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Utah Red Raspberry Jam: The Effects of Formulation, Heating, and Time on Color, Flavor, Texture, and Antioxidant Capacity

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Utah Red Raspberry Jam: The Effects of Formulation, Heating, and Storage on Color, Flavor, Texture, and Antioxidant Content

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

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The amount and type of antioxidants present in raspberries is dependent upon cultivar, ripeness, and growing conditions. Previous research on raspberry jam has reported some color, antioxidant, and flavor loss after processing and storage, though it is unknown to what extent similar changes will occur in raspberries grown in Utah. Sugar concentration and heating temperature as well as storage time were evaluated in an effort to maximize color retention, flavor, antioxidant content, shelf life, and consumer acceptance of Utah-grown raspberry jam. Four types of jams were processed in two batches each: low-sugar (40-42° Brix) and typical sugar (65-68° Brix) at 85 and 95 °C, from two separate farms in Utah. Oxygen Radical Absorbance Capacity (ORAC), total anthocyanin content (TAC), color, headspace, °Brix, pH, consistency, and water activity were measured in fresh jam, and after one and three months of typical storage (dark, room temperature) to evaluate changes after storage. A sensory analysis compared three-month stored jam to fresh jam made from the same berry crop. ORAC significantly declined in all jams during storage. Fresh low-sugar jam was found to contain higher ORAC values than high-sugar jam after processing and after three months of storage. All jams retained their initial anthocyanins over the first month and significantly lost an average of 28.8% anthocyanins between months 1 and 3 of storage. Color loss was found to be less pronounced than anthocyanin degradation, though nearly all jams maintained initial L^*, C^*, and h^* values over the first month then significantly decreased by the third month of storage. When comparing fresh and three-month jam, significant sensory differences were found in color, overall acceptability, flavor, and texture. All parameters scored higher for freshly-made jam, though three-month stored jam was still found to be acceptable to consumers. In summary, after three months of storage, significant nutrient quantity and sensory quality remains in Utah raspberry jam, despite significant declines in several assays and significant differences between treatments.

Abbreviations: SE, standard error; ORAC, Oxygen Radical Absorbance Capacity; WGJ, white grape juice concentrate; HS, high-sugar; LS, low-sugar; HT, high temperature; LT, low temperature; J, Cornaby Farms; K, Berries By The Bay; RT, room temperature.

Keywords: raspberries, jam, processing, heating, temperature, antioxidant content, ORAC, color, anthocyanins, storage, stability

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Utah Red Raspberry Jam:
The Effects of Formulation, Heating, and Storage on Color, Flavor, Texture, and Antioxidant Content

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Abstract

The amount and type of antioxidants present in raspberries is dependent upon cultivar, ripeness, and growing conditions. Previous research on raspberry jam has reported some color, antioxidant, and flavor loss after processing and storage, though it is unknown to what extent similar changes will occur in raspberries grown in Utah. Sugar concentration and heating temperature as well as storage time were evaluated in an effort to maximize color retention, flavor, antioxidant content, shelf life, and consumer acceptance of Utah-grown raspberry jam. Four types of jams were processed in two batches each: low-sugar (40-42° Brix) and typical sugar (65-68° Brix) at 85 and 95 °C, from two separate farms in Utah. Oxygen Radical Absorbance Capacity (ORAC), total anthocyanin content (TAC), color, headspace, °Brix, pH, consistency, and water activity were measured in fresh jam, and after one and three months of typical storage (dark, room temperature) to evaluate changes after storage. A sensory analysis compared three-month stored jam to fresh jam made from the same berry crop. ORAC significantly declined in all jams during storage. Fresh low-sugar jam was found to contain higher ORAC values than high-sugar jam after processing and after three months of storage. All jams retained their initial anthocyanins over the first month and significantly lost an average of 28.8% anthocyanins between months 1 and 3 of storage time. Color loss was found to be less pronounced than anthocyanin degradation, though nearly all jams maintained initial *L*, C*,* and *h** values over the first month then significantly decreased by the third month of storage. When comparing fresh and three-month jam, significant sensory differences were found in color, overall acceptability, flavor, and texture. All parameters scored higher for freshly-made jam, though three-month stored jam was still found to be acceptable to consumers. In summary, after three months of storage, significant nutrient quantity and sensory quality remains in Utah

raspberry jam, despite significant declines in several assays and significant differences between treatments.

Abbreviations: SE, standard error; ORAC, Oxygen Radical Absorbance Capacity; WGJ, white grape juice concentrate; HS, high-sugar; LS, low-sugar; HT, high temperature; LT, low temperature; J, Cornaby Farms; K, Berries By The Bay; RT, room temperature.

Keywords: raspberries, jam, processing, heating, temperature, antioxidant content, ORAC, color, anthocyanins, storage, stability

Introduction

The amount and type of antioxidants present in raspberries is dependent upon cultivar, ripeness, and growing conditions. Existing research has focused on raspberries that were grown in Finland, Spain, or the Northeastern or Midwestern United States (Ozgen et al. 2008; Anttonen et al. 2005; Kähkönen et al. 2001; Liu et al. 2002). These raspberries are often harvested in the spring from floricane (spring bearing) plants in temperate environments, and are of widely varying cultivars. Raspberry antioxidant data in existing literature has limited application to producers in Utah because of these differences. Most of the raspberries commercially grown in Utah are red raspberries harvested in the fall from primocane (fall bearing) plants grown in hot, arid conditions that have been nourished by irrigation and fertilization throughout the summer.

Due to the short shelf life of fresh raspberries, preservation, usually through processing into jams, is an important part of developing this specialty crop in Utah. Previous research (Zafrilla et al. 2001; Kim and Padilla-Zakour 2004; Hager et al. 2008) on raspberry jam has reported some color, antioxidant, and flavor loss after processing and storage. Since raspberry cultivar and growing conditions result in large differences in antioxidant activity (Ozgen et al. 2008), it is unknown to what extent similar changes will occur in raspberries grown in Utah. Processing may have different effects on Utah raspberries. Previous work (Freeman et al. 2011) found that Utah unprocessed raspberries have a higher antioxidant capacity [oxygen radical absorbance capacity (ORAC)] than commercial varieties grown in California. It is unclear to what extent this higher antioxidant content will be retained after processing.

Thermally processed Utah red raspberry jam appears to suffer significant color and flavor loss, especially with light and heat exposure during storage (personal communication, Janet Stocks, Cornaby Farms). Color and antioxidant loss may be directly related, though this has not

been confirmed in Utah raspberries. In addition to the raspberry cultivar used to produce a jam, different variables during processing affect color, antioxidant, and flavor loss as well, including heating temperature, cooking time, sugar concentration, type of sweetener, and type of preservative used (Garcia-Viguera et al. 1998). Many jams on the market are produced using long cooking times and boiling temperatures in order to prevent spoilage during storage, but these processing methods result in a more browned and "cooked" flavor, which can reduce the flavor of the raspberry (personal communication, Janet Stocks, Cornaby Farms). Some jams sold commercially are processed at lower temperatures because they have been advertised but unsubstantiated as a better method to minimize nutrient loss. High temperatures used in jam processing aid in chalcone formation, produced from anthocyanins, which help cause undesired brown pigment formation in jams (Kim and Padilla-Zakour, 2004). A higher concentration of fruit in the jam should directly correlate with more nutrients per serving, and can be marketed as such, but may affect storage outcomes.

The objectives of this study were to vary factors (sugar concentration, heating temperature, and storage time) that may affect jam quality in an effort to maximize color retention, flavor, antioxidant content, shelf life, and consumer acceptance. It was hypothesized that there would be a significant loss in color, flavor, and nutritional antioxidant content with processing of raspberries compared to previously published values for fresh berries. Increasing the temperature during cooking will result in significantly higher loss of color, flavor, and antioxidant content. Raspberry jam with more fruit and less sugar will have higher values per serving for color, flavor, and nutritional antioxidant content, but values will degrade faster over storage time than with high-sugar jam. The purpose of the sensory panel was to determine if consumers could see or taste a difference in the jam as it aged and to compare a panelist's

opinion about flavor and color changes between the various types of jam. Lastly, it was hypothesized that raspberry jam heated at a higher temperature and compared to a freshly-made jam will be significantly less acceptable when measured by sensory analysis.

Materials and Methods

Study Design

Two berry farms in separate locations in the state of Utah were selected (**Table 1**). All berries used were Caroline cultivar red raspberries harvested in Utah in the 2011 season using conventional (non-organic) agricultural methods. Cornaby Farms, located in Salem, Utah, and Berries by the Bay, located in Willard, Utah, are over one hundred miles apart. Despite both farms being subject to a similar Utah climate, the two locations differ topographically, meteorologically, and in soil conditions, which ultimately produce different berry lots (Pereira et al. 2006). Growing methods on Cornaby Farms included raised beds, underground fertigation, and a trellis system. Methods at Berries by the Bay involved a drip irrigation system, a two-application fertilization at the beginning and middle of the growing season, and a mix of raised and non-raised beds. Fresh raspberries were harvested when ripe and immediately frozen to -20°C to preserve the fruits. Previous research (Freeman et al 2011; Garcia-Viguera et al. 1998) has shown no antioxidant differences between fresh and freshly frozen raspberries.

Boiling (95 °C) and a reduced temperature (85 °C) were used for maximum heating temperatures. Two sugar concentrations were used in order to compare jam with a high fruit:sugar ratio (40-42 °Brix), which would potentially contain a higher amount of antioxidants and other nutrients per g, to a high-sugar:fruit jam (65-68 °Brix). Sugar concentrations were determined based on common and required industry practices. The standard of identity for jam

requires a 65-68 °Brix. Low-sugar jam or fruit spread will commonly have 40-42 °Brix. Storage conditions were the same for all jams, room temperature, 20°C (RT) in the dark. Heating and holding times for both temperatures and sugar concentrations during jam production were also approximately the same. Heating time is the amount of time required to reach the desired temperature; holding time is the time the jam batch is held at that desired temperature during processing (2 minutes).

Table 1. Berry location, heating, and formula variations used for the production of raspberry jam.

Farm Location	Max. Heating Temp. (°C)	Sugar Concentration
Salem, Utah	85	Low
		Typical
	95	Low
		Typical
Willard, Utah	85	Low
		Typical
	95	Low
		Typical

Jam Ingredients

Cane sugar was purchased at a local grocery store (see **Table 2**). Powdered citric acid was purchased from Jungbunzlauer Suisse AG (Neuton Centre, MA). The pectin used for the high-sugar batches of jam was TIC Pretested® HM Rapid Set Pectin (White Marsh, MD). The low-sugar jam was made using a proprietary mixture of low-methoxy pectin and other gelling agents used by Cornaby Farms for their low-sugar jams. The low-sugar jam also contained water and a small amount of white grape juice concentrate in place of sugar. White grape juice

concentrate (WGJ, Tree Top Inc., Selah, WA) was added as a cane sugar alternative for marketing to the consumer perception of better health over cane sugar. WGJ is a typical addition to low-sugar jams and was added to the low-sugar jams in this study in order to best represent industry practices. For the current study, it is assumed that the addition of white grape juice concentrate does not have an effect on antioxidant decay any more than sugar; the juice provides an insignificant amount of antioxidants to the jam (Mullen et al. 2007; Dávalos et al. 2004b). Water was added for textural purposes and is typically used in low-sugar raspberry jam (Janet Stocks, Cornaby Farms). An antifoaming agent (DOW Food Grade Antifoam, Pacific Pectin, Inc., Oakhurst, CA) was used to minimize foaming that occurs during the cooking and boiling of both jams.

Table 2. Formulas for high- and low-sugar raspberry jams (w/w).

