44	11.45	0.24
6	4	Pasteurized (1)	10.87 11.22 10.96	11.02	0.18
6	4	Pasteurized (2)	10.96 10.96 11.22	11.04	0.15
6	4	Pasteurized (3)	11.04 11.44 11.26	11.25	0.20
6	21	Unpasteurized	10.69 10.95 10.95	10.87	0.15
6	21	Pasteurized (1)	10.69 10.95 11.33	10.99	0.32
6	21	Pasteurized (2)	10.91 10.95 11.12	10.99	0.11
6	21	Pasteurized (3)	10.35 10.74 10.65	10.58	0.20
6	38	Unpasteurized	10.95 11.12 11.16	11.08	0.11
6	38	Pasteurized (1)	11.42 11.42 11.42	11.42	0.00

**Table A.2.3 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Phenolics (GAE g/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
6	38	Pasteurized (2)	10.95 11.12 11.42	11.16	0.24
6	38	Pasteurized (3)	11.33 10.74 10.78	10.95	0.33
12	4	Unpasteurized	10.73 10.64 10.39	10.59	0.18
12	4	Pasteurized (1)	10.52 10.17 10.56	10.42	0.21
12	4	Pasteurized (2)	10.13 10.22 10.30	10.22	0.09
12	4	Pasteurized (3)	10.39 10.52 10.43	10.44	0.07
12	21	Unpasteurized	10.26 10.56 10.39	10.40	0.15
12	21	Pasteurized (1)	10.77 10.94 11.20	10.97	0.21
12	21	Pasteurized (2)	9.96 10.26 10.39	10.20	0.22
12	21	Pasteurized (3)	10.34 10.22 10.52	10.36	0.15
12	38	Unpasteurized	11.24 11.63 11.46	11.44	0.19
12	38	Pasteurized (1)	12.48 12.48 12.65	12.54	0.10
12	38	Pasteurized (2)	12.31 13.16 12.99	12.82	0.45
12	38	Pasteurized (3)	11.88 12.31 12.48	12.22	0.31

**Table A.2.3 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Phenolics (GAE g/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
18	4	Unpasteurized	12.09 12.09 11.91	12.03	0.11
18	4	Pasteurized (1)	12.55 12.55 12.18	12.43	0.21
18	4	Pasteurized (2)	12.46 12.18 12.64	12.43	0.23
18	4	Pasteurized (3)	11.96 12.28 12.55	12.26	0.30
18	21	Unpasteurized	12.74 13.09 13.17	13.00	0.23
18	21	Pasteurized (1)	13.43 13.70 13.87	13.67	0.22
18	21	Pasteurized (2)	12.82 12.91 12.91	12.88	0.05
18	21	Pasteurized (3)	13.78 13.35 13.17	13.43	0.31
18	38	Unpasteurized	12.48 12.30 12.56	12.45	0.13
18	38	Pasteurized (1)	13.96 13.43 13.43	13.61	0.30
18	38	Pasteurized (2)	13.26 13.52 13.17	13.32	0.18
18	38	Pasteurized (3)	13.96 13.70 13.52	13.73	0.22
24	4	Unpasteurized	10.79 11.14 10.97	10.97	0.18
24	4	Pasteurized (1)	11.06 10.97 10.79	10.94	0.14

**Table A.2.3 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Phenolics (GAE g/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
24	4	Pasteurized (2)	10.83 10.97 10.70	10.83	0.13
24	4	Pasteurized (3)	10.48 10.57 10.34	10.46	0.11
24	21	Unpasteurized	10.88 10.97 10.97	10.94	0.05
24	21	Pasteurized (1)	10.30 10.39 10.34	10.34	0.04
24	21	Pasteurized (2)	11.28 11.50 11.37	11.38	0.11
24	21	Pasteurized (3)	11.81 11.23 11.32	11.46	0.31
24	38	Unpasteurized	13.69 12.88 14.31	13.63	0.71
24	38	Pasteurized (1)	14.22 14.84 14.67	14.58	0.32
24	38	Pasteurized (2)	14.67 14.67 14.49	14.61	0.10
24	38	Pasteurized (3)	14.22 14.22 13.77	14.07	0.26
36	4	Unpasteurized	10.92 11.13 11.26	11.10	0.17
36	4	Pasteurized (1)	11.26 11.52 11.43	11.40	0.13
36	4	Pasteurized (2)	11.09 11.17 11.30	11.19	0.11
36	4	Pasteurized (3)	11.05 11.35 11.64	11.35	0.30



**Table A.2.3 Continued**

<b>Storage Period (Weeks)</b>	<b>Storage Temperature (°C)</b>	<b>Processing</b>	<b>Total Phenolics (GAE g/liter tart cherry juice concentrate)</b>	<b>Average</b>	<b>Standard Deviation</b>
36	21	Unpasteurized	11.26 11.47 11.47	11.40	0.12
36	21	Pasteurized (1)	11.60 11.64 11.73	11.66	0.07
36	21	Pasteurized (2)	11.43 11.73 11.60	11.59	0.15
36	21	Pasteurized (3)	11.43 11.81 11.60	11.62	0.19
48	4	Unpasteurized	11.17 11.26 11.17	11.20	0.05
48	4	Pasteurized (1)	11.00 11.35 11.09	11.15	0.18
48	4	Pasteurized (2)	11.43 11.43 11.52	11.46	0.05
48	4	Pasteurized (3)	10.92 11.17 10.70	10.93	0.24
48	21	Unpasteurized	11.39 11.52 11.64	11.52	0.13
48	21	Pasteurized (1)	11.81 11.99 11.99	11.93	0.10
48	21	Pasteurized (2)	11.73 11.73 11.90	11.79	0.10
48	21	Pasteurized (3)	11.60 11.81 11.81	11.74	0.12

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