Polyphenol stability and antioxidant capacity of various sweetened dried cranberries and cranberry supplements

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B.S., University of New Haven, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Approved by:

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Abstract

Cranberries are fruits rich in polyphenols known to promote health in various ways, from urinary tract health to cardiovascular and oral health. As consumers look to reduce calories and sugar and move to more convenient snacking and bioactive food forms, research on different forms of cranberries and stability of bioactive compounds over time becomes more important. There is a lot of research on stability of polyphenols in cranberry juice drinks, however, similar research on sweetened dried cranberries and cranberry supplements is a needed research area. This research focused on the effects of product matrix, processing, and packaging on polyphenol stability and antioxidant capacity of various sweetened dried cranberries and cranberry supplements over time.

Across the sweetened dried cranberry treatments, it was found that product matrix significantly influenced not only initial polyphenol content but also degradation over time. Sliced cranberries had higher polyphenol content than whole cranberries due to higher infusion rate via increased surface area. Infusion ingredients also impacted polyphenol content and stability, with additional bulking agents, such as apple concentrate or soluble corn fiber, providing enhanced insulation and stability of polyphenols during processing compared to sucrose-only treatments.

Compared to raw cranberries, sweetened dried cranberries are significantly lower in polyphenols. Measurement of antioxidant capacity via DPPH assay found that sweetened dried cranberries had similar antioxidant capacity to raw cranberries, though other studies analyzing cranberries via ORAC assay resulted in higher antioxidant values for raw cranberries demonstrating the importance of consistency in assay when comparing values.

Across the cranberry supplement treatments, it was found that the spray-dried treatment had significantly higher initial and final polyphenol content as well as stability for all analyses

over time compared to gummy treatments. Compared to raw cranberries, the spray-dried treatment had significantly more polyphenols and antioxidant activity, further signifying that spray-drying stabilizes polyphenols due to lower moisture content and A_w, leads to a higher concentration of active components due to water removal, and the capsule form further protects polyphenols from degradation. Across gummy treatments, product matrix and packaging impacted not only initial polyphenol content but also stability over time. The gummy treatment with fewer filler ingredients contained a higher ratio of polyphenols and higher stability (except for proanthocyanidins) due to being packaged as individual wrapped gummies compared to bulk for the other gummy treatment.

Overall this research demonstrates that product matrix in conjunction with processing parameters and packaging for sweetened dried cranberries and cranberry supplements significantly impacts polyphenol content and stability. The product with the highest polyphenol content and most stability over time was the spray dried supplement, though as consumers seek bioactive foods instead of supplements the sliced soluble corn fiber, glycerin, sucrose, and sucralose infused sweetened dried cranberry and gummy with few filler ingredients may be preferable options. Further research should be done to evaluate how different processing conditions, packaging, and other product matrix attributes may improve polyphenol content and stability of non-spray dried cranberry products over time.

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Acknowledgements

I would like to thank Dr. Hughes for not only guiding me through this journey, but also serving as a great mentor academically, professionally, and personally – I could not have done this without your patience, expertise, and compassion. I would like to thank my committee members Dr. J. Scott Smith and Dr. Yucel for their suggestions and guidance during this process and ensuring my success from afar.

I would like to show gratitude and thanks to Ocean Spray Cranberries for allowing me to continue my education, showing support along the way, and fostering a culture of growth and development.

Lastly, I would like to thank my family for their support throughout the long nights and long journey that this has been.

Chapter 1 - Literature Review

Introduction

Consumer views on foods have shifted to becoming more health forward and health conscious (Kell, 2016). With this trend becoming more prevalent, there is a need for the food industry to provide consumers with products that not only taste good but are good for them. Within the food industry it is no longer just acceptable to have products that taste good and have no added value to a person.

In the United States chronic diseases are highly prevalent – according to the CDC one-third of adults from 2011-2014 were considered obese (Ogden, et al., 2015), diabetes is the leading cause of kidney failure (Centers for Disease Control and Prevention, 2011), and heart disease and cancer accounted for 46% of all deaths in 2014 (Centers for Disease Control and Prevention, 2015). Many of these chronic diseases are linked to either over consumption of sodium rich foods (Jackson, et al., 2016), under consumption of fruits and vegetables (Centers for Disease Control and Prevention, 2017), or a lack of physical activity (US Department of Health and Human Services, 2018). In 2015 40% of adults admitted to eating fruits and vegetables less than once a day (Centers for Disease Control and Prevention, 2017). These epidemics in the United States cause consumers and government bodies to push for healthier food options and move away from heavily processed food products.

In 1994, the USDA created the Center for Nutrition Policy and Promotion (CNPP) to help promote the healthy life-style of Americans through diet and nutrition (United States Department of Agriculture, 2018). The construction of the CNPP addressed concern over obesity and diabetes in the United States and the USDA's attempt to limit obesity and

diabetes by recommending healthier options. In 1994 48 states cited obesity in adults under 18% and 49 states cited diabetes under 6% (Center for Disease Control Division of Diabetes Translation, 2018). The CNPP initiatives such as MyPlate aimed to keep obesity and diabetes percentages low in the United States. However, in 2015, 0 states could cite obesity under 18% and 0 states could site diabetes under 6% (Center for Disease Control Division of Diabetes Translation, 2018). In 2015 36 states cite obesity over 26% and 27 states cite diabetes over 9% (Center for Disease Control Division of Diabetes Translation, 2018). Even though healthy initiatives were pushed from the government, obesity and diabetes did not decline as had been the intention of the CNPP.