	High Sugar Jam	**Low Sugar Jam**
	Percent, % (w/w)	Percent, % (w/w)
Raspberries	40.37	47.49
Cane Sugar	58.88	26.35
Gelling agents[a]	0.41	1.65
Citric Acid	0.34	0.42
WGJ[b]	0.00	8.72
Water	0.00	15.36
TOTAL	100.00	100.00

[a]Pectin differs for the two jam formulas as required for different sugar concentrations; the low-sugar pectin formula is proprietary. [b]WGJ = white grape juice concentrate.

Formula Modifications

An industry-standard jam formulation and protocol (Bishop's Storehouse Services Processing Procedures, LDS Welfare Services, Salt Lake City, UT) was used for the high-sugar

raspberry jam batches. The jam protocol was modified for use with the processing equipment and facility structure available.

A low-sugar jam formula used by Cornaby Farms for raspberry jams sold commercially was used for this study. Slight modifications to the pectin concentration were made due to the smaller batch size from the original formula. Both formulas were modified from the originals so that both jams were as similar as possible in consistency to each other. This was measured by a spoon-test after cooking the jam and immediately before pouring into jars as well as by a consistometer after the jam had been poured into jars and cooled to room temperature.

Jam Processing

Upon receipt, berries were kept frozen (-20 °C) in their original plastic containers, then defrosted at room temperature prior to jam production. All batches were processed at the Brigham Young University pilot plant. Ingredients were pre-weighed and kept separate. Both low-methoxy and high-methoxy pectins were premixed with a portion of the sugar to properly disperse the pectin.

Industry-standard processing methods (Downing, 1996) were followed as closely as possible, but were modified for the facilities available. As shown in **Figure 1**, pre-weighed and freshly-thawed raspberries were placed into an unheated stainless steel, jacketed steam kettle and mashed with a large hand-masher until no whole berries were visible. Two drops of DOW Food Grade antifoaming agent were added to the berries. Pre-mixed sugar and pectin (and WGJ and water for the low-sugar jam) were slowly added with continuous stirring and mashing. This method was identical for all jam variations.

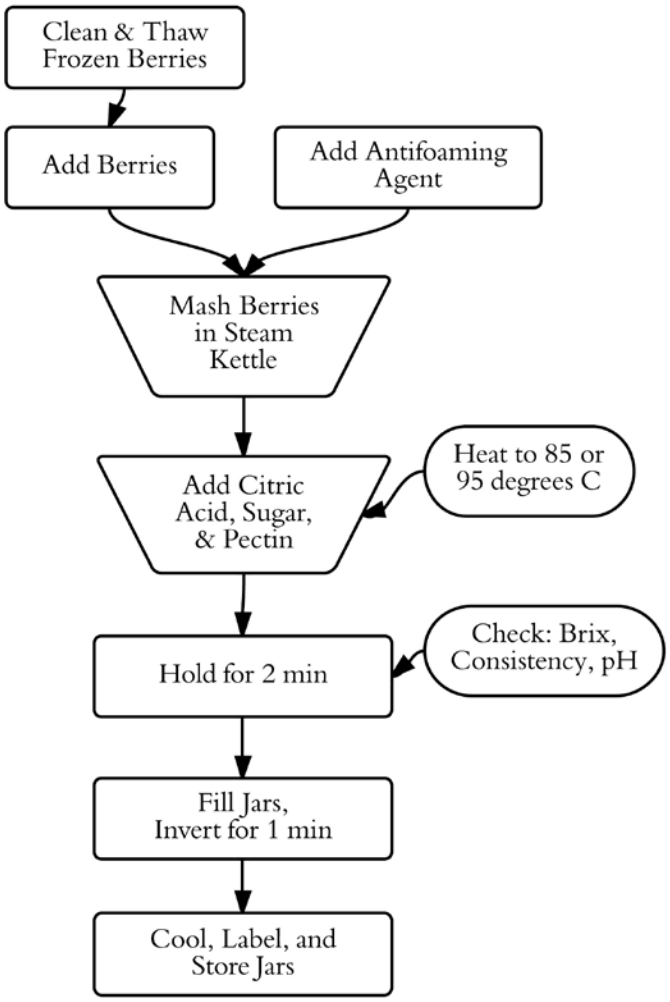

Figure 1. Flow chart depicting the process used to make jam. Ovals represent check points, trapezoid shapes represent the kettle and items placed into the kettle or actions done in the kettle, rectangles represent items placed into the kettle or steps in the jam-making process.

After all ingredients but citric acid were added, the steam kettle was turned on. The mixture was heated to the treatment temperature as quickly as possible with continuous stirring and a thermometer was held in the center of the steam kettle.

When the batch reached approximately 74°C, citric acid was added while stirring. Heating of the jam continued until the jam reach the desired temperature, and the jam was held at

that temperature for two minutes. A small sample of the jam was removed, placed in a sample cup and rapidly cooled in a bowl of ice water. The pH range needed to be within 2.9-3.1 to ensure pectin formation, correct flavor and to prevent microbial growth; it was adjusted with citric acid if needed. The pH was measured using a Denver Instruments UltraBASIC pH meter (Bohemia, NY). Degrees Brix was analyzed using a Reichert AR200 Refractometer (Depew, NY). For low-sugar jams and high-sugar jams, the °Brix needed to be approximately 40-42° and 65-68°, respectively. Additional sugar was added if needed. Consistency was analyzed by an industry standard spoon test. A sample of jam was placed in a spoon, allowed to cool, and observed for how well the jam remains suspended on the spoon.

Jars and lids were sterilized in a steam box (Brigham Young University Machine Shop) to match the temperature of the jam. After the jam was cooked and held for 2 min, each jar was filled with jam to a 0.60-0.70 cm headspace. Immediately after filling, lids were placed on the jars, tightened by hand, and inverted for 30-45 seconds in order to sterilize the jar lid. Each jar was labeled and placed in a dark cabinet for storage (RT, ~25 °C) for three months.

Chemicals

Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Acros Organics, Geel, Belgium), fluorescein (Na salt), K_2HPO_4, KH_2PO_4,12 M HCl, methanol, and acetone was purchased through Fisher Scientific Inc. (Fair Lawn, NJ). AAPH (2,2′-Azobis(2-methylpropionamidine) dihydrochloride) was purchased from Wako Chemicals Inc. (Richmond, VA). Dithiothreitol (DTT) was purchased through Sigma-Aldrich (St. Louis, MO). Ascorbic acid was purchased through Mallinckrodt Chemicals (Hazelwood, MO).

Post-processing

Water activity, consistency, vacuum headspace, °Brix, pH, color, total anthocyanin content, ORAC, and ascorbate were all measured immediately after processing, at 1 month, and 3 months of room temperature and dark storage. °Brix and pH were also measured during processing.

Water activity was measured using an AquaLab Water Activity Meter (Riverside, CA). The instrument was calibrated using a standardized NaCl solution at 6.0 M as well as distilled water. Sample cups were filled halfway with jam and placed into the chamber; the chamber was sealed and the sample's water activity was read and recorded.

Consistency was measured using a Bostwick Consistometer (CSC Scientific Company, Fairfax, VA). The instrument was first calibrated to the proper angle, the gate was closed, and the reservoir was filled with the sample of jam. The gate was then opened and the jam ran freely along the slope. The consistency was measured as the distance (in cm) that the product flowed in 30 seconds.

A vacuum gauge (Dixie Canner Co., Athens, GA) was used to ensure proper sealing of the jars. A calibrated gauge was placed on the lid about two-thirds from the center, a thorough seal was made, and the gauge was firmly pressed down on the lid and the measured vacuum was recorded. Values were reported in inches Hg.

The refractometer described above was used to measure °Brix. The refractometer was initially calibrated with distilled water, then the dried well was filled with jam and read. This was performed in duplicate for each batch of jam.

In order to account for variations due to temperature differences during cooking, pH was measured during both processing and at a separate time at room temperature storage using a

Denver Instruments UltraBASIC pH meter (Bohemia, NY) to ensure the pH maintained the desired value after cooling .

Color was assessed using a Hunterlab Colorflex colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). L*a*b* values were measured by adding enough jam to cover the bottom of a 3 oz. glass sample cup and measured. L*a*b* were then converted to L*C*h using Chroma (C*) = $(a^2 + b^2)^{1/2}$ and hue angle (h) = arctangent (b/a). Chroma measures the intensity of a color, with a loss indicating the color became less vivid and darker, or a loss in intensity of redness in raspberry jam. The hue angles *h** were expressed on a 360° color wheel where 0° represents purplish-red, 90° yellow, 180° blue-green, and 270° blue.

Total Anthocyanin Content (TAC) was determined by the combined and modified methods of Pirie and Mullins (1976), and Mejia-Meza et al. (2010) on a BioTek Synergy 2 plate reader using 96-well black side with clear bottom plates (Corning Inc., Corning, NY). Acidified aqueous methanol (50% CH_3OH, 0.05% 12 M HCl, 49.95% ddH_2O) was used as the extraction solvent. Samples were extracted three times (150 μL of solvent each). The combined extracts of the jam were measured at an absorbance of 520 nm, in duplicate. Measured anthocyanin values were converted to mg cyanidin-3-glucoside equivalents per 100 g fresh weight using a molecular weight of 449.42 and a molar extinction coefficient of 26900/M/cm (Moyer et al., 2002) and a path length of 0.29 cm.

Antioxidant content was assessed using the Oxygen Radical Absorbance Capacity (ORAC) assay (Dávalos et al. 2004a, Freeman et al., 2011). Modifications to the Dávalos method by Freeman et al. were used for this analysis. Extractions were performed three times on each jam from different jars. Samples and standards were loaded into wells of Corning Costar 96-well black side clear bottom plates (Fisher Scientific) via a Precision Micropipettor (BioTek

Instrumetns, Inc., Winooski, VT). The outside wells on the 96-well plate were filled with blanks in order to minimize variation due to temperature. Samples were then read every minute for 120 min in a BioTek Synergy 2 fluorescence plate reader (BioTek Instruments, Inc.). ORAC values were expressed as Trolox equivalents per g (TE/g) of raspberry jam as well as per g of raspberry when appropriate.

Ascorbate content was determined using High Performance Liquid Chromatography according to Parker et al. (2010). Jam (0.1 mL) was diluted with 0.1 mL DTT solution (4.2 mM DTT in 0.1 K_2HPO_4, pH 7.0) and vortexed. Next, 0.1 mL of this mixture was mixed with 0.1 mL of 4.5% *m*-phosphoric acid, centrifuged, transferred to HPLC vial inserts and injected onto the HPLC. Absorption spectra were recorded from 190-600 nm for all peaks. Peak separation was accomplished with an Agilent Zorbax SB-Aq. 4.6x150 mm, 3.5 μm column.

Sensory Panel Design

A 58-person taste panel was contractually performed by the Brigham Young University Consumer Sensory Laboratory (Provo, UT). The panel included 30 males and 28 females with 11 members under age 20, 11 ages 20-29, 10 ages 30-39, 12 ages 40-49, and 12 ages 50-60, and 2 above 60 years. All panelists were found to like and consume raspberry jam.

Panelists were given pairs of jams that were identical in formulae but made on different dates, with one being freshly-made and one having been stored for three months There were eight total pairs of jam each panelist rated (see **Table 1** for all variations). Each panelist received two jams at a time. One jam sample was prepared in June and stored for three months in the dark at room temperature, and the second batch was made fresh in September only a few days prior to the sensory panel. To prevent sensory fatigue, sampling the eight pairs was accomplished

by each panelist over three separate sessions on consecutive days, three pairs twice and two pairs the last time. Sample order (fresh or stored jam) was rotated between pairs and the order of pairs received were randomized among all sessions.

