Processing and storage effects on the polyphenolic content and antioxidant capacity of conventional and sugar-free blueberry jams

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Recommended Citation

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ABSTRACT

Fresh blueberries have received much attention due to their positive role in human health and disease prevention. The abundance of polyphenolics, namely anthocyanins and procyanidins, is thought to play an important role in health promotion. Due to seasonal availability and limited shelf-life, blueberries are commonly preserved and consumed in various thermally processed forms (jams, juices, canned whole fruit, and purées). Both conventional high sugar and sugar-free blueberry jams are available on the market, but no information is available on how different formulations, processing conditions, and storage of processed jams affect the retention of polyphenolics and antioxidant capacity found in fresh berries. In this study, fresh blueberries were processed into conventional and sugar-free jams, and stored for 6 months at 4°C and 25°C. Jams were analyzed 1 d after processing and after 2, 4, and 6 months of storage for anthocyanins and procyanidins by HPLC, percent polymeric color, and oxygen radical absorbing capacity (ORAC). Anthocyanins in conventional jams were more susceptible to degradation and polymerization during storage than anthocyanins in sugar-free jams, which may be associated with elevated sugar content. Higher levels of polymers in conventional jams resulted in higher ORAC values, indicating that the polymers formed during storage possess potent antioxidant capacity. However, more research is needed to characterize the anthocyanin polymers and assess their bioavailability. Anthocyanin pigments were much more stable in sugar-free jams indicating that the low calorie jams are a healthy alternative to conventional jams. Anthocyanins, procyanidins, and ORAC were better retained in jams stored at 4°C than at 25°C indicating blueberry jams should be stored under refrigeration in order to maximize retention of health-promoting antioxidants.

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MEET THE STUDENT- AUTHOR

I am from Little Rock and graduated from Mt. St. Mary Academy in 2005. I completed my freshman year of college at Miami University (of Ohio), and then transferred to the University of Arkansas. Since declaring my food technology major at the end of my sophomore year, I have loved every part of it. I have been awarded several departmental and university scholarships and honors, such as the Ozark Food Processors Association Scholarship, the Mid-South Institute of Food Technologists Scholarship, the Alumni Society Ring Scholar Award.

Upon my transfer, I founded the University of Arkansas Tennis Club and continued as club president through my senior year. The club has become very successful, with an active recreational team and a competitive team that travels the country playing other college club tennis teams. I am also active within Bumpers College and serve on the Student Advisory Board.

I plan to begin my M.S. degree program in food science here at the U of A in fall 2009 working under Dr. Andy Proctor, and I aspire to have a career product development with a major food corporation. I would like to thank Dr. Luke Howard, my honors mentor, and his lab team for all of their support these past few years.

Chelsey Castrodale

INTRODUCTION

Antioxidants in foods have become a topic of great interest as a result of documented health-protective benefits. Blueberries have received much attention due to their high concentrations of polyphenolics including anthocyanins, procyanidins, and antioxidant activity. Berry anthocyanins and procyanidins are not only potent antioxidants, but they also contribute to the colors and astringency of the fruit. Antioxidants are beneficial to health due to their ability to scavenge free radicals and reduce oxidative stress. Oxidative stress is associated with many serious health conditions such as heart disease, cancer, Alzheimer's, and Parkinson's disease (Wu et al., 2004). Due to seasonal availability and limited shelf life, berries are commonly processed and consumed in various forms, including canned products, jams, jellies, juices, and syrups. Unfortunately, polyphenolic and antioxidant degradation is prevalent with processing of these products (García-Viguera et al., 1998; García-Viguera et al., 1999; Cabrita et al., 2000; García-Viguera and Zafrilla, 2001; Skrede and Wrolstad, 2002; Wicklund et al., 2004; Giusti and Jing, 2007).