One initiative that the CNPP established was the MyPlate food guidance system which aims to develop healthy dietary segments for a balanced diet of fruits, vegetables, grains, protein, and dairy. The MyPlate initiative recommends that half of a plate should consist of whole fruits and a variety of vegetables (United States Department of Agriculture, 2018). Fruits and vegetables are nutrient dense and are healthy food sources, which is why they are recommended to make up half of the plate. Whole fruits, whether fresh, frozen, prepackaged, or dried, are recommended due to their nutritive content such as potassium or fiber. These whole fruits are recommended due to their higher nutritive values compared to juice drinks (typically less than 100% juice with added sugars and a lack of dietary fibers) and 100% juices (typically have no added sugar but still lack dietary fibers) (United States Department of Agriculture, 2018). Vegetables are recommended to make up slightly more than one quarter of the plate due to low fat content, low calories, zero cholesterol, and their rich nutritive content including dietary fiber, potassium, and vitamins and minerals (United State Department of Agriculture, 2018). Vegetables are recommended to make up a slightly larger

majority of the plate than fruits because of the low to no sugar content found in vegetables compared to fruits.

Chronic diseases and obesity have increased even with governmental guidance for consumers to eat healthier. It is not that consumers are not aware of healthy foods and the need for them in their diet, but consumer lifestyles have changed. More consumers are eating on-the-go and live a fast-paced lifestyle, which makes eating whole fruits and vegetables less conducive to their busy lifestyle. Companies that produce and market vegetable products have innovated towards consumers with busy lifestyles. Consumers can buy packs of baby carrots or celery to eat with hummus and bring on-the-go, or they can buy prepackaged and prepared vegetable based foods that make cooking easier and healthier (precut vegetables in the produce department for easy additions to meals or vegetable noodles and riced vegetables for healthier pasta and rice replacements). Companies that manufacture fruit based products on the other hand have not done innovations in the same way, and there are rarely any prepackaged fruits or pre-peeled fruits that would be easier to take on-the-go. A part of this may be that there is more involved to consume fresh fruits, such as needing to peel oranges, de-core an apple, or peel bananas, and that fresh fruit tends to go bad more quickly than vegetables, such as bananas ripening quickly and apples bruising easily when taken on-the-go.

Dried fruits can provide the convenience of on-the-go type snacks while still maintaining the nutrition that consumers look for in fresh fruits. In fact, the dried fruit market is anticipated to increase 5.7% between 2018-2026 due to consumer need for healthier food options in conjunction with consumer need for easier and more convenient foods (Transparency Market Research, 2018). The downside to dried fruits, however, is that when

the fruits are dried to remove water, other ingredients are generally added like sugar to increase sweetness in fruits like pineapples and cranberries, or browning agents in dried bananas to mitigate browning reactions that occur during drying. Addition of ingredients like added sugars and anti-browning agents can reduce the health perception of dried fruits in relation to their whole fruit counterparts. Consumers need more healthy options that encompass the health benefits of whole fruits while still being easy to bring on-the-go and require less preparation. Consumers who do not choose whole fresh fruits can alternatively get the nutrients of whole fruits through other forms such as nutraceuticals or supplements, functional consumer goods, or dried fruits.

Nutraceuticals

Nutraceuticals have no regulatory standing in the United States, but they are recognized by the government as functional foods which aim to prevent diseases (Kalra, 2003). Nutraceuticals harness the biologically active compounds in whole fruits, for example, in a nonconventional form of a food such as a pill or supplement. Ginger shots and extracts are used to relieve symptoms of nausea (Semwal, et al., 2015), and cranberry extract supplements can aid in urinary tract health via bacterial anti-adhesion effects (Singh, et al., 2016). Nutraceuticals are aimed at consumers who may want to self-diagnose the need for select bioactives, however the FDA requires companies to place disclaimers on their packaging to alert the public that the claims that the products are making have not been evaluated by the FDA and are not intended to replace pharmaceutical medications. An example is that a claim suggesting that a product may aid in urinary tract health is allowed, however, a claim stating that a product cures UTIs is not allowed. The statement required is

the following: “This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease” (21CFR101.93).

Nutraceuticals are an easy option for consumers compared to the rigor that comes with preparing meals or the amount of a whole fruit you would need to consume to get the same benefits as an extract, which removes the water in the fruit to concentrate bioactive compounds in a more convenient form. However, Millennials (born 1981-1996) may not find nutraceuticals as enticing as the older generations because they are not food products and they come with a level of dissociation from the experience of eating food (Loria & Lee, 2018).

Millennials make up the largest age group in the United States and thus drive a large majority of the eating trends in the United States (Rosenbloom, 2018). Eating healthy by preparing meals at home is expensive and time consuming. A 2017 study found that 55% of Millennials are driven by the convenience of a food over the foods’ taste (Rosenbloom, 2018). With this large demographic wanting convenience in their food choices nutraceuticals may seem like a good option as they are easy to consume and require no preparation, unlike making meals at home. However, the word nutraceutical is a combination of the words nutrition and pharmaceutical and while the word nutrition is on the minds of consumers and seen positively, pharmaceutical is not thought of in such a positive light. In fact, Millennials ranked the pharmaceutical industry as the least trusted health institution in the United States (Patel, 2017). This perception steers Millennials away from traditional means of medicine and towards holistic and more natural approaches to give their bodies the nutrients that are needed to thrive and to promote self-care. Millennials spend a great deal of money on themselves in order to promote wellness (of body and mind) and a 2017 study by Charles

Schwab found that Millennials spend more than both Gen X and Baby Boomers on items and luxuries that provide them with comfort and convenience (Elkins, 2017). Moving away from nutraceuticals and towards food products with bioactive compounds is one way to capture Millennial consumers.

Bioactive Compounds

Label Claims for Bioactive Compounds

A bioactive compound has effects on a persons' biological system and promotes a persons' health, such as flavonols in berries which act as antioxidants and lower total cholesterol (Kris-Etherton, et al., 2002). For food manufacturers to make claims on bioactive compounds in their products there must be scientific evidence which links the bioactive compounds in question to specific health benefits. These bioactive compounds also must be quantified by the food manufacturer and substantiated that they remain throughout the products' shelf life. Health claims can come in the form of nutrient content claims or structure/function claims. These claims differ from pharmaceutical claims in that they do not make a statement that they will cure or prevent any disease or ailment but can be statement of fact on nutrients contained in the foods or can attest to functionality of the food product in support of health. The Nutrition Labeling and Education Act of 1990 (NLEA) allows food manufacturers to make nutrient content claims on their labels as long as they can prove that that amounts of nutrients they are claiming are actually present in the food product (United States Department of Health and Human Services, 2018). Labels may use terms such as "high in" or "low in" to describe the amounts of the nutrient in the food relative to the Reference Daily Intake (RDI) or percentage of the daily value (%DV) of certain nutrients

based on a 2000 calorie diet, as regulated by the FDA (U.S. Food & Drug Administration, 2018).