Panelists sat in separate booths that had standard fluorescent lighting. Participants were first asked demographics questions: age category, gender, and attitudes toward raspberry jam.

Trays with samples were presented to the panelists via pass-through compartments on the counter in front of them. Each tray had two samples of room-temperature jam (30 g) in plastic containers labeled with 3-digit codes. The tray also had an unsalted saltine cracker, and a cup of room-temperature filtered water, and two spoons. In order to evaluate the jams, they answered a series of questions. They used a 9-point hedonic scale to evaluate color, overall acceptability, flavor, and texture of the samples, with 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely. They used a 5-point just-about-right test to evaluate thickness,, size of fruit pieces, and sweetness. A score of 3 was "just about right," a 5 was rated as definitely too much, and a 1 was definitely too little for that parameter.

When the panelist completed the questions for the two samples of jam in front of them, they returned the tray via the pass-through compartment, and a different set of jam was presented to them again and they answered the same questions for the next pair of samples. They received a maximum of three pairs of samples (six samples total) during a session.

Statistics

All data besides sensory data were collected in duplicate or triplicate. A full factorial design with four factors was used to collect the data. The factors were farm location (two levels: K, Berries by the Bay; J, Cornaby Farms), maximum heating temperature (two levels: 95 °C; 85

°C), sugar concentration (two levels: low-sugar; typical-sugar), and time (2 or 3 levels: fresh or three months stored; months June, July, and September). Original analytical and sensory data were analyzed using a normal linear model which considered the main effects, all two and three-way interactions, and the four way interaction for each factor in Statistical Analysis System Version 9.2® software (SAS Institute Inc., Cary, NC). Variable selection was conducted using an F-test, which tests for the significance of each variable. Insignificant predictor variables were eliminated from the model at the $\alpha = 0.05$ significance level. Tukey adjusted comparisons were then used to determine significant pair-wise differences between and within the remaining factors.

Results and Discussion

Formulation, Cooking, and Storage Effects on ORAC

Significant changes in ORAC were observed due to sugar concentration (**Figure 2**). Initial ORAC values for these jams were 18.9 and 23.4 for high and low-sugar formulations, respectively, and were significantly different ($p<0.0001$) from each other. The higher percentage of fruit in the low-sugar jam likely accounted for the differing initial ORAC values. Over the course of three months, high- and low-sugar jams significantly decreased in ORAC value by 29.8 and 27.5%, respectively. Low-sugar jams were found to have higher ORAC values per g jam initially, but after 1 month of storage, values were comparable to high-sugar jams. Low-sugar jam did not significantly change from months 1-3, with only a 1.3% loss in value, while high-sugar continued to decrease, resulting in a significantly lower value after three months compared to low-sugar jam.

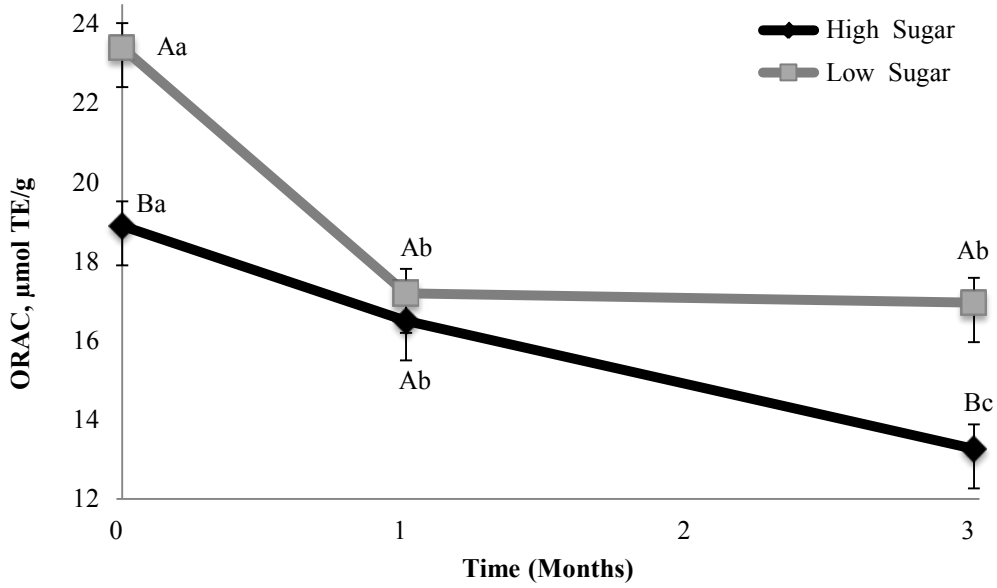

Figure 2. Change in ORAC over three months of storage by sugar concentration. Different capital letters within the same time and lowercase letters within the same sugar concentration indicate a significant difference ($p<0.05$). Data are expressed as mean values in µmol Trolox equivalents per g of jam ± SE with farm location and heating temperature included in the analysis.

These results agree with those reported by Zafrilla et al. (2011) who found significant decreases overall in phenolics in stored raspberry jams. When analyzing ORAC values in high-sugar and sugar-free blueberry jams, Howard et al. (2010) found significant decreases in the first 2 months of storage, consistent with the current study. Looking at low-sugar strawberry and cherry jams, Poiana et al. (2011) observed a loss in antioxidant activity of 4-7% after one month and 11-19% after three months of storage, which is overall less than that found in low-sugar raspberry jams in the current study.

There is a market demand for foods with reduced sugar for the perceived health benefits. The present study (see **Figure 2**), with the advantage of comparing low-sugar and high-sugar jams from the same raspberries, found that reducing the sugar reduces the nutrient preservation after 1 month. However, overall antioxidant capacity of low-sugar jam was significantly higher than high-sugar jam after 3 months storage ($p<.0001$).

There were no significant differences in ORAC values between the two heating temperatures (**Figure 3**). Significant changes were observed over storage time, however. Initial ORAC values for these jams were 21.4 and 20.9 µmol TE/g for high- and low-temperature jams, respectively. Both high- and low-temperature jams significantly decreased in ORAC values over time, with 27.3 and 29.8% overall losses, respectively, after three months.

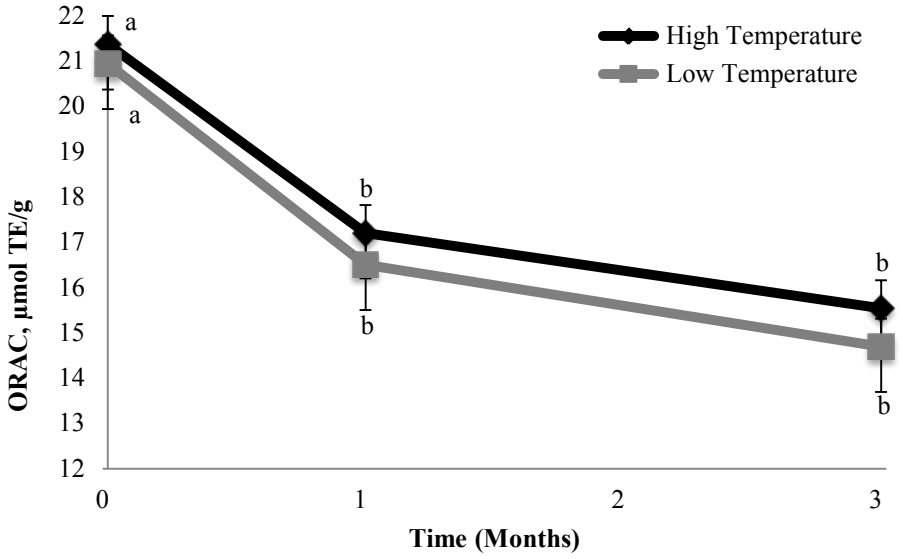

Figure 3. Change in ORAC over three months of storage by heating temperature. Different lowercase letters within the same processing temperature indicate statistical significance ($p<0.05$). Data are expressed as mean values in µmol Trolox equivalents per g of jam ± SE with farm location and sugar concentration included in the analysis.

The enzyme polyphenol oxidase (PPO) has been shown to play an important role in the enzymatic browning of fruits, though the enzyme is destroyed by heating, as occurs in jam processing. Chutintrasri and Noomhorm (2005) found that PPO is thermally inactivated, but the amount destroyed varies with temperature. When analyzing PPO in pineapples, they found that at 85°C, 7% of PPO remained after 5 minutes of processing. Garcia-Viguera et al. (1998) saw a loss in anthocyanin content in their raspberries because of the presence of polyphenol oxidase in raspberries, thus demonstrating that the results from the study by Chutintrasri and Noomhorm can be applied to the current study. In the current study, low-temperature jam was processed for only 2 minutes at 85 °C, making it highly likely that the low-temperature jams contained higher amounts of PPO. 90 °C was the highest temperature Chutintrasri and Noomhorm studied; they found only 1.2% of PPO remained after 5 minutes of processing. Though high-temperature jams in this study were processed at 95 °C, PPO was likely to have been completely inactivated. High-temperature jam processing may have more effectively inactivated PPO, though the ORAC difference between processing temperatures was not significant.

Total Anthocyanins in Jams

There were significant differences overall between farm location and processing temperature (data not shown) ($p<0.05$). Variations due to farm location, even though the same cultivar was selected, agree with other researchers (Ozgen et al., 2008). There was a significant decrease after three months of storage in all four farm*temperature combinations (**Figure 4**). Initial TAC of the raspberries was between 17.8 (farm J, LT processing) and 27.5 (farm K, LT processing) mg cyn-3-glu eq./100 g. Over storage time, all jams retained their initial

anthocyanins after the first month and significantly lost TAC between months 1-3 of storage time, with a 28.8% loss on average.

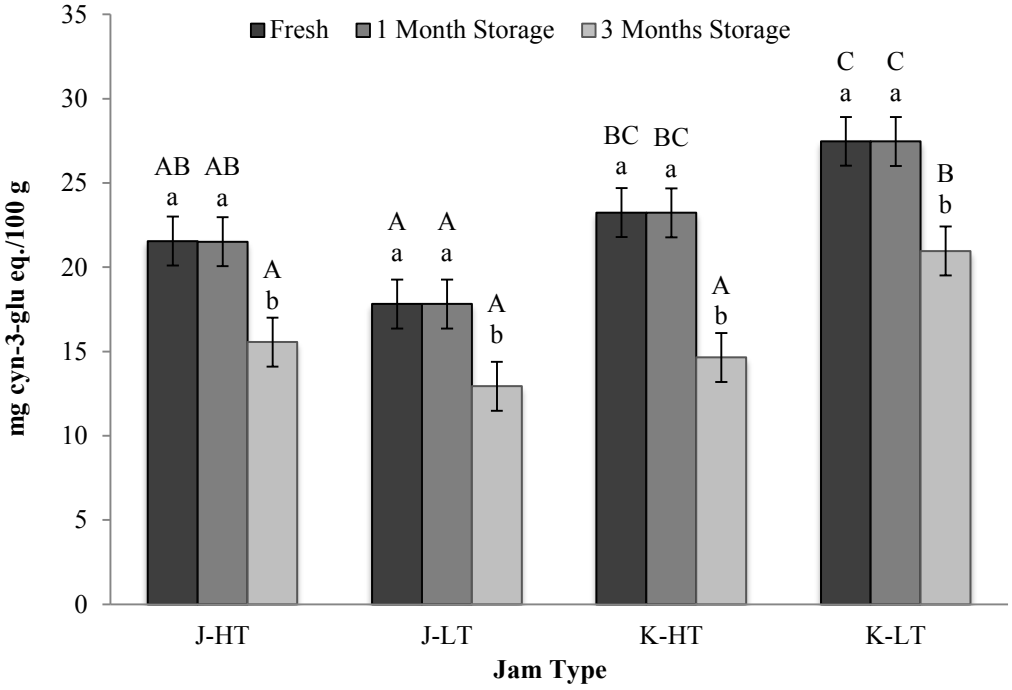

Figure 4. Change in Total Anthocyanin Content (TAC) in jams over three months of storage by farm and processing temperature. J = Cornaby Farms, K = Berries by the Bay; HT = High-temperature processed (95 °C) jam, LT = low-temperature processed (85 °C) jam. Different capital letters within the same time (same color bars) and lowercase letters within the same jam grouping indicate statistical significance ($p<0.05$). Data are expressed as mean values in TAC (mg cyn-3-glu eq./100 g jam) ± SE with sugar concentration included in the analysis.