There are many aspects of jam manufacturing that can have an adverse effect on berry polyphenolics and antioxidant capacity. Processing of raspberry jam resulted in anthocyanin losses of 21 to 39% in two cultivars of raspberries (García-Viguera and Zafrilla, 2001). Storage temperature of processed jams can also impact anthocya-nin retention and antioxidant activity. Raspberry (García-Viguera et al., 1998) and strawberry (García-Viguera et al., 1999) jams stored at refrigerated temperature retained much higher levels of anthocyanins than jams stored at ambient temperature. Similarly, the antioxidant content of strawberry jam was more stable when stored at 4°C than at 20°C (Wicklund et al., 2004). The effect of sugar content on anthocyanin stability is controversial. High sugar levels were found to protect anthocyanins from degradation during storage of frozen strawberries (Wrolstad et al., 1990), which may be the result of reduced water activity (Skrede and Wrolstad, 2002). Conversely, studies on strawberry jams and strawberry preserves found that dehydration due to high sugar content initiated polymerization of anthocyanins with other polyphenolic compounds (García-Viguera et al., 1999; Abers and Wrolstad, 1979). The high temperature processing of jam inactivates enzymes involved in degradation of polyphenolics, therefore the only reactions taking place during storage are non-enzymatic. The primary non-enzymatic mechanism responsible for anthocyanin degradation during storage of jams involves a condensation reaction between anthocyanins and procyanidins that results in the formation of high molecular weight polymeric pigments. Unfortunately, it is not known whether the polymeric pigments are absorbed or have similar health benefits as the monomeric anthocyanins. However, if not completely inactivated by thermal treatments, polyphenol oxidase can react with anthocyanins to

produce polymeric compounds. This reaction decreases anthocyanin content and results in browning of pigments (Wesche-Ebeling and Montgomery, 1990; García-Viguera et al., 1999).

The objectives of this study were to determine the effects of jam processing and storage at ambient and refrigerated temperatures on the anthocyanin, procyanidin, and percent polymeric color of blueberry jam. The effects of sugar (conventional vs. sugar-free) on the degradation of polyphenolics during processing and storage were also determined. Antioxidant capacities of berry jams and other products have been examined, but in the only known study on blueberry jam (Kalt et al., 2000) the brand of jam, blueberry cultivar, and product formulation were unknown. Also, blueberries are only seasonally available, and it may be possible to obtain the health-promoting antioxidant properties through consumption of blueberry jam when fresh berries are unavailable. In addition, there is a lack of information on the effects of sugar on anthocyanin and antioxidant content of jams.

MATERIALS AND METHODS

Blueberry Jam Preparation and Processing. Conventional jams were prepared following the recipe on the Sure-Jell® Premium Fruit Pectin package for cooked blueberry jams. Sugar-free jams were prepared following the recipe on the Sure-Jell® Premium Fruit Pectin No Sugar Needed package for cooked no-sugar-added triple berry jams. Jars and screw bands were washed in a dishwasher before use. Lids were prepared by pouring boiling water over the lids and allowed to stand until use. Fresh blueberries were crushed using a stainless steel masher. For conventional blueberry jam, crushed fruit (1040 g) and 1 box of Sure-Jell® Premium Fruit Pectin (49 g) were added to a 6 qt (6.6 l) saucepot. The mixture was heated on high heat until it reached a full boil, then 900 g of sugar were added. The mixture was brought to a boil again and held for exactly 1 min. For sugar-free jams, crushed fruit (810 g) and 1 box of Sure-Jell® Premium Fruit Pectin No Sugar Needed (49 g) were added to a 6 quart (6.6 l) saucepot. The mixture was heated on high heat until it reached a full boil. After 1 min, 15 g of granulated Splenda were added. The jams were dispensed into 8-ounce glass jars, immediately capped, and pasteurized in boiling water for 10 min. Half of the conventional and sugar-free jams were stored at 25°C and the other half at 4°C.

Extraction of Anthocyanins. Jams were pureed using a common household blender (Black & Decker, Towson, Md.). Anthocyanins were extracted using the method of Hager et al. (2008) with modifications. Pureed jam (5 g) was homogenized with 20 mL of methanol/water/formic acid (60:37:3 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, Ohio). Fresh blueberries were pureed in a household blender (Black & Decker, Towson, Md.), and then 10 g of puree were homogenized using the same solvent. The samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.), filter cakes were isolated, and the extraction repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent.