Unlike nutrient content claims, structure/function claims on food products reference how a nutrient in a food product will effect body functions once ingested (United States Department of Health and Human Services, 2018). These claims also must be backed by scientific evidence to support the claims being made. An example of a nutrient content claim might be that a food product is high in calcium (based on %DV), whereas a structure/function claim might be that the product contains calcium to promote or support bone health. Another example of a nutrient content claim in cranberry juice may be that the juice product contains polyphenols, whereas a structure function claim would be that the juice is able to support urinary tract health. Structure/function claims are more difficult to prove as they require more scientific testing to link the mechanism of the bioactive compound to an effect on the body, whereas a nutrient content claim can just quantify the amount of the nutrient in the food product without any link to mechanisms in the body.

Antioxidants

Antioxidants and Their Mechanisms

Antioxidants are generally defined as those compounds that can inhibit oxidation through free radical scavenging (Decker, et al., 2010). Free radicals are molecules that contain an unpaired electron (Lobo, et al., 2010). Free radicals can originate in the human body from human metabolic processes or from external forces such as chemical exposure or air pollution (Lobo, et al., 2010). Free radicals are negative in humans because they are highly reactive and can cause damage to DNA, cells, and proteins (Lobo, et al., 2010). Free radical scavengers (FRS) are the

most common form of food product antioxidants and include polyphenols, ascorbic acid, and carotenoids (Decker, et al., 2010). By donating hydrogen molecules to compounds that are oxidizing, FRS can scavenge free radicals to limit biological damage to cells and tissues by decreasing energy in the form of an electron (Decker, et al., 2010) (Yeo, et al., 2010). In humans, lipoxygenases are enzyme types that create oxidation of low-density lipoprotein and oxidation reactions (Rosenson, 2009). Anthocyanins have been shown, using an *in vitro* model of soybean lipoxygenase-1, to inhibit lipoxygenase, with delphinidin 3-O-galactoside and delphinidin 3-O-glucoside being the most effective anthocyanins at inhibiting lipoxygenase compared to other anthocyanins (Knaup, et al., 2009). In that study, the anthocyanins were isolated and extracted from lowbush wild blueberries (delphinidin 3-O-glucoside, delphinidin 3-O-galactoside, delphinidin 3-O-arabinoside, malvidin 3-O-glucoside), aronia concentrate (cyanidin 3-O-glucoside, cyanidin 3-O-galactoside, cyanidin 3-O-arabinoside), and cranberry juice (peonidin 3-O-glucoside, peonidin 3-O-galactoside, peonidin 3-O-arabinoside) (Knaup, et a., 2009).

Less commonly than FRS, antioxidants can also come in the form of quenchers and chelators. Quenchers prevent oxidation by quenching singlet oxygens and lipid oxygen products, and lowering excited oxygen molecules to their ground state (Yeo, et al., 2010) (Tsao, 2015). Chelators act in the presence of transition metals and prevent metals such as iron or copper from redox reactions, which in turn prevents oxidation from occurring in food products (Allen, 2015) (Yeo, et al., 2010).

In food products oxidation is a negative attribute because it contributes to off-flavors, odors, color changes, and nutrient degradation (Skibsted, 2010). In order to mitigate these changes, antioxidants can be added to products to maintain the overall quality of the food as well as the integrity of the bioactive compounds. An example is in the addition of apple polyphenols

to red pitaya fruit, which delayed fruit discoloration, microbial growth, and softening of the fruit (Fan, et al., 2018). In food products, oxidation reactions occur from free radicals in the food product or by free radical formation via redox reactions (Lobo, et al., 2010). When foods contain antioxidant compounds such as vitamin A (beta-carotene), vitamin C (L-ascorbic acid), or plant phenolics, however, the antioxidant compounds can inhibit the free radicals from harming the food product. Vitamin A (beta-carotene) works in food products to scavenge singlet oxygens to create a nonradical and therefore by inhibiting the formation of free radicals it inhibits further oxidation, however this process can also change the color of the food due to bleaching of the beta-carotene compounds (Decker, et al., 2010). Vitamin C (L-ascorbic acid) is seen as a potent antioxidant, however, this depends on other competing reactions. Vitamin C can reduce transition metals but, in the process, enables hydrogen molecules generated to become free radicals which in turn promote oxidation reactions (Decker, et al., 2010). Plant phenolics such as carnosic acid and rosmarinic acids in rosemary are added to meat and oil emulsions to limit lipid oxidation (Decker, et al., 2010). In the process of protecting the foods from oxidation reactions and free radicals, the antioxidant species are used up. If too many antioxidant species are used up and free radicals begin to form in an imbalance to antioxidants, oxidative stress occurs which can breakdown lipids, proteins, and nucleic acids in the foods, like how antioxidants and free radicals work in the human body (Lobo, et al., 2010). Oxidative stress can occur by free radical formation by oxidation of amino acids, cleavage of peptide bonds, and by cross-linkages formed via lipid peroxidation (Lobo, et al., 2010). In plants and fruits where there are no lipids or proteins, oxidation can occur via polyphenol oxidase (PPO) enzymes or reactions with phenols innate to the plant (Pourcel, et al., 2007).