TAC in this study was measured on the Caroline berry cultivar. Results from Kim and Padilla-Zakour (2004) showed total anthocyanins in fresh Prelude cultivar high-sugar raspberry jam to have 30.4 mg cyn-3-glu eq./100 g jam. They selected Prelude raspberries for their highly

colored skin and flesh, resulting in a higher anthocyanin content than found in the present study. Anthocyanin values for the raspberry jam in the current study were comparable to previous work in our laboratory (Snyder et al., 2012) using the Caroline cultivar. Jam cooking was not expected to cause appreciable losses in total phenolics of the fruit as was indicated by previous researchers (Kim and Padilla Zakour, 2004; Amakura et al., 2000). Results from Garcia-Viguera et al. (1998) showed a 66% loss in total anthocyanins during the first three months of storage at 20 °C in red raspberry jams made with Zeva and Heritage varieties. In a similar study of low-sugar strawberry, sweet and sour cherry jams (Poiana et al., 2011), the three jams stored over 3 months lost 21.6-33.1% monomeric anthocyanins, which is on average higher than losses in low-sugar jams in the current study, which ranged from 23.7-27.4%. Differences are expected with different fruit compositions and the structures in which the anthocyanins reside, which could provide protection or exposure to various anthocyanins (Hager et al., 2008).

Color Quality Alteration

Significant changes in L^* were observed due to sugar concentration (**Table 3**, $p<0.05$). All jams retained their initial values over the first month. Over months 1-3 of storage, high-sugar jams significantly decreased L^* values, indicating a darkening of the jam, and low-sugar jams significantly increased L^* values, indicating a lightening of the jam. The different concentrations of berries and sugar may have different effects on color over time. Garcia-Viguera et al. (1998) showed no significant changes in L^* color for 63°Brix raspberry jams stored at 20°C for three months, but there was a slight decrease in L^* color value over time, which is consistent with the decrease in L^* color values for high-sugar jams in this study.

Table 3. Significant color changes in L*, chroma (C*), and hue (h*) over three months of storage.[1]

	L*	
	HS	**LS**
Fresh	6.3Aa[2]	10.3Ba
1 Month	6.5Aa	10.0Ba
3 Months	5.3Ab	11.0Bb
SE	0.2	0.2

	Chroma			
	J-HT	**J-LT**	**K-HT**	**K-LT**
Fresh	25.6Aa	25.2Aa	25.8Aa	26.6Aa
1 Month	23.2Aa	23.4Aa	23.9ABab	26.2Ba
3 Months	19.4Ab	20.6ABb	23.1Bb	22.3Bb
SE	0.7	0.7	0.7	0.7

	Hue			
	J-HT	**J-LT**	**K-HT**	**K-LT**
Fresh	71.7Aa	70.7Aa	70.6Aa	73.1Aa
1 Month	69.7Aa	69.1Aab	67.9Ab	73.2Ba
3 Months	66.1Ab	66.6Ab	68.6ABab	70.4Bb
SE	0.5	0.5	0.5	0.5

[1] J = Cornaby Farms, K = Berries by the Bay; HS = High-sugar jam, LS = Low-sugar jam; HT = High-temperature processed (95 °C) jam, LT = low-temperature processed (85 °C) jam.

[2]Different capital letters within rows and lowercase letters within columns indicate significant differences ($p<0.05$).

Significant changes in chroma (*C**) color values differed by farm*processing temperature (**Table 3**). All jams retained their initial *C** color values over the first month, but during months 1-3 of storage, all jams significantly lost *C** color values, with a 16.7% average loss over storage time.

Significant changes in *h** color values differed by farm × processing temperature (**Table 3**). A loss in hue value with a loss in C* would also indicate a change in color towards a more

red-brown color. Three of the jam types retained their initial hue values over the first month aside from K-HT. During months 1-3 of storage, all jams beside K-HT significantly lost *h** values, with losses ranging from 2.8% to 7.8%. Berries from farm K retained their color better than berries from farm J.

Overall, the rate of color loss was slower and less pronounced than the rate of anthocyanin degradation, which agrees with results from other studies (Koca & Ustun, 2009; Poiana et al., 2011; Garcia-Viguera et al., 1999). Over storage time, the concentration of polymeric pigments in juices increase, which influences the juice color (Withy et al., 1993). Color differences during jam storage were likely to have been caused by the polymerization phenomena as well as losses in anthocyanin color compounds.

Although previous studies have reported that higher cooking temperatures resulted in increased brown pigmentation (Kim and Padilla-Zakour, 2004), the heating temperatures used in the current study didn't appear to generate overall color differences in fresh jam. When comparing all freshly-made jams over the statistical interaction between farm*temperature*time, no jam was significantly different from any other jam for L ($p>0.10$), Chroma ($p>0.89$), or hue ($p>0.07$). Thus, although these reactions likely occurred, they appear to have occurred in both high- and low-temperature jams in similar amounts and therefore processing temperature was not as important as other factors, such as increasing the overall fruit content in the jam.

Sensory Scores

There were statistically significant decreases in sensory hedonic scores when comparing the fresh and stored jams (**Figure 5**). No two- or three-way interactions were found to be significant, so all jam variations were combined into one graph. Color and overall acceptability

scores decreased by 4.1 and 4.3%, respectively. Flavor and texture scores decreased by 6.1 and 4.5%, respectively. These changes were statistically significant and may be of practical significance, though older jams were still acceptable to the consumer. These changes do imply an overall loss in likeability as the jam is stored, which correlates to storage degradation data presented above, including polymerization, Maillard browning reactions, and loss of anthocyanins and other nutrients, all of which alter the appearance, flavor, and texture of the jams.

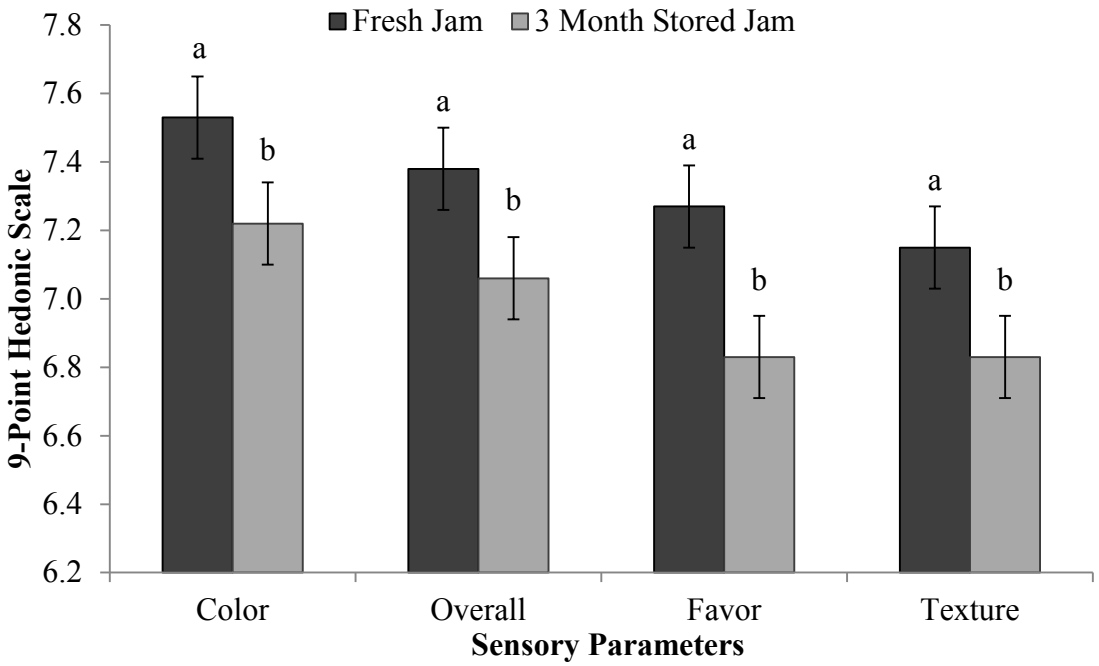

Figure 5. Comparison of composite sensory scores for fresh jam and jam stored for 3 months for overall score, flavor, color, and texture. Lowercase letters within the same sensory parameter that are different indicate statistical significance ($p<0.05$). Data are expressed as mean values on a 9-Point Hedonic Scale ± SE with sugar concentration data included in the analysis.

Many comparisons in fruit size and thickness levels were found to be statistically significant but were not of practical significance and are therefore not a concern for the purposes of this study. For example, although there was found to be a significant difference ($p < .0001$) in fruit size for the low-sugar/low-temp/farm K jam from fresh to stored, values were 3.35 and 2.71, respectively. For the same jam, thickness values were equally significant ($p<0.0001$) and had a similar change from fresh to stored, 3.86 to 2.94, respectively. This pattern was observed in all jam batches. It can be reasonably concluded that variations during preparing and cooking the jam would affect the perceived variations in thickness and fruit size, but are assumed to cause negligible differences in data. After accounting for jam batches, the panelists did not find any samples to be significantly different in sweetness acceptance.

Conclusions

Utah-grown Caroline raspberries processed into jam showed high levels of ORAC and TAC, demonstrating that many phenolic compounds and anthocyanins were retained during cooking. Values decreased over storage time, primarily between 1 and 3 months, and thus jam provides more nutrients when consumed fresh. However, nutrients can still be provided by jams that have been stored for three months. Sensory analysis demonstrated that fresh and stored jams could be differentiated but both were accepted by the consumer. This is the first report of these changes in Utah raspberry jam.

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Appendices

Appendix A: Extended Literature Review

Review of Literature

There is rising concern over health and food safety, which has led to research into the natural chemicals present in food and the effects they may have on the human body. Environmental conditions such as pollution, stress, or poor lifestyle choices cause increased oxidation within the body and create free radicals which can lead to degenerative diseases like cancer, heart disease, Alzheimer's, and Parkinson's Disease (Halliwell 1994). These age-related degenerative diseases have been linked with overwhelming levels of reactive oxygen species that exceed the normal defensive capacity of inherent antioxidant systems. Reactive oxygen species are free radicals that involve oxygen and include the hydroxyl radical, peroxyl radical, and superoxide radical anion and are physiologically generated during metabolic activity (Kim and Padilla-Zakour 2004).