HPLC Analysis of Anthocyanins. Anthocyanins were analyzed by HPLC using the method of Cho et al. (2004). Individual anthocyanin monoglycosides and acylated anthocyanin derivatives were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves of a mixture of anthocyanin glucosides obtained from Polyphenols Laboratories AS (Sandnes, Norway). Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides and acylated anthocyanin derivatives, with results expressed as mg per 100 g of original berry.

Extraction and Purification of Procyanidins. Jams were pureed using a common household blender (Black & Decker, Towson, Md.). Procyanidins were extracted using the method of Gu et al. (2002) with modifications. Pureed jams (5 g) were homogenized with 20 mL of acetone/water/acetic acid (70:29:0.5 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, Ohio). Fresh blueberries were pureed in a household blender (Black & Decker, Towson, Md.), and then 10 g of berry puree were homogenized using the same solvent. The samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.), filter cakes were isolated and the extraction repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent. Samples were purified by solid phase extraction using Sephadex LH-20. After equilibrating 3 grams of the Sephadex LH-20 with water overnight, the absorbent material was manually packed into 6 x 1.5 cm columns. Samples were prepared for purification by evaporating 25 mL of the crude extract under vacuum in a SpeedVac® (SPE 1010, Thermo Savant, Holbrook, N.Y.) at 25°C to approximately 7.5 mL. The concentrated extract was loaded onto the LH-20 column and the column was washed with 40 mL of 30% methanol in water to remove interfering compounds. Procyanidins were then eluted by washing the column with 80 mL of 70% acetone in water. The eluted procyanidins fraction was evaporated to dryness using a SpeedVac® and reconstituted with 2 mL of acetone/water/acetic acid (70:29.5:0.5 v/v/v). The reconstituted samples were filtered through 0.45 μm PTFE syringe filters prior to HPLC analysis.

HPLC Analysis of Procyanidins. Procyanidins were analyzed by HPLC using the method of Kelm et al. (2006). The procyanidin peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm

using a Waters Model 474 fluorescence detector (Milford, Mass.). Individual procyanidins with degrees of polymerization from DP1 (monomer) through DP8 (octamer) were quantified using external calibration curves of cocoa procyanidin standard obtained from Mars Incorporated (Hackettstown, N.J.). Total procyanidins were calculated as the sum of individual procyanidins with results expressed as mg per kg of original berry.

Polymeric Color Analysis. Percent polymeric color was determined using the method described by Giusti and Wrolstad (2001). Sample extracts were diluted with deionized water to obtain an absorbance reading between 0.5 and 1.0 at 512 nm using an 8452A Diode Array Spectrophotometer (Hewlett Packard, Palo Alto, Calif.). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL diluted sample (bisulfite bleached sample) and 0.2 mL of deionized water was added to 2.8 mL diluted sample (non-bleached, control sample). After equilibrating for 15 min, samples were evaluated at = 700, 512, and 420 nm. Color density was calculated using the control sample according to the following formula:

Color density = $[(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})] \times$ dilution Factor.

Polymeric color was determined using the bisulfite-bleached sample using the following formula:

Polymeric Color = $[(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})]$.

Percent polymeric color was calculated using the formula:

% Polymeric Color = (polymeric color/color density) $\times 100$.

Determination of Antioxidant Capacity. Antioxidant capacity was measured using the oxygen radical absorbing capacity (ORAC) assay of Prior et al. (2003). The assay was carried out using a FLUOstar microplate reader with fluorescein as fluorescent probe. Results were expressed as µmoles of trolox equivalents per g of original berry. *Calculations.* Anthocyanin, procyanidin, and ORAC levels in jams were converted to original berry weight using the following calculation:

$C_{product} * R = C_{berry}$,

where, $C_{product}$ = concentration of jam, R = ratio of mass of product produced to the mass of the original berry, and C_{berry} = concentration based on original berry weight. This conversion allowed for concentration and dilution effects to be accounted for and all products to be compared on an equivalent basis.