Determination of Antioxidant Capacity

It is important to measure antioxidant capacity and activity in food products both as a manufacturer and as a researcher. As a manufacturer, it is important to validate nutrient content claims, and as a researcher it is important to look at the antioxidant potential of different compounds. Antioxidant activity references whether a compound can act as an antioxidant. Antioxidant capacity of a compound refers to how much (or to what degree) the compound is acting as antioxidant. Antioxidant capacity therefore can give more quantifiable values whereas antioxidant activity would simply refer to whether the compound can act as an antioxidant. There are various methods of analyzing antioxidant capacity, including ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity assay (ORAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric assay (Popovic, et al., 2012). These methods are differentiated as either being electron transfer or hydrogen atom transfer assays. Electron transfer assays such as DPPH, FRAP, and TEAC are color reactions due to the reduction of oxidants by an electron transfer, while hydrogen atom transfer assays such as ORAC are caused by competing reactions mechanisms of an antioxidant and the substrate such as sodium fluorescein which results in a fluorescein decay curve which can be measured against a blank (Popovic, et al., 2012) (Roy, et al., 2010).

While these assays can measure antioxidant capacity of food products, antioxidants do not all work in the same way. Some antioxidants react due to multiple mechanisms while others may only react due to a single reaction, and some antioxidants may react to certain free radical species but not others (Prior, et al., 2005). For example, phenolic compounds quench peroxyl radicals better than carotenoids but carotenoids are better as quenching singlet oxygen species (Prior, et al., 2005). The different combinations of reactions and free radical species for

antioxidants to react with means that while assays can accurately measure antioxidant capacity, no assay will be able to accurately measure all the different scenarios that may occur in a food matrix (Prior, et al., 2005). A single food matrix can vary based on ingredients used, processing parameters and conditions, and storage and packaging conditions. These variables will also impact how the antioxidants in the food are able to be measured. Processing conditions may damage antioxidants if they are too harsh (long processing times or high processing temperatures), if there are too few antioxidants to be able to insulate from degradation, and improper packaging and storage may let in oxygen which may speed up degradation. It is important for food manufacturers to understand how different processing conditions and food matrices may affect antioxidants and the bioactive compounds in the food products. Testing assays based on the different antioxidant species and reaction mechanisms can help food manufacturers to better understand how antioxidants are functioning in their food products.

Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay is measured by a reaction between an electron donor antioxidant species and Fe3+ to produce Fe2+, which produces a color change when reacted with 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (Martins, et al., 2013). FRAP assay measures antioxidant capacity based on the ferric ion, which may not be directly applicable to how antioxidants react in biological matrices because the ferric ion is not available as an antioxidant species in humans. A disadvantage to the FRAP assay is that the reaction is read spectrophotometrically within 6 minutes of the reaction which assumes that all reduction reactions will be complete within 6 minutes (Prior, et al., 2005). This assumption may not always be true as polyphenols and other phenolic compounds have longer reaction times, up to multiple hours, due to reduction reactivity, so the FRAP assay may

result in underestimation of antioxidants in these cases (Prior, et al., 2005) (Zhong & Shahidi, 2015).

Trolox Equivalent Antioxidant Capacity Assay (TEAC)

The TEAC assay is a reaction between the radical 2-2'-azino-bis(3-ethylbenxothiazoline-6-sulphonic acid) (ABTS) and antioxidants. Antioxidant compounds decrease the color created during the reaction by scavenging the free radicals and the antioxidant capacity can be measured (Prior, et al., 2005). TEAC is a simple assay which utilizes a 30 minute reaction time and can be used on products with varying pH ranges (Prior, et al., 2005). One disadvantage to this assay is that the radical used for the reaction (ABTS) is not found in humans or other mammals, so it is not representative of antioxidants in the human body (Prior, et al., 2005). Since this assay is not representative of antioxidants in the human body, the assay is useful for overall antioxidant quantification and not correlation with antioxidants in relation to biological functions.

Oxygen Radical Absorbance Capacity Assay (ORAC)

ORAC utilizes a reaction between antioxidants based on inhibition of oxidation by peroxyl radicals which results in a fluorescence curve which can be used to calculate antioxidant capacity based on product formed and rate of the reaction (Roy, et al., 2010)(Prior, et al., 2005). However, in 2012 the USDA chose to remove ORAC values for foods from the Nutrient Data Laboratory (NDL) because of scientific evidence indicating that ORAC values could not be linked to bioactive compounds in humans (United States Department of Agriculture, Agricultural Research Service, 2010). ORAC values were previously used by the USDA to quantify antioxidant capacity of foods and by food manufacturers to talk about the antioxidant benefits of

their products. However, since ORAC values cannot be directly translated to biological functions in the human body, the values can be misleading to consumers since even though a food contains antioxidants, they may not be recognized by the FDA to make claims (United States Department of Agriculture, Agricultural Research Service, 2010). The ORAC method also needs to be done in a temperature-controlled environment and utilizes a fluorometer rather than a spectrophotometer, which may not be available in many food laboratories (Prior, et al., 2005).

2,2-diphenyl-1-picrylhydrazyl Assay (DPPH)

DPPH is a dark purple radical which when reacted with antioxidants will lighten in color due to electron transfer to the DPPH free radical, and the color change can be characterized with a spectrophotometer (Roy, et al., 2010). DPPH assay has increased in use in the food industry because it is a simple test, which requires commerically available reagents and a spectrophotometer to run the reaction (Roy, et al., 2010). DPPH assay though can have trouble if the test compounds in question have spectra that will absorb at the same wavelength that the DPPH reaction is read (515 nm). This is the case with carotenoids, so care needs to be taken if samples containing carotenoids are to be used (Prior, et al., 2005).

ORAC and DPPH have been used to assess antioxidant capacities in fruit products such as plums, Chinese bayberries, cranberries, grape seeds, and banana peels (Almeida, et al., 2016) (Yang, et al., 2009) (White, et al., 2010) (Babbar, et al., 2011). In these products, substances such as phenolic compounds, carotenoids, and ascorbic acid can contribute to antioxidant capacity. Not all fruits have the same chemical makeup, so the contributing compounds differ in different food products. In berries and darker fruits, the polyphenols are important compounds

that maintain fruit integrity against oxidation and act as antioxidants (Chen, et al., 2014) (Hassan & Abdel-Aziz, 2010).