The effects of reactive oxygen species have been shown in numerous epidemiological studies to be counteracted by the consumption of a diet rich in fruits, vegetables, legumes, and whole grains (Steinmetz 1991; Block and Patterson 1992), but the exact reason why these foods are beneficial is still uncertain (Lau et al. 2006). From the apparent epidemiological benefit of these foods, antioxidant supplements continue to grow in popularity. In a meta-analysis review (Bjelakovic et al. 2007) of 68 randomized trials with almost a quarter of a million participants analyzing the effects of antioxidant supplements on mortality, no studies were able to prove that dietary supplementation with synthetic antioxidants were significantly beneficial. Sometimes, the results showed that purified stand-alone antioxidants were actually harmful and resulted in increased mortality rates by at least 5%. These findings contradict the general claims that antioxidants, particularly single compound antioxidant supplements, improve health. In a study of male smokers, β-carotene supplements resulted in 18% higher lung cancer rates and an 8%

increase in total mortality (Albanes et al. 1996). The limited knowledge of antioxidant compounds has resulted in using solely a few known antioxidants (such as vitamins E, C or β-carotene in large doses), whereas a whole fruit or other food contains a wide range of antioxidants and other nutritive phytochemicals (Halvorsen et al. 2002).

Antioxidants may have far greater effects when consumed in combination with other antioxidants and nutrients (Hercberg et al. 1998), which is how they are found in a whole food, such as a berry. In one study (Lau et al. 2006), these positive effects were tested using a dietary supplementation of blueberries, spinach, or strawberries in rats. The study showed a significant reduction in neuroinflammation and oxidative stress and vulnerability to them, improved short-term memory and cognitive function, and improved motor function. Lau et al. postulated that the significantly improved motor and cognitive function and reduced cell decay that occurred in their study was due to a multiplicity of actions from the antioxidant-rich foods.

One meta-analysis found statistically significant protective effects from fruit and vegetable consumption in 128 of 156 dietary studies, and found that persons consuming high amounts of fruit and vegetables halved their risk of cancer compared to those with low intakes (Block and Patterson 1992). Steinmetz (1991) conducted a review on scientific literature using 206 human epidemiologic studies and 22 animal studies, and found results consistent with the meta-analysis by Block and Patterson. In summary, consumption of phytochemicals when eaten in a natural, whole food and not taken as a supplement appears to be the best way to increase health and longevity.

Different foods have varying profiles and concentrations of antioxidants (Halvorsen et al. 2002). There is more than a 1000-fold difference between total antioxidants in various dietary plants Berries have been shown to have some of the highest levels of polyphenols per g of fruit,

being one of the top sources of total phenolics and antioxidant capacity [oxygen radical absorbance capacity (ORAC)]. Of the approximately 150 dietary plants analyzed in one study cited by Halvorsen et al., berries were 8 of the 12 plants found to contain >5 mmol total antioxidants per 100 g wet weight. Berry fruits contain total antioxidant levels up to 4 times greater than other fruits, 10 times greater than vegetables, and 40 times greater than cereals (Halvorsen et al. 2002). Different berry species contain a wide variety of phenolic phytochemicals.

Raspberries provide a good source of antioxidants. They were found to contain an average of 3.44 mmol antioxidant per 100 g of wet berry from sources in Norway, Poland, and Holland (Halvorsen et al. 2002). When comparing the antioxidant capacity of seven common fruits and vegetables, raspberries had the highest overall antioxidant content (AOC) followed by strawberries, kiwi, broccoli, leek, and apple, with tomatoes having the lowest AOC (Beekwilder et al. 2005). The phenolic profile in red raspberries consists of 50% ellagitannins, 25% anthocyanins, and 20% vitamin C. Raspberry polyphenols are primarily anthocyanins and hydrolysable tannins, and they provide a rich source of cyanidin glycosides. Raspberries are also one of the main sources of ellagitannins, which are typically uncommon in plant foods (Rao and Snyder 2010). Ellagitannins hydrolyze into ellagic acid in the stomach and gut and are then converted to urolithin-A type metabolites by gut microbiota, which may persist in the colon through enterohepatic circulation (Sharma, 2010). Ellagitannins have been found to exhibit anti-tumor and anti-HIV activities as well as radical-scavenging activities. Ellagitannins are also more effective at inhibiting lipid peroxidation than any other type of tannin (Okuda et al, 1989). Consumption of them provides unique and potentially health promoting phytochemicals.

One problem with analyzing raspberries for their nutritional values is the wide variability in quantities of phytochemicals in a raspberry based on both growing conditions and cultivar. Hydrolyzed ellagic acid content from four cultivars from Spain ranged from 20.7 to 24.4 mg/100 g (de Ancos et al. 2000), whereas 17 cultivars grown in Finland contained anywhere from 38 to 118 mg/100 g, and wild raspberries in Finland had even higher values at 156.0 mg/100 g of raspberries (Rao and Snyder 2010). Such a large range of values clearly demonstrates that different cultivating techniques and berry cultivar change the fruit's phytochemical content. Wild berries likely have significantly higher values than domesticated cultivars because the use of pesticides reduces the plants' survival need to produce deterrents such as ellagitannins (Rao and Snyder 2010).

As a fruit ripens, the phenolic composition of berries changes. Tannin levels decrease as a raspberry matures, and anthocyanin concentrations sharply increase when the raspberry turns red and becomes fully ripe. Variations in harvesting time (spring, summer, or fall) also affect concentrations of various phytochemicals in raspberries. Of the four Spanish-grown cultivars previously discussed, the late harvest cultivars contained a greater amount and increased complexity of anthocyanins (de Ancos et al., 2000). For these reasons, specifically analyzing Utah-grown fall-harvested raspberries in our study is of great interest.

Raspberries are a healthy food choice, containing only 52 kcal per 100 g serving and high amounts of dietary fiber (6.5 g/100g). The seeds contain 97.8% unsaturated fatty acids, and provide essential fatty acids (USDA National Nutrient Database, 2008). The fatty acid composition of raspberry seed oil is 55% linoleic, 29% α-linolenic, and 12% oleic acid (Oomah et al. 2000). The seeds contain fat-soluble vitamins such as carotenoids and tocopherols, and the flesh contains high levels of vitamin C (26.2 mg/100 g). Raspberries and other berries are

traditionally used in desserts worldwide and are considered by many to be a desirable fruit to eat due to their attractive color and superior flavor (Amakura et al. 2000). Due to their extremely delicate nature and short shelf life, about 85% of all commercially-grown raspberries grown in the United Stated are processed in some way (Sinha, 2007). They are either frozen, turned into fruit puree, or processed into jam, with 40% of all commercially grown raspberries processed into jam.

Fortunately, short and long-term frozen storage of raspberries has been shown to have minimal effects on phytochemical composition (Rao and Snyder 2010; de Ancos et al. 2000b), and because freezing is typically the first step in most commercially-grown raspberries, it is valuable to know few nutrients are lost. Over short term periods of freezing, studies found no significant loss of phytochemical contents, and a significant increase in anthocyanins (Rao and Snyder 2010). This is most likely caused by to cell rupture, and when frozen and thawed prior to pressing, the fruit juice yield is increased, which then increases anthocyanin availability (de Ancos et al., 2000). Primocane raspberries frozen over a 3 month period showed no significant changes on Oxygen Radical Absorbance Capacity (ORAC) or total phenolic content (Freeman et al. 2011). Over long-term periods, samples frozen at -20°C for 12 months had no significant decreases in total phenolic content or antioxidant capacity, but the samples did lose 14-21% ellagic acid and 33-55% of its vitamin C over this storage period (de Ancos et al. 2000).

Because such a large amount of commercially-grown raspberries are processed into jam, studying their processing methods and changes in the fruit's composition is of great importance for those who produce and consume raspberry products. The explanation of the formation of jam is well-described by Downing (1996) and will be summarized in the following paragraphs. The

formation of jams and jellies requires the presence of four ingredients: pectin, sugar, acid and water.

Making a good gel for the jam primarily depends on the pH and the correct concentration of sugar and pectin (Downing 1996). Pectin serves as the gelling agent and transforms the fruit and sugar syrup into a more solid jam. Pectin undergoes a physical transformation as it dissolves and is heated, bonding with the sugar at the correct pH (made precise by the addition of an acid). Water is needed to dissolve the sugar, acid, and fruit, and disperse the pectin. In order to have consistent jam formation with starting ingredients that are typically inconsistent, such as the fruit, the jam batch is typically cooked to an established degree Brix (percent soluble solids). In this way, the concentration of sugar is known and can be consistent with each batch. Sugar concentration needs to be high enough (above 55%) to dehydrate the dissolved pectin molecules, precipitating them out of the jam solution.

The pH in each jam batch can vary significantly (Downing 1996). Fruits are acidic, but their levels of acidity will vary from type of fruit, cultivar, level of ripeness, harvesting season, etc. In order to maintain a consistent pH, the active acidity of the batch is measured, and an acid (typically citric acid) is added when needed in order to arrive at the desired pH, which is typically around 2.95-3.00. The pH needs to be low enough to reduce the negative charge on the pectin molecules, allowing them to hydrogen bond to adjacent pectin molecules, forming a lattice and trapping water and solutes in the pectin network. The low pH also inhibits the growth of anaerobic bacteria.

When processing jams, federal regulations require strict standards in order to minimize risk of microbial growth (Downing 1996). The containers need to be preheated prior to filling so that the maximum temperature difference between the container and the jam is less than 15.6°C.

Before filling, preserves go into a holding pan for cooling and checking. Filling temperature can be a problem in jams with fruit pieces because a hot temperature can result in floating fruit pieces. In these instances, producers fill at temperatures around 57°C, but then the containers need to be pasteurized after sealing at 90°C in order to bring the coldest part of the jam up to 82°C.

After the containers are filled they are sealed with a hermetic seal using steam vacuum sealing equipment (Downing 1996). They then undergo a holding period prior to cooling for three to five minutes in order to sufficiently sterilize the exposed surfaces in the headspace. The jams are then pasteurized by a hot water bath, hot water sprays, or steam at 91°C until the coldest spot in the container reaches 82°C. Cooling is then done as rapidly as possible to maximize color and flavor of the jam, which can be done by immersion cooling, and are finally labeled.

Jam differs from a jelly in that jams are typically less firm and use less pectin (Downing 1996). Jam contains fruit solids, unlike jelly, and the fibers from those solids contribute to the consistency of the jam. Two of the spreads processed at Cornaby Farms that will be used in this study are not actually "jam" by legal definition due to their significantly lower sugar concentrations. They are instead labeled as "spreadable fruit." These spreads are only 40° Brix, rather than 65°-68° Brix as are jams by federal definitions. The 25 calories per tablespoon spreadable fruits consists of 25% sugar, whereas jams are typically about 48% or more sugar.

Alternatives to standard jam include low-sugar and all-fruit jams. Low sugar jams are made using low-methoxy (LM) pectin, which do not require sugar for gel formation and are not as sensitive to pH as is high-methoxy pectin, which is commonly used in jam processing. LM pectin gels work only in the presence of a polyvalent ion, such as calcium, which can either be

found naturally in the fruit or is added to the jam (such as a calcium salt). LM pectin can produce a jam with fewer calories and either less sugar or no added sugar (Downing 1996).

The processing of fruit into jam has a significant effect on the anthocyanin and color stability of raspberries (Garcia-Viguera et al. 1998). The temperature and time of processing have been found to greatly affect anthocyanin stability, and browning reactions occur in the raspberry products during storage. Other factors that affect the stability of the jam include pH and acidity, phenolic compounds present in the jam, sugars and sugar degradation products, oxygen concentration, ascorbic acid, and fruit maturity. Not only are these factors important to maximize the nutritive value of the jam, but flavor and color loss will likely reduce consumer acceptance of the product.

Different varieties of raspberries have had significantly different reductions in anthocyanin content per g of fresh fruit (Garcia-Viguera et al. 1999). The anthocyanin content of the Heritage raspberry cultivar was reduced by 17-24% during processing, whereas the anthocyanin content of the Zeva raspberry cultivar was reduced by 37-40%. When processing jam using frozen raspberries, the anthocyanin content was 9-24% lower than in jams made using fresh raspberries. This is believed to be caused by the disruption of the cell structures by ice crystals, and during thawing the oxidative enzymes and vacuolar substrates (phenolics) interact, causing a loss of phenolic pigments due to this enzymatic process. This effect may vary based on speed of freezing and strength of the drupelets by cultivar.