Sampling. Three jars each of conventional and sugar-free jams stored at 25°C and 4°C were sampled 1 d after processing, and after 2, 4, and 6 mo of storage.

Statistical Analysis. Effects of jam type, storage time, and storage temperature on total anthocyanins, total procyanidins, polymeric color, and ORAC were analyzed by analysis of variance (ANOVA) using JMP® software (SAS Inst. Inc., Cary, N.C.).

RESULTS AND DISCUSSION

Total Anthocyanins. Anthocyanin content of the jams was affected by storage time (P<0.0001), storage temperature (P<0.0002), and jam type (P<0.0221). Percent retention of anthocyanins compared to levels found in fresh blueberries was calculated to determine changes in total anthocyanin content of conventional and sugar-free blueberry jams (Fig. 1). One day after processing, 81% of the total anthocyanins in conventional blueberry jam were retained. This was consistent with a previous study on raspberry jam that reported anthocyanin retentions of 76 to 83% (García-Viguera et al., 1998). In sugar-free jam, 83% of total anthocyanins were retained after processing. After 2 mo of storage, there was a clear temperature effect on anthocyanin retention. Jams stored at 4°C retained 77% and 72% anthocyanins for conventional and sugar-free jams, respectively. Jams stored at 25°C had much lower retentions of 65% and 62% for conventional and sugar-free jams, respectively. After 4 mo of storage, an effect of sugar content on anthocyanin retention was evident, as sugar-free jam stored at 4°C had the highest retention of 64%. The remaining jams had similar retentions; conventional jam stored at 4°C and sugar-free jam stored at 25°C both retained 56% of anthocyanins, and 25°C conventional jam retained 55%. At the end of the 6 mo storage period, both temperature and sugar content had a significant effect on anthocyanin retention. Sugar-free jam stored at 4°C retained the greatest amount of anthocyanins (71%). The degradation of anthocyanins caused by the higher storage temperature of sugar-free jam seemed to be lessened by sugar content, because this jam had the same retention as conventional jam stored at 4°C (52%). Conventional jam stored at 25°C had the lowest anthocyanin retention (36%). Anthocyanins were found to be more stable when jams were stored at lower temperatures. These results were consistent with previous studies on strawberry and raspberry jam, where anthocyanins were found to be more stable when stored at lower temperatures (García-Viguera et al., 1998; García-Viguera et al., 1999). Additionally, it was found that sugar increased anthocyanin degradation, as sugar-free jams retained higher levels than conventional jams.

Total Procyanidins. Total procyanidin content of the jams was affected by storage time (P<0.0001) and storage temperature (P<0.0001), but not by jam type (P=0.1614). Procyanidin degradation was also determined by percent retention compared to fresh berries, and the trend over the 6 mo was similar to that of anthocyanins (Fig. 2), except sugar content had no effect on procyanidins. After initial

processing 80% of procyanidins were retained in conventional jam, and 89% were retained in sugar-free jam. Temperature effects were noticeable early with 4°C retentions of 62% for conventional jams and 64% for sugar-free jams after 2 mo. Jams stored at 25°C showed lower retentions of 53% for conventional and 51% for sugar-free jam. After 4 mo, procyanidin retention was significantly greater for jams stored at 4°C compared to those stored at 25°C. After 6 mo, procyanidin retention was affected by storage temperature. Sugar-free jam stored at 4°C had the highest retention (41%), and conventional jam stored at 4°C had the next highest retention (28%). These values were higher than the 25°C jam retentions of 16% and 20% for conventional and sugar-free jams, respectively. As with anthocyanins, higher storage temperature was detrimental to procyanidins.