The various methods for antioxidant capacity measurement in food products differ in how their mechanisms allow them to act as antioxidants. Therefore, it is hard to directly compare different methods if they do not inhibit free radical formation in the same ways which makes it increasingly hard for scientists to use these methods to make claims on food products and rather have to substantiate with human trials.

Legal Status of Antioxidants in the United States

In the United States, food manufacturers may make antioxidant claims in the form of nutrient content claims. The FDA will approve a product with an antioxidant nutrient content claim so long as the antioxidant has an established RDI level and has scientifically proven antioxidant activity in the form of human clinical studies. The level at which the antioxidant is used may additionally qualify for good source (10% RDI), excellent source (20% RDI), 100%, "low in" (5% DV or less), or "high in" (20% DV or more), which are easy for the consumer to understand (U.S. Food & Drug Administration, 2018). Currently there are only three antioxidants in the United States that have an established RDI backed by human clinical trials: vitamin A (beta-carotene or retinol), vitamin C (L-ascorbic acid), and vitamin E (gamma-tocopherol or alpha-tocopherol). Despite scientific studies which demonstrate that polyphenols have antioxidant properties in food matrices (Chen & Yu, 2017) (Kim, et al., 2003) (White, et al., 2010), the FDA has not established RDI values for polyphenols as antioxidants due to the complexity of validating polyphenol antioxidant properties in humans. The RDI is based off of the estimated average requirement for the nutrient (amount estimated to meet nutritional

requirements for half the population in a group) plus either a standard deviation or 10% coefficient of variance (Institute of Medicine (US) Food and Nutrition Board, 1998). The estimated average requirement is based on peer-reviewed scientific literature which displays reduction of certain diseases.

There are no studies which indicate a necessary quantity of polyphenols that must be ingested to reduce disease in humans. There is also a lack of knowledge on which polyphenols are specifically responsible for acting as antioxidants. Polyphenols are a class of compounds, and while individual compounds may be effective as individual antioxidants, there is no evidence that the whole class of compounds acts as antioxidants. Similarly, vitamins are a class of compounds which as a class do not have an RDI level, but the individual vitamins each have an RDI. The mechanisms of vitamins A, C, and E as antioxidants are well supported by scientific literature, thus they meet the FDA requirements for being labeled as an antioxidant. If more clinical studies are conducted on specific polyphenols, they could have the potential to be qualified as an antioxidant with an RDI level for food claims. Nutrient content claims such as good source of specific polyphenols such as proanthocyanins, anthocyanins, and carotenoids would then be able to be used on label for consumers interested in health forward products. However, the complexity of human clinical trials reduces the ability for studies to be successfully done on polyphenol antioxidants. Although single isolated polyphenols like anthocyanins or proanthocyanidins may exhibit antioxidant capacity *in vitro*, once they are introduced into human subjects, which differ in health status and gut microflora, the compounds may not behave as they would in a controlled *in vitro* model, which demonstrates the differences in an antioxidant compound having capacity regardless of the matrix versus its availability in different formats (Manach, et al., 2004).

Polyphenols

One of the main groups of bioactive compounds in fruits are polyphenols, a classification of phytochemicals. Polyphenols have an aromatic ring and at least one hydroxyl group that includes a derivative such as an ester of glycoside (Gnanavinthan, 2013). Polyphenols are the most widely consumed antioxidant in the human diet and are made up of flavonoids, tocopherols, phenolic acids, lignan, stillbens, tannins, and coumarins (Gnanavinthan, 2013). These groups of polyphenols have natural antioxidant capacity and have been found to reduce oxidative stress caused by cancers and heart disease in *in vitro* models (Manach, et al., 2004) (Eaton, et al., 1996) (Scalbert, et al., 2005). Most polyphenols are absorbed in the small intestine (Donovan, et al., 2006) with the remaining portion broken down and absorbed in the large intestine (Del Rio, et al., 2010). Polyphenol activity in humans can differ depending on the type of phenolic compound and its intrinsic biological activity, poor absorption of certain compounds, high metabolism rate of certain compounds, or because some compounds are more rapidly eliminated in humans than others (Manach, et al., 2004). Bioactivity of polyphenols is the effect that they have on a persons' body whereas the bioavailability of polyphenols is the amount of the compounds that are capable of being absorbed and used in the body. While many compounds are bioactive, their bioavailability may be very small in the human body and their effects might not be great. In human studies therefore it can be difficult for polyphenols to be quantified and validated. Different polyphenols will be more readily available in humans while others will rapidly breakdown. Also, since most polyphenols are absorbed in the intestines, many compounds do not make it through the digestive system to be studied in human trials, further limiting the reliability of *in vitro* polyphenol studies.

Flavonoids

Polyphenolic compounds are primarily composed of a group of compounds called flavonoids. Flavonoids are composed of flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (Benzie & Wachtel-Galor, 2013) (Manach, et al., 2004), with all of these having the same chemical foundation in common: 2 aromatic rings bound by 3 carbons which form a heterocycle (Manach, et al., 2004). Flavonoids play a role in the biological functions of plants by acting as FRS, maintaining plant structure, providing UV protection for plants, and regulating plant physiology and reproduction (Flacone Ferreyra, et al., 2012). All of these biological activities are fundamental for plant survival, which is why flavonoids are so widely found in nature.

Flavonols are the most common of the flavonoids, flavones are only typically found in parsley and celery, flavanones are found in high amounts in citrus, isoflavones are relatives of estrogen compounds found in the legumes, flavanols are found in tea as catechins and proanthocyanidins in fruits, and anthocyanins are pigments in fruits and vegetables (Manach, et al., 2004). A USDA report of flavonoid content of commonly consumed foods showed fruits such as berries contain the highest flavonoid contents, with blueberries (raw highbush variety) containing 180 mg/100 g of total flavonoids (90% of which are anthocyanins), cranberries (raw) containing 132 mg/100 g of total flavonoids (79% of which are anthocyanins), and cherries (sweet, raw) containing 44 mg/100 g of total flavonoids (70% of which are anthocyanins) (Bhagwat, et al., 2013). As in the case of the blueberries, cranberries, and cherries, anthocyanins make up more than 70% of the total flavonoids, making anthocyanins a vital component of many fruits.