In a study on the effects of jam processing (Kim and Padilla-Zakour; 2004), total phenolic levels of raspberry jam did not significantly change in concentration after processing when compared with cherries and plums. During food processing, a lower pH was found to give better thermal stability, but because jam processing requires a very narrow pH range (Mok and

Hettiarachy; 1991), pH optimization is not a changeable variable in jam-making. Because jams have a low pH (2.95-3.20), the pH provides some protection during processing (Kim and Padilla-Zakour. 2004).

Storage also affects the anthocyanin content and color stability of the jam. Variables during storage include pH of the jam, light, oxygen, metal ions, enzymes, sugars, temperature, and time (Kim and Padilla-Zakour. 2004). One study (Garcia-Viguera et al. 1998) found increased storage temperature to be the primary cause for anthocyanin degradation and loss over time. The greatest loss in pigment composition at warmer storage temperatures (30 and 37°C) occurred during the first month, while at cooler temperatures (20°C), the greatest pigment loss occurred over the first three months. This same study also found that over storage time, jam prepared with frozen fruit degraded similarly (no significant difference) to that made with fresh fruit. Flavonol composition of fresh berries was not significantly reduced during processing. The rate of color loss in jam was found to be much slower than the rate of anthocyanin degradation. Garcia-Viguera et al. postulated that the decrease in color that occurred during storage was most likely due to polymerization phenomena, which occurs in wines and juices. Enzymatic browning is not a likely cause of jam discoloration because those enzymes are inactivated during processing (Kim and Padilla-Zakour. 2004). Brown pigment formation may also occur because of the formation of chalcones (Kim and Padilla-Zakour. 2004). High temperatures used during jam processing drive the equilibrium reaction between chalcone and anthocyanins toward chalcones, increasing their concentration. Anthocyanins were found to be more thermally stable at the low pH of jams (2.95 to 3.20) when compared to pH 5.0 (Mok and Hettiarachchy 1991).

Appendix A References

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Appendix B: Detailed Materials and Methods

Study Design

Two berry farms in separate locations in the state of Utah were selected (**Table 1 in manuscript**). Cornaby Farms, located in Salem, Utah, and Berries by the Bay, located in Willard, Utah, are over one hundred miles apart. Despite a similar Utah climate, the two locations differ topographically, meteorologically, and in soil conditions. Willard, Utah, is just east of the northeastern-most edge of the Great Salt Lake and west of the Wasatch Mountain Range and Cache National Forest. Micrometeorology variations in Willard, Utah, would be impacted by lake effect precipitation, the increase in salt in the surrounding air, and orographic uplift caused by the farm's proximity to a large mountain range, which would help the area receive more rainfall. Salem, Utah, is located in a valley about 10 miles southeast of Utah Lake with lower-elevation mountains cupping the eastern side of the valley. For these topographical and meteorological variations, these two farms were selected and deemed appropriate representations of berry farms in Utah.

Two sugar concentrations were used in order to compare a high fruit jam, which would potentially contain a higher amount of antioxidants and other nutrients per g, to a high-sugar jam, which would potentially be better preserved due to the reduced water activity levels. Boiling and a reduced temperature (85 °C) were used for maximum processing temperatures. In addition, there is a trend in the market toward foods with reduced sugar for their health benefits; the concern is that with reduced sugar, the jam is not preserved as effectively and therefore may have reduced nutrients over storage time.

Variations between the processing methods will only include processing temperature and sugar content. Processing and holding time for both processing temperatures and sugar concentrations were the same. In summary, there are two variables for berry source, sugar

concentration, and processing temperature, which result in eight different variations of jam, as can be seen in **Table 1 in manuscript**.

Berry Collection

Same as manuscript. See page 7.

Jam Ingredients

Cane sugar was purchased at a local grocery store. (see **Table 2 in manuscript**). Food-Grade citric acid was provided by Jungbunzlauer Suisse AG (Neuton Centre, MA). The pectin used for the high-sugar batches of jam was TIC Pretested® HM Rapid Set Pectin (White Marsh, MD). The low-sugar jam batches used a proprietary mixture of low-methoxy pectin and gels used by Cornaby Farms for their low-sugar jams. Low-methoxy pectin is used because a high-sugar content is not needed to form a gel as is required for high-methoxy pectin.

Pectin serves as the gelling agent and transforms the fruit and sugar syrup into a gel. Pectin undergoes a physical transformation as it dissolves and is heated, bonding with the sugar at the correct pH. For high-methoxy pectin, sugar concentration needs to be high enough to dehydrate the dissolved pectin molecules, precipitating them out of the jam solution. Low sugar jams were made using low-methoxy (LM) pectin as well as other proprietary gelling agents, which do not require sugar for gel formation (Downing 1996). LM pectin gels require a polyvalent ion, (calcium was used), which was premixed in the low-methoxy pectin.

The low-sugar jam formula also contained water and a small amount of white grape juice concentrate (WGJ, Tree Top Inc., Selah, WA) in place of sugar. The water was added for textural purposes and is typically used in low-sugar raspberry jam (Janet Stocks, Cornaby Farms); without it, the berry concentration is too high and the jam is too seedy for the consumer.

White grape juice concentrate is also a typical addition to low-sugar jams to increase the sweetness without additional sugar.

An antifoaming agent (DOW Food Grade Antifoam, Pacific Pectin, Inc., Oakhurst, CA) was used to minimize foaming that occurs during the cooking and boiling of both jams. This was a minimally used ingredient and is typically used in industry for jam production.

Jam Formulae

Same as manuscript. See page 8.

Jam Processing

Upon receipt, berries were kept frozen (-20 °C) in their original containers, then defrosted prior to jam production. All batches were processed at the Brigham Young University pilot plant. Ingredients were pre-weighed and kept separate. Both low-methoxy and high-methoxy pectins were premixed with a portion of the sugar to properly disperse the pectin.

Immediately before jam processing, jars and lids were sterilized in a steam box (Brigham Young University Machine Shop) to match the temperature of the jam.

Industry-standard processing methods (Downing, 1996) were followed as closely as possible, but were modified for the facilities available at Brigham Young University. Pre-weighed and freshly-thawed raspberries were then placed into an unheated steam kettle and mashed by hand until no whole berries were visible. Two drops of antifoaming agent was added to the berries. Pre-mixed sugar and pectin were slowly added with continuous stirring and mashing. This method was identical for all jam variations.

Specifically for the low-sugar jams, pre-weighed white grape juice concentrate and water were added to the mixture at this time in place of sugar and mixed.

After all ingredients but citric acid were added, the steam kettle was turned on and the mixture was heated to desired temperature as quickly as possible. During heating, the mixture was constantly stirred. A thermometer was held in the center of the steam kettle and monitored. For the low-temperature jam batches, the heating power of the steam kettle was lowered when the mixture reached approximately 10° below the desired temperature because the temperature continued to rise without additional heat. This was likely caused by a delay in the thermometer as well as a slight delay in heat transfer from the edges of the kettle to the center of the kettle.

When the batch reached approximately 74 °C, citric acid added while stirring. Heating of the jam continued until the jam reach the desired temperature (boiling for high-sugar and 85°C for the low-temperature jam), and the jam was held at that temperature for two minutes. A small sample of the jam was taken out to test the jam's pH, sugar concentration and thickness. The jam was placed in a sample cup and rapidly cooled in a bowl of ice water. The pH range needed to be within 2.9-3.1 and was measured using a Denver Instruments UltraBASIC pH meter. °Brix was analyzed using a Reichert AR200 Refractometer. For low-sugar jams and high-sugar jams, the °Brix needed to be approximately 40-42° and 65-68°, respectively. Consistency was analyzed by human measurement using a spoon and stirring the jam. All batches were found to be satisfactory for pH and °Brix without needing to add more sugar or citric acid than what had already been pre-weighed and added to the batch.

After the jam was sufficiently cooked, each jar was filled with jam with a ¼" headspace. Immediately after pouring, lids were placed on the jars and tightened by hand and inverted for 30-45 seconds in order to sterilize the headspace. The jars were allowed to cool, and each jar was

labeled and placed in a dark cabinet for storage. All samples were stored for three months and analyzed periodically during storage.

Post-processing

Same as manuscript. See page 11.

Sensory Panel Design

A 52 person taste panel was contractually performed by the BYU sensory laboratory. The panel included 30 males and 28 females with 11 members under age 20, 11 ages 20-29, 10 ages 30-39, 12 ages 40-49, and 12 above 50. The purpose of the sensory panel was to determine if consumers could see or taste a difference in the jam as it aged. The goal was to compare a panelist's opinion about flavor and color changes between the various types of jam. High-sugar jam has a lower water activity level and is better preserved than low-sugar jam, so comparing a panelist's opinion about each batch of jam can compare flavor and color changes between the various types of jams. The challenge was to structure the panel in such a way to compare change based on age of the jam specifically, both in how to accomplish this with the jam and how to obtain these answers from the panelists without telling them that they were analyzing jam based on the age of the jam.

Panelists were given pairs of jam that were identical in batch formula and processing method, but one jam sample was prepared in June and stored for three months in the dark at room temperature, and the second batch was made fresh in September only a few days prior to the sensory panel. There were eight pairs of jam the panelist rated, and this was done in three separate sessions in order to prevent sensory fatigue. Panelists were presented with a set of two

samples of jam at a time on a tray with a cracker and a cup of water. They answered a series of questions, and when they completed that round, they returned the tray, and a different set of jam was presented to them again. They received a maximum of three pairs of samples during a session. Because of the work involved, panelists were paid $10 upon completion of all samples of jam.

Participants of the sensory evaluation panel were first asked demographic-based questions in order to determine age category, gender, attitudes toward raspberry jam. All panelists were found to like raspberry jam. They used a 9-point acceptance test to evaluate color, overall acceptability, flavor, and texture of the samples, with 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely. They used a 5-point just-about-right test to evaluate thickness, size of fruit pieces, and sweetness. A score of 3 was "just about right," a 5 was rated as definitely too much, and a 1 was definitely too little for that parameter. For each pair, the jams were ranked in order of preference.

Sweetness, fruit piece size, thickness, and texture were measured in order to ensure the jams were similar to the panelist. Finding differences in fruit sizes, for example, would signify that the berries were not broken down to equal sizes. Age-related differences included color, overall acceptability, and flavor. Finding statistically significant changes in the score from the three-month old jam to the freshly-processed would show a change based on storage time.

The color acceptance scores obtained from the sensory panel can be correlated to color values obtained from the colorimeter. For example, a decrease in redness colorimeter value may correlate with decreased color acceptance for a batch of jam.

Appendix B References

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Appendix C: Additional Tables and Figures

Table A-1. Just-About-Right[1] sensory analysis for thickness and fruit size[2].

Farm	Sugar	Temp.	**JAR Question**	**Fresh**	**Stored**	P
J	**HS**	**HT**	Thickness	2.80[3]	3.15	*
			Fruit Size	2.76	3.17	*
J	**HS**	**LT**	Thickness	2.86	2.46	*
			Fruit Size	2.88	2.74	
J	**LS**	**HT**	Thickness	3.38	3.08	*
			Fruit Size	3.28	2.96	*
J	**LS**	**LT**	Thickness	2.83	3.02	
			Fruit Size	2.87	3.04	
K	**HS**	**HT**	Thickness	2.92	3.58	*
			Fruit Size	2.83[#]	3.40[#]	*
K	**HS**	**LT**	Thickness	3.12	2.35	*
			Fruit Size	2.94	2.65	
K	**LS**	**HT**	Thickness	2.78	3.86	*
			Fruit Size	2.65	3.47	*
K	**LS**	**LT**	Thickness	3.86	2.94	*
			Fruit Size	3.35	2.71	*

[1]A score of 3 is the ideal value; 1 is definitely too little, 5 is definitely too much.