Percent Polymeric Color. Percent polymeric color was measured to determine the amount of polymerized anthocyanins resistant to bleaching with sodium metabisulfite (Fig. 3). Higher percent polymeric color indicated the possible formation of anthocyanin-procyanidin polymers. Polymeric color was affected by storage time, storage temperature, and jam type (P<0.0001). One day after processing, all of the jams had approximately 7% polymeric color. After 4 mo of storage, a difference in storage temperatures was observed. Jams stored at 4°C had polymeric color values of 9% and 10% for conventional and sugar-free jams, respectively; but jams stored at 25°C had higher values of 17% and 13% for conventional and sugar-free jams, respectively. After 6 mo, it was evident that the presence of sugar resulted in a significant increase in anthocyanin polymerization though storage temperature was also causative. The 25°C conventional jam had the highest percent polymeric color of 42%. Conventional jam stored at 4°C had a polymeric color value of 26%, which was much lower than that of the 25°C jam, but still higher than the values of sugar-free jams. These data suggest that jams stored at 25°C had lower anthocyanin retentions as a result of the formation of anthocyanin-procyanidin polymers as indicated by percent polymeric color. Sugar also seemed to promote polymerization, as conventional jams had higher percent polymeric color values than sugar-free jams. Additionally, it is possible that Maillard Browning reaction products could have been formed in the presence of sugar and contributed to increased polymeric color values.

Antioxidant Capacity. Antioxidant capacity was measured by Oxygen Radical Absorbance Capacity (ORAC), and percent retentions were calculated to determine changes in antioxidant capacity (Fig. 4). ORAC was affected by storage time (P<0.0114), storage temperature (P<0.0001), and jam type (P<0.0011). After initial processing, retentions were minimally affected, with 90% and 79% retentions in conventional and sugar-free jams, respectively.

The conventional jam stored at 4°C had the highest retention of antioxidant capacity over the 6 mo storage time, retaining 82% after 2 mo, and 85% after 6 mo. Sugar-free jam stored at 4°C also had somewhat high retentions after 2 mo (74%), and after 6 mo (80%). After 2 mo of storage, 25°C jams retained 70% and 61% of antioxidant capacity for conventional and sugar-free jams, respectively. These retentions rose slightly after 6 mo to 75% for conventional and 65% for sugar-free jams. Overall, conventional jams retained more antioxidant capacity than sugar-free jams, but 4°C jams retained more than 25°C jams. Even though conventional sugar jams showed higher anthocyanin degradation and percent polymeric color, they also had higher antioxidant capacities after 6 mo. It is possible that the antioxidant capacity lost from anthocyanin degradation was compensated by anthocyanin polymers formed, which still display antioxidant properties. However, these polymers are most likely large molecular weight compounds, and it is not known if they can be absorbed into the body to provide antioxidant benefits. This finding is in agreement with a study by Brownmiller et al. (2008), which found that antioxidant capacity of blueberry juices was stable during storage, during which time significant losses in total anthocyanins occurred, but polymeric color increased. Additionally, some Maillard browning compounds that could have formed may have antioxidant properties (Yilmaz and Toledo, 2005).

SUMMARY

Anthocyanins in conventional jams were more susceptible to polymerization during storage than anthocyanins in sugar-free, which may be associated with elevated sugar content. Higher levels of polymers in conventional jams resulted in higher ORAC values, indicating that the polymers formed during storage possess potent antioxidant capacity. However, more research is needed to characterize the anthocyanin polymers and assess their bioavailability. Anthocyanin pigments were much more stable in sugar-free jams indicating that the low-calorie jams are a healthy alternative to conventional jams. Blueberry jams should be stored under refrigeration in order to better retain polyphenolics and antioxidant capacity.