Anthocyanins

Anthocyanins are a class of flavonoid which give pigmentation to fruits and vegetables, usually in the form of reds, blues, purples, and blacks (Steyn, 2009). Copigmentation is important for anthocyanins because it is a pigment and copigmentation protects anthocyanins from degradation. Copigmentation occurs when anthocyanin molecules stack on top of each other causing insulation of the compounds from oxidation reactions and pH changes which may degrade the anthocyanins (Gordillo, et al., 2012). This insulation through copigmentation not only leads to increased anthocyanin stability, but the different stacking combinations lead to the different variety of colors that consumers may see in fruits from anthocyanins (raspberry pigmentation versus strawberry pigmentation versus cranberry pigmentation) (Gordillo, et al., 2012). Of the over 700 anthocyanins found, 90% of them are made up of 6 anthocyanidin bases (Wallace & Giusti, 2015). These anthocyanidin bases (which lack a sugar substituent) are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvinidin, which are differentiated by the 2 substituents attached to one of three phenolic rings (Wallace & Giusti, 2015). A 2006 study looking at the concentrations of anthocyanins in 100 commonly consumed U.S. foods found that only 24% contained anthocyanins (16% fruits and 7% vegetables), suggesting that fruits contain more anthocyanins than vegetables (Wu, et al., 2006). The USDA analyzed anthocyanin content in 2013, showing that fruits such as bilberry (285.21 mg/100 g), chokeberries (349.79 mg/100 g), elderberries (485.28 mg/100 g), plums (558.19 mg/100 g), and black raspberries (671.79 mg/100 g) all have high anthocyanin content compared to navel oranges (0 mg/100 g), red delicious apples (2.97 mg/100 g), and bananas (7.39 mg/100 g) (Bhagwat, et al., 2013). These fruits are associated with red, blue, purple, or black pigmentation, which is why they are very high in anthocyanin content compared to orange and yellow fruits.

The same can be said for vegetables, where red cabbage (209.95 mg/100 g) and radicchio (134.67 mg/100 g) also contain high anthocyanin content compared to celery and carrots, which all have 0 mg/100 g of anthocyanins (Bhagwat, et al., 2013).

Anthocyanins as Antioxidants

There are many studies which assess anthocyanins and antioxidant capacity (Chen, et al., 2014) (Skrovankova, et al., 2015) (Hernandez-Herrero & Frutos, 2015). An *in vitro* Chinese study looking at twelve different types of berries showed a positive relationship between anthocyanin content and antioxidant capacity (Chen, et al., 2014). In this study, blue-berried honeysuckle (dark red in color) had the highest anthocyanin level (68.11 mg/kg) and antioxidant capacity (103.63 mg/kg via DPPH assay) while the Chinese dwarf cherry and raspberry (both pink in color) had the lowest anthocyanin levels (3.96 mg/kg and 2.58 mg/kg, respectively) and also had lower antioxidant capacity (70.29 mg/kg and 39.42 mg/kg via DPPH assay, respectively) (Chen, et al., 2014). The data show a positive correlation between total phenolic content and antioxidant capacity (r=0.809), and overall those fruits with higher anthocyanin content had higher antioxidant capacity (Chen, et al., 2014). Other berries like strawberries, blackberries, blueberries, and cranberries have antioxidant capacity and also have high anthocyanin content (Skrovankova, 2015). While berries contain many bioactive compounds that could be attributed to antioxidant capacity, there appears to be a positive correlation between anthocyanins specifically and antioxidant capacity which further needs to be explored through their functionality in the human body.

Anthocyanins as Colorants

The association of anthocyanins with pigmentation make them a viable option as a natural colorant in food products. Consumers are beginning to move away from synthetic ingredients including sweeteners, preservatives, and colorants as natural alternatives are becoming available and being used by manufacturers to meet consumer demand for more natural ingredients. A 2014 study found that consumers are less tolerant of synthetic colorants than synthetic preservatives or sweeteners (Bearth, et al., 2014). Synthetic colorants that are deemed safe by the FDA are regulated under the 1938 Federal Food, Drug, and Cosmetic Act which labels certified colorants as food, drug, and cosmetic compliant (FD&C), drug and cosmetic compliant (D&C) or external drug and cosmetic compliant (Ext. D&C) (Barrows, et al., 2003). Examples of these colorants permitted for use in foods include FD&C Blue #1, FD&C Red #40, and FD&C Yellow #6 (U.S. Food & Drug Administration, 2017). Food manufacturers add colorants to foods to extend shelf-life, make the products more visually appealing, and to make up for color deterioration that may result from processing. Consumers are driven by visually appealing food products and are more likely to eat foods that are red over green due to the prehistoric human association with red as an indication of freshness and calories in foods (Cantu & Griskevicius, 2012). Foods that do not have naturally appealing colors or have colors that may fade or degrade during processing or over the course of the products shelf life need to add color, and as consumers are less accepting of FD&C colorants (Bearth, et al., 2014), natural colorants are a good option for the food industry.

Anthocyanins are water-soluble and provide pigmentation, so they are a good option for many food matrices (Mateus & de Freitas, 2008). Grapes are a common source of anthocyanins in the food industry (Mateus & de Freitas, 2008) but other fruits and vegetables also contain

anthocyanins such as blueberries, raspberries, cranberries, and red cabbage (Mateus & de Freitas, 2008). Anthocyanins change color reversibly with increased pH and appear red in acidic pH solutions, purple in neutral pH solutions, and blue in basic pH solutions (Khoo, et al., 2017). A 2015 study showed purple black carrot (*Daucus carota L.*) anthocyanins are stable as a natural food color extract and that the color change that occurs based on the pH of the product could be a potential useful indicator of food spoilage (Masoodi, et al., 2016). If a food matrix changes pH to a pH more suitable for pathogen and microbial growth, the anthocyanin would react in the food matrix and change color, alerting the consumer and manufacturer of potential hazards (Masoodi, et al., 2016). Color changes based on pH may be a potential useful indicator of food safety, but it has also been a limiting factor in being able to use anthocyanin derived food colorants. Many food applications that use anthocyanins as a food colorant do so because the pH of their product is low enough to maintain the original anthocyanin color.