[2] Sweetness was not significant and not included in the table.

[3]Standard error for all values were between 0.14 and 0.15 except for K-HS-HT Fruit Size (SE=0.58, marked by # symbol).

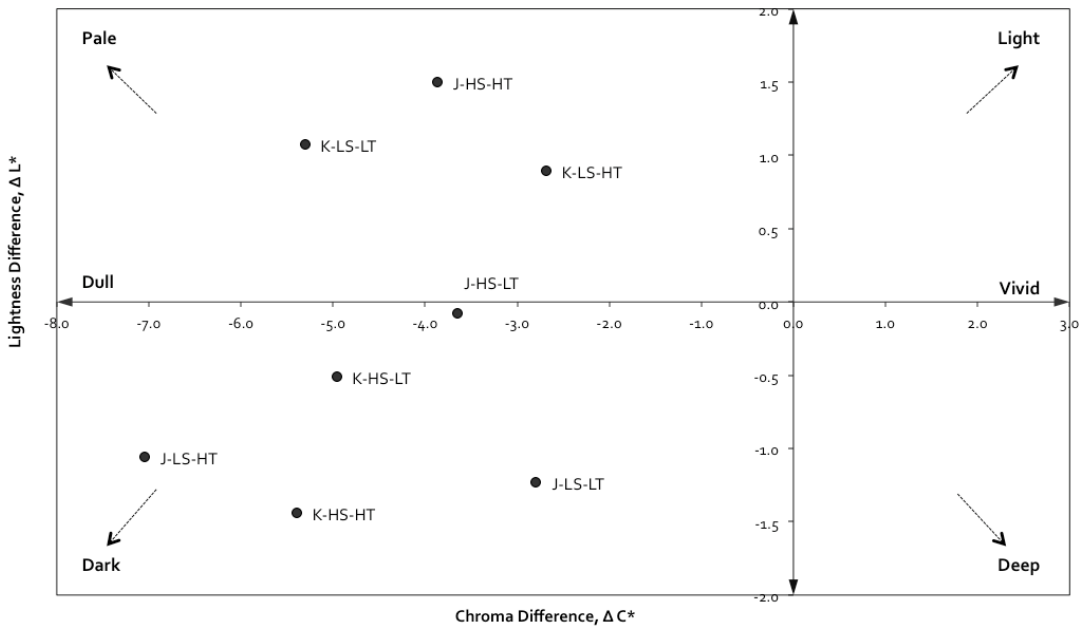

Figure A-1. Difference in value for both L* and C* from time 0 to time 3 months of storage. J = Cornaby Farms, K = Berries by the Bay; HS = High-sugar jam, LS = Low-sugar jam; HT = High-temperature processed (boiling) jam, LT = low-temperature processed (85°C) jam. All C* and L* values significantly changed for all jams over this time. Data are expressed as mean values for both L* and C*.

Color Quality Alteration of C* and L*

Figure 1 describes changes in chroma and lightness over the three-month storage period for each jam. Jams that increased in lightness values but lost chroma values are described as becoming more pale and dull; jams that decreased in both lightness and chroma are described as becoming more dark and dull (Konica Minolta Sensing 2007). Few consistent conclusions can be drawn from this regarding individual types of jams, but all jams became more dull. Jams had no apparent consistency in their likelihood of becoming more pale or dark and no further conclusions can be made.

ORAC Extraction and ORAC Protocol for Raspberry Jam

Modified from Dávalos et al., 2004a; Wu et al., 2004

Updated June 2012

Reagents for ORAC Extraction:

1. Raspberry Jam
2. AWA—acetone:ddH_2O:acetic acid 70:29.5:0.5

Reagents for ORAC Protocol:

Ensure blanks, standards and samples are all prepared in AWA.

1. 75 mM phosphate buffer (PB), pH 7

   Add 3.7152 g KH_2PO_4 and 10.8637 g K_2HPO_4 and make to ~1 L with ddH_2O, check pH

   This buffer needs no refrigeration and will last long-term.

2. 0.4 mM Trolox (250.29 g/mol) initial [ ]

   Add 0.010 g Trolox to 100 mL 70:29.5:0.5 AWA in a dark container. Scrape off the top layer and take Trolox from a lower layer to ensure a higher quality. Dissolve by swirling or by using a stir bar. To make 80 µM Trolox: Add 1 mL Trolox solution and fill to 5 mL with 70:29.5:0.5 AWA. Store at –80°C (stable for 1 month). Use the following directions to make each concentration to be used in the standard curve.

   1- Make up samples (~10) of 80 µL (60 µL is used for each plate + 20 µL extra) of each of the following:

      a. 80 µM Trolox final [ ]

      b. 40 µM Trolox final [ ]

c. 20 μM Trolox final [ ]

d. 10 μM Trolox final [ ]

3. 70.3 nM Fluorescein (FL) (376.28 g/mol) final [ ]

   Add 0.0225 g FL to 50 mL dark flask and fill with PB. Dilute 24.5 μL of concentrated solution to 250 mL with PB, avoiding light exposure. Aliquot to 16 ml samples and store at –20°C (small freezer)-stable for 1 month.

4. 12 mM AAPH (271.17 g/mol) final [ ]

   Add 0.1085 g AAPH to 10 mL warm (44˚C) PB in 10mL flask, but do not add PB until immediately before each run to ensure freshness. Store at room temp. To be made fresh daily.

5. Acetone:ddH_2O:Acetic acid—70:29.5:0.5 (AWS)

Notes:

1. When no dark containers are available, wrap volumetric flasks in aluminum foil. Minimize light use.
2. The solutions should experience as few freeze-thaw periods as possible. Freeze-thaw cycles affect the antioxidant capacity of some compounds. Ideally the samples should only be frozen once. Be sure to allow enough time to make and properly dilute the solutions so they are not frozen more than is necessary. In addition, avoid excess light exposure.

Sample Extraction

1. Set the sonicator water bath temp to 37° C.

2. Extract the mixture by weighing out roughly 0.40 g of the fruit mixture into labeled Eppendorf tubes. For a guideline, weights should be within 0.01 g of the target weight. Write down all weights.
3. Add 300 µl of AWA to the weighed fruit mixture, cap, vortex for 30 seconds, sonicate at 37 °C for 5 degs at 60 sonics/minute, centrifuge at 20000 x g for 2 min, and pipette the supernatant to a new, labeled set of Eppendorf tubes. These new tubes will contain the final extraction, so label them accordingly.
4. Repeat step 3 two additional times, adding the supernatants to the same tube as the original supernatant. After the third addition of AWA and subsequent vortexing and sonicating, centrifuge at 20000 x g for 2 min.
5. In the final extraction tube there will be approximately 900 µl of AWA plus the liquid initially in the fruit mixture. If you will be running your ORAC plate later, store samples in freezer ASAP.

Autopipettor Setup

1. The two autopipetting protocols used are fluorescein.PGM and aaph-fast.PGM. They are found in the default folder. Have both open and ready to load. To do so:
    a. Open “Precision Power V2” application on the desktop.
    b. Once loaded, click “Open”.
    c. Open the file named “fluorescein.PGM”
    d. Click “Prepare First Run”. The program will ask from which column the pipette tips should be taken. Select the column of choice and click “Okay”. Do not click “Start” yet.

e. Follow the same guidelines for opening the file "aaph-fast.PGM." Note: Multiple files can be open at a time, but only one file can be loaded at any given time. Once finished with the fluorescein file, click "Unload Program" to load the aaph-fast file.

Procedure

1. Turn on hot water bath and set temperature to 45°C (allows for some cooling during transport and while wells are filled). It may be useful to initially fill the hot water bath with warm sink water to speed up the warming process.
2. Thaw fluorescein while heating phosphate buffer (to add to AAPH just before run begins) together in hot water bath.
3. Remove sample extractions and Trolox (80 μM) from freezer to thaw.
4. Create a plate layout/excel document for data analysis using the template file D:\Documents and Settings\All Users\Documents\ORAC Template\ ORAC Template 11-19-2010.xlsx
    - Each sample will require three wells, as shown in the plate layout below.
5. Preheat BioTek Synergy 2 (BioTek Inc., VT) fluorometer to 37 °C for ~30 min before beginning analysis.
6. Turn on the tungsten lamp. To do so:
    a. Turn on the *BioTek Synergy 2* machine.
    b. Open *Gen 5* application on desktop.
    c. Select your experiment from the list.

d. On the top bar of the Gen 5 software interface, click on the image with the temperature reading
e. Under the "Pre-Heating" tab, make sure the requested temperature is set to 37°C, then check the "On" box.
f. Under the "Tungsten Lamp" tab, click "Turn Lamp On".

7. Protocol for *Gen 5* (this should already be set): FL 96 well plate (black plates with see through wells on bottom- NOT the clear plates). PRT, emission wavelength = 528/20, excitation wavelength = 485/20, shake plate for 3s at medium intensity before each reading, read every minute for 2 hrs (reading may be stopped earlier, when points are flat along the bottom, though be consistent across runs). Temperature set at 37 °C. Sensitivity set at 60.
8. Print off a copy of your plate layout or organize your samples/Trolox so that you can pipette them into the correct wells.
9. Wash any needed instruments.
10. Pre-label all Eppendorf tubes.
11. Vortex thawed sample extractions and Trolox. Centrifuge at 10000 x g for 30 sec.
12. Prepare Trolox standards first. Dilutions of 80, 40, 20, &10 µM make for a good standard curve.
13. Perform a single dilution from the original extracted sample of 1:100, 1:150, and 1:200 from original extraction.
14. Once the correct sample dilution(s) has(have) been determined and created, add 20 µl of each of your four Trolox dilutions (80, 40, 20, and 10 µM) to the correct wells on your

plate. Then add 20 µl each of your samples to the corresponding wells indicated on your plate layout.