ACKNOWLEDGMENTS

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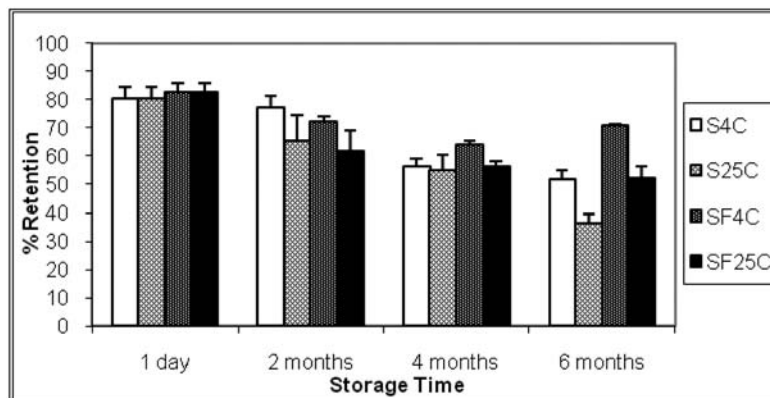
Fig. 1. Percent retention of total monomeric anthocyanins of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C.
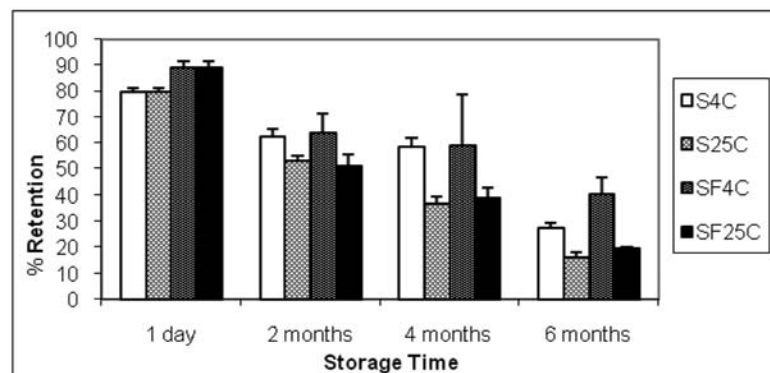

Fig. 2. Percent retention of total procyanidins of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C

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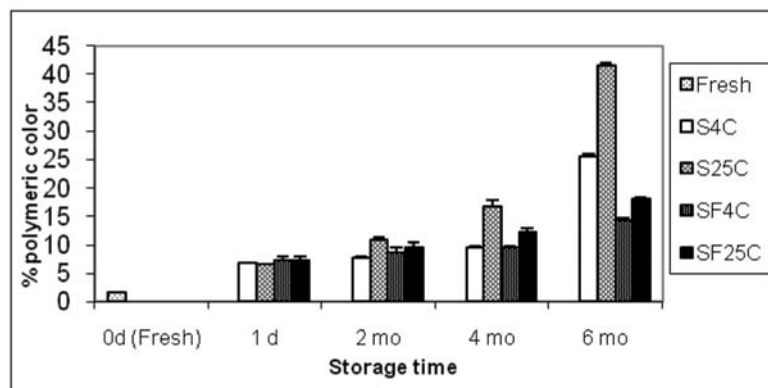
Fig. 3. Percent polymeric color of fresh blueberries and blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C.
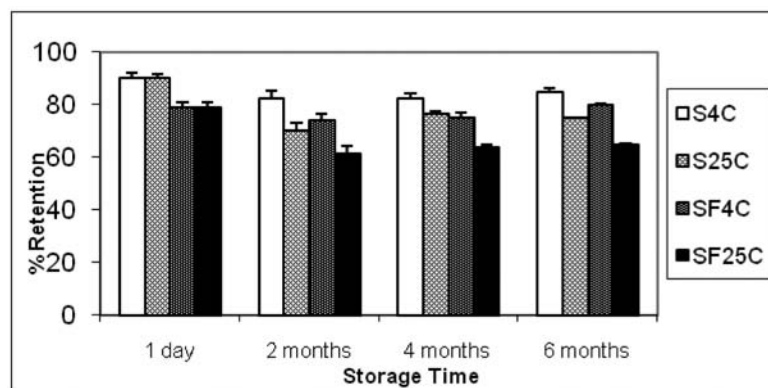

Fig. 4. Antioxidant capacity (ORAC) of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C