Another drawback to anthocyanins as food colorants is that they degrade quickly due to processing and storage conditions. Thermal processing of blueberry purees and blueberry juice (clarified by centrifugation at 6000 x *g* for 10 minutes) have shown that processing decreases anthocyanin content (Cesa, et al., 2017) (Brownmiller, et al., 2008). In cranberry juice processing, blanching of the fruit resulted in significant reduction of total anthocyanin content (White, et al., 2011). Three different processing conditions were looked at prior to the addition of pectinase and juice clarification, one in which ground fruit was blanched at 95^{o}C for 3 minutes, one in which ground fruit was unblanched, and one in which whole fruit was blanched at 95^{o}C for 3 minutes, and it was found that anthocyanins were retained at 39%, 53.1% and 42.4% respectively (compared to the frozen initial fruit) (White, et al., 2011). The range of colors produced by anthocyanins not only has to do with the pH of the solution as well as the

processing stability, but copigmentation of anthocyanins plays a large role. Copigmentation of anthocyanins causes stability of the anthocyanins and enhances their color through an absorption shift (5-20 nm) either intramolecularly or intermolecularly (Gomez-Miguez, et al., 2006) (Rustioni, et al., 2012). Intramolecular interactions occur via interaction of the central anthocyanin molecule and the aromatic acyl compounds linked to them, where intermolecular interactions occur between anthocyanins and other non-pigmented compounds, usually in the form of phenolic compounds (Gomez-Miguez, et al., 2006).

Other Potential Health Benefits of Anthocyanins

Aside from the antioxidant capacity of anthocyanins, there have been studies with mulberry extracts fed to mice and rats as well as introduced to human cancer cells that show that anthocyanins (cyanidin-3-glucose, cyanidin-3-rutinoside, and peonidin-3-glucoside) possess other beneficial health attributes, especially in relation to cancer prevention (Huang, Chang, Wu, Hung, & Wang, 2011) (Long, et al., 2018) (Li, et al., 2016). A 2011 study showed anthocyanin extracts from mulberries (cyanidin-3-glucoside and cyanidin-3-rutinoside) were effective in inhibiting gastric cancer cells in mice by inducing apoptosis in the cancerous glandular cells, thus reducing the amount of cancer cells that could grow via p38 caspase 8 signaling and p38 Bac signaling pathways (Huang, Chang, Wu, Hung, & Wang, 2011). Anthocyanin induced apoptosis of cancer cells was seen again with mulberry anthocyanins in relation to thyroid cancer where mulberry anthocyanins acted as thyroid tumor suppressors by deactivating Akt/mTOR signaling in cancer cells (Long, et al., 2018). Anthocyanins induce cell death in cancer cells independent of where the cells are derived from. In patients with trastuzumab-resistant breast cancer, anthocyanin extracts of mulberry (cyanidin-3-glucoside and peonidin-3-glucoside) were

once again shown to induce apoptosis in the cancerous cells which limited cancer cell migration and overall growth, both *in vitro* and *in vivo* (Li, et al., 2016).

A 2017 study evaluated the effect of anthocyanins on human pancreatic cancer cells and found that when healthy individuals consumed a grape and bilberry juice that was high in anthocyanin content (841 mg/L of total anthocyanins), pancreatic cancer cells (derived in the study from pancreatic carcinoma cell lines PANC-1 and AsPC-1) were unable to migrate due to reduction of radical oxygen species by the anthocyanins (Kuntz, Kunz, & Rudloff, 2017). Even though there are studies that validate specific anthocyanins' role as antioxidants there is still no legal guidance from the FDA which allows anthocyanins to be called out in food products as a source of antioxidants. More work needs to be done to link anthocyanins as a group of compounds and their effectiveness as antioxidants. Since there is no guidance on anthocyanins as a group of compounds, there is no recommendation as to the amounts that would be needed to be consumed for antioxidant benefits in humans. One drawback is that unlike vitamin C, there are no illnesses that would result from not consuming enough anthocyanins in a persons' diet. Even though studies show that anthocyanins can cause apoptosis in cancer cells and can limit cancer cell growth, there is no evidence that without consumption of anthocyanins humans would develop cancers. So even though there are no identified illnesses that would be prevented by the absence of anthocyanins in a persons' diet, anthocyanin consumption can aid in prevention or reduction of chronic diseases such as cancer (Kuntz, Kunz, & Rudloff, 2017) (Huang, Chang, Wu, Hung, & Wang, 2011) (Li, et al., 2016).

Proanthocyanidins

Proanthocyanidins are a part of the flavonoid group also known as condensed tannins (Figure 1) (Gnanavinthan, 2013). They are polymers of flavan-3-ols and are commonly consumed parts of the human diet (Santos-Buelga & Scalbert, 2000). Proanthocyanidins are produced when flavanol monomers are polymerized (Beecher, 2003). This polymerization can occur naturally within the plant or from oxidation during processing (Beecher, 2003). As antioxidants, polyphenol compounds such as proanthocyanidins will defend the plant against oxidation in nature and will also do this when oxidation reactions occur when food products are processed to protect the food. Proanthocyanidins are found as either B-type carbon-carbon linkages or A-type carbon-oxygen. Figure 2 shows a comparison of proanthocyanidin dimers between a B-type linkage from a granny smith apple and an A-type linkage from red peanut skin (Figure 2) (Xu, et al., 2015). The main difference between type-A and type-B linkages as illustrated is the addition of an ether bond within the A-type linkages. Foods such as grapes and blueberries contain the widely appearing B-type linkages, while cranberries and plums contain A-type linkages (Xu, et al., 2015) (Howell, 2007) (Gu, et al., 2004). A-type linkages are associated with anti-adhesion activity (Howell, 2007) (Gu, et al., 2004). Cranberries have A-type proanthocyanidins and the anti-adhesion properties associated with A-type proanthocyanidins make cranberries good promotors of urinary tract health (Howell, 2007). Anti-adhesion properties make it so that harmful bacteria such as P-fimbriated uropathogenic *E. coli* cannot adhere within the body and cause infections (Howell, 2007). This occurs because the A-type proanthocyanidins bind to *E. coli* fimbrial tips which inhibits the bacteria from adhering (Hisano, et al., 2012).

A 2003 study found that B-type proanthocyanidins from apple juice, green tea, and dark chocolate provided no anti-adhesion *in vitro* while A-type proanthocyanidins from cranberry juice cocktail showed *in vitro* anti-adhesion (Howell, et al., 2005). While A-type proanthocyanidins are more noted for their anti-adhesion properties in urinary tract health, a 2010 study found that A-type proanthocyanidins are responsible for the antioxidant capacity of lychee seeds *in vivo*, and proanthocyanidin litchitannin A2 exhibited anti-CVB3 (coxsackie virus B3) activity *in vitro*, indicating that A-type proanthocyanidins are beneficial to health as an anti-viral as well as for anti-adhesion (Xu, et al., 2010).

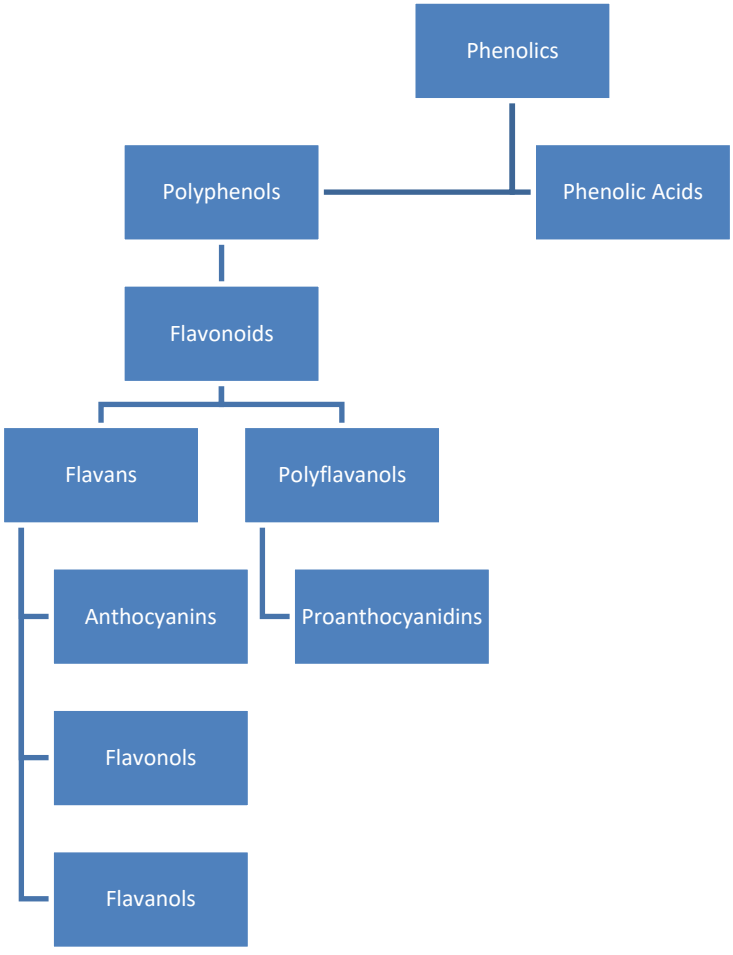

Figure 1.1 Phenolic compounds found in fruits and vegetables (Gnanavinthan, 2013)

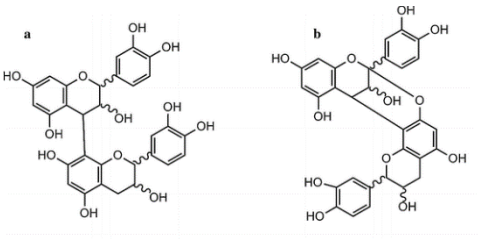

Figure 1.2 a) B-type epicatechin dimer from granny smith apples. b) A-type epicatechin dimer from red peanut skin (Xu et al., 2015)

Inherent Characteristics of Food Products

Moisture Content and Water Activity

Moisture content refers to the total amount of water in a food system whereas water activity (A_w) refers to the unbound or free water in a food system. Both moisture content and A_w are used in the food industry for product shelf life considerations as well as food safety parameters for the inhibition of microbial growth (Bell, 2007) (Chirife & Fontana Jr., 2007). Low moisture content and A_w can limit microbial growth and reduce chemical reactions in food products. Chemical reactions in foods such as oxidation are facilitated by water content in the products. Therefore, when the total amount of water (moisture content) in products is reduced, those reactions will be diminished (Bell, 2007). A_w is also an indicator because microbial reactions are hindered at low A_w and chemical reactions such as oxidation are related to A_w levels (Perera, 2005).

To achieve a low A_w in products processing needs to take place to remove most of the water. Water removal can occur by methods such as freezing, concentration, thermal processing, or addition of ingredients such as salt or sugar to bind water. Considerations need to be taken

when selecting methods by which to lower A_w as there usually are consequences to the product that will result from the different techniques. While lowering the A_w by thermal processing will increase stability, shelf life, and limit microbial growth (Perera, 2005), it also can be harmful to a product with respect to browning, crystallization, and degradation of bioactive compounds. As previously stated, thermal processing degrades polyphenols which are the main bioactive components of fruits (Ioannone, et al., 2015) (Mendez-Lagunas, et al., 2017) (Beecher, 2003). On the other hand, products naturally low in A_w, such as grains, do not have the same issues of browning and bioactive compound degradation as products with high A_w as they do not need a thermal process to achieve low A_w.

Polyphenols can act as antioxidants by protecting the food product by scavenging free radicals. In doing so the antioxidant species that scavenge the free radical are no longer active as an antioxidant species and therefore the polyphenol