15. Place the plate on the Autopipettor machine.
16. Remove the thawed fluorescein from the hot water bath and pour into the trough placed in B-3. Remove lid covering pipette tips.
17. Click "Start" on the *Precision Power V2* "Fluorescein" program. It should already be loaded and ready to go. This will add 120 µL of fluorescein to the 20 µl of diluted sample extraction/ Trolox already in the plate.
18. Place the prepared plate into the fluorometer for 15 minutes to warm the wells up to 37 °C. During the 15 minutes:
    - Weigh out AAPH pellets (.1085g) and place in 10 mL flask using a glass funnel.
    - Prepare autopipettor for AAPH pipetting:
        - Use the "aaph-fast" protocol in the "Precision Power" software, which will fill each well with 60uL AAPH solution.
        - Click "Prepare First Run", select row of tips to be used, click "Okay", and then click "Start"
        - Click "Pause" as pipettor (with tips on) moves toward empty trough B-4
19. After the 15 minutes have elapsed, the 10 mL flask with AAPH pellets must be filled to exactly 10 mL with warmed phosphate buffer. Mix thoroughly by shaking and inverting with cap on.
    - WARNING: avoid contact between skin and AAPH, as it is carcinogenic.
20. Eject warm plate from plate reader and place into autopipettor, closing the ejected plate reader tray upon removal of plate so as to maintain temperature within

21. Pour AAPH into B-4 reagent trough of the autopipettor (see autopipettor layout), making sure the well has been washed and dried. Resume pipetting in Precision Power. The autopipettor takes roughly one minute to fill the wells.
22. While AAPH is being added to plate:
    - Create new plate in Gen5 and name it appropriately
    - Click “Plate”, “Add Plates…” Now, in the “Plate” drop down menu, click “Read”, then click the “READ” button. This will not start the plate reading, but will eject the empty plate tray and alert you that the machine is ready. DO NOT CLICK “OK” until the AAPH is in the plate and the plate is placed on the plate tray of the fluorometer.
    - Because AAPH begins creating radicals instantly, as soon and as quickly as possible transfer the plate to the fluorometer and begin the run by pressing “ok” on the prompt spoken of in the previous step. You want to wait until it is done pipetting because if the plate reader is left open it will cool off and will have to heat back up for a few minutes before it will start reading, which will throw off your data.
23. Set timer for 1 hour (when you will start this cycle again)
24. Analyze previous plate
25. Clean dishes

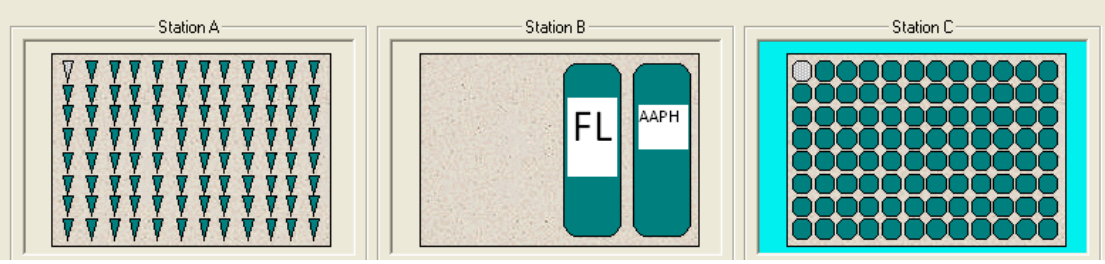

Figure A-2. Depiction of the layout for the autopipettor. Station A (left) contains clean tips from which the autopipettor will take and use tips; station B (middle) depicts the proper location of the troughs containing fluorescein (FL) and AAPH; station C (right) depicts the 96-well plate containing samples of the extracts and into which the autopipettor will place fluorescein and AAPH.

Set up as follows in a plate layout Excel spreadsheet. Having the plate layout in Excel format will be important in the data analysis steps to follow.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		10uM	20uM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	BlankB	
C		10uM	20uM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	BlankC	
D		10uM	20uM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	BlankD	
E		40uM	80uM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	BlankE	
F		40uM	80uM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	BlankF	
G		40uM	80uM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	BlankG	
H												

Figure A-3. 96-well plate layout. The outer row, filled with blank solution, is depicted by the black border. Inside the border, every two columns represents different concentrations of either Trolox standard (first two columns) or different samples. The first three rows of the first column being filled with 10 µM solution and the second three rows with 40 µM solution. The first three rows of the second column is filled with 20 µM solution and the second three rows is filled with 80 µM solution. This pattern continues until column 11 which is filled with blanks.

References

1. Dávalos A, Gomez-Cordoves C, Bartolome B. 2004a. Extending applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. J Agric Food Chem. 52:48-54.

2. Wu, X, Gu L, Holden J, Haytowitz DB, Gebhardt SE, Beecher G, Prior RL. 2004. Development of a database for total antioxidant capacity in foods: a preliminary study. J Food Compos Anal 17:407-422.

Total Anthocyanin Content Protocol for Raspberry Jam

Modified from Giusti MM, Wrolstad RE. 2001.

Current Protocols in Food Analytical Chemistry

Updated June 2012

Reagents

0.1 N HCl in methanol

To make 10mL: 5000.0 μL CH_3OH, 5.0 μL HCl, 4995.0 μL dH_2O

Sample Preparation

1. Obtain six varieties of raspberries.
2. Label Eppendorf tubes> 6 varieties > 3 sample types (juice, pulp, seed) > 6 tubes per sample type per variety (108 total tubes)

Determination of Total Anthocyanin Capacity (TAC)

Open Gen 5 > experiment > Anthocyanin.prt

Jam

1. Transfer .2 g jam into a fresh Eppendorf tube
2. Add 150 μL 0.1 N HCl in methanol
3. Vortex until mixed
4. Sonicate for 5 minutes
5. Vortex until mixed

6. Centrifuge for 2.0 minutes at 10 000 x *g*
7. Decant supernatant into another fresh Eppendorf tube
8. Repeat steps (5-10)
9. Repeat steps (5-10) again, except increase centrifuge speed to 20 000 x *g*
10. Vortex supernatant mixture, then pipette 100 μL of supernatant into black 96-well plate in duplicate

Update wavelength read value (520) in Gen 5 program.

After all wells are occupied with sample, place plate into BioTek reader and push the read button.

Save data in appropriate file before exiting Gen 5 program

Calculating TAC

Measured anthocyanin values were converted to mg cyanidin-3-glucoside equivalents per 100 g fresh weight using a molecular weight of 449.42 and a molar extinction coefficient of 26900/M/cm (Moyer et al., 2002) and a path length of 0.29 cm.

References

1. Giusti MM, Wrolstad RE. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Current protocols in food analytical chemistry; Wrolstad, RE, Ed, New York: John Wiley & Sons.

Sensory Panel Sample Ballot

Welcome to the Food Science Sensory Laboratory. A copy of the form titled "Consent to Be a Research Subject" is posted in each booth. Please read it carefully before continuing. By signing your name below, you acknowledge that you have read and understand the consent form, and desire of your own free will and volition to participate in this study. You may withdraw at any time without penalty. Please inform the receptionist if you wish to withdraw.

Name_____ Signature_____

In this session, you will evaluate eight samples of raspberry jam in pairs of two, side by side. Please read all instructions and questions carefully. Before you receive your samples, please answer these questions by checking the appropriate circles.

* What is your age category?

  - O Under 20 years
  - O 20 – 29 years
  - O 30 – 39 years
  - O 40 – 49 years
  - O 50 – 60 years
  - O Over 60 years

* What is your gender?
  - O Female
  - O Male

* What is your attitude about raspberry jam?
  - O I like it
  - O I neither like nor dislike it
  - O I dislike it

Press the green READY light to call for your samples. If at any time during the test you need help, press the button by the HELP light to the right of the screen.

Turn the page to continue

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

DO NOT taste the samples yet.

* How much do you like or dislike the **COLOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Now taste the samples from left to right as they are arranged for you on the tray. Take a sip of water and take a bite of cracker between samples to refresh your sense of taste.

* What is your FIRST IMPRESSION of the **OVERALL ACCEPTABILITY** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Turn the page to continue.

* How much do you like or dislike the **FLAVOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How much do you like or dislike the **TEXTURE** of each sample?

Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How do you feel about the **THICKNESS/THINNESS** of each sample?

Definitely too thick	O	O
Slightly too thick	O	O
Just about right	O	O
Slightly too thin	O	O
Definitely too thin	O	O

Turn the page to continue

* How do you feel about the **SIZE OF FRUIT PIECES** in each sample?

	Sample Number (please fill in)	
	_____	_____
Definitely too large	O	O
Slightly too large	O	O
Just about right	O	O
Slightly too small	O	O
Definitely too small	O	O

* How do you feel about the **SWEETNESS** of each sample?

Definitely too sweet	O	O
Slightly too sweet	O	O
Just about right	O	O
Slightly too tart	O	O
Definitely too tart	O	O

When you are done with the samples, please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "NEXT" LIGHT**. Wait for the next pair of samples to arrive.

Turn the page to continue

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

DO NOT taste the samples yet.

* How much do you like or dislike the **COLOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Now taste the samples from left to right as they are arranged for you on the tray. Take a sip of water and take a bite of cracker between samples to refresh your sense of taste.

* What is your FIRST IMPRESSION of the **OVERALL ACCEPTABILITY** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Turn the page to continue.

* How much do you like or dislike the **FLAVOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How much do you like or dislike the **TEXTURE** of each sample?

Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How do you feel about the **THICKNESS/THINNESS** of each sample?

Definitely too thick	O	O
Slightly too thick	O	O
Just about right	O	O
Slightly too thin	O	O
Definitely too thin	O	O

Turn the page to continue

* How do you feel about the **SIZE OF FRUIT PIECES** in each sample?

Definitely too large	O	O
Slightly too large	O	O
Just about right	O	O
Slightly too small	O	O
Definitely too small	O	O

* How do you feel about the **SWEETNESS** of each sample?

	Sample Number (please fill in)	
	_____	_____
Definitely too sweet	O	O
Slightly too sweet	O	O
Just about right	O	O
Slightly too tart	O	O
Definitely too tart	O	O

When you are done with the samples, please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "NEXT" LIGHT**. Wait for the next pair of samples to arrive.

Turn the page to continue

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

DO NOT taste the samples yet.

* How much do you like or dislike the **COLOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Now taste the samples from left to right as they are arranged for you on the tray. Take a sip of water and take a bite of cracker between samples to refresh your sense of taste.

* What is your FIRST IMPRESSION of the **OVERALL ACCEPTABILITY** of each sample?

	Sample Number (please fill in)	
	_____	_____
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Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Turn the page to continue.

* How much do you like or dislike the **FLAVOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How much do you like or dislike the **TEXTURE** of each sample?

Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How do you feel about the **THICKNESS/THINNESS** of each sample?

Definitely too thick	O	O
Slightly too thick	O	O
Just about right	O	O
Slightly too thin	O	O
Definitely too thin	O	O

Turn the page to continue

* How do you feel about the **SIZE OF FRUIT PIECES** in each sample?

Definitely too large	O	O
Slightly too large	O	O
Just about right	O	O
Slightly too small	O	O
Definitely too small	O	O

* How do you feel about the **SWEETNESS** of each sample?

	Sample Number (please fill in)	
	_____	_____
Definitely too sweet	O	O
Slightly too sweet	O	O
Just about right	O	O
Slightly too tart	O	O
Definitely too tart	O	O

When you are done with the samples, please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "NEXT" LIGHT**. Wait for the next pair of samples to arrive.

Turn the page to continue

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

DO NOT taste the samples yet.

* How much do you like or dislike the **COLOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Now taste the samples from left to right as they are arranged for you on the tray. Take a sip of water and take a bite of cracker between samples to refresh your sense of taste.

* What is your FIRST IMPRESSION of the **OVERALL ACCEPTABILITY** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

Turn the page to continue.

* How much do you like or dislike the **FLAVOR** of each sample?

	Sample Number (please fill in)	
	_____	_____
Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How much do you like or dislike the **TEXTURE** of each sample?

Like extremely	O	O
Like very much	O	O
Like moderately	O	O
Like slightly	O	O
Neither like nor dislike	O	O
Dislike slightly	O	O
Dislike moderately	O	O
Dislike very much	O	O
Dislike extremely	O	O

* How do you feel about the **THICKNESS/THINNESS** of each sample?

Definitely too thick	O	O
Slightly too thick	O	O
Just about right	O	O
Slightly too thin	O	O
Definitely too thin	O	O

Turn the page to continue

* How do you feel about the **SIZE OF FRUIT PIECES** in each sample?

Definitely too large	O	O
Slightly too large	O	O
Just about right	O	O
Slightly too small	O	O
Definitely too small	O	O

* How do you feel about the **SWEETNESS** of each sample?

	Sample Number (please fill in)	
	_____	_____
Definitely too sweet	O	O
Slightly too sweet	O	O
Just about right	O	O
Slightly too tart	O	O
Definitely too tart	O	O

You are finished. Please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "FINISHED" LIGHT**. Please give this questionnaire to the receptionist. **THANK YOU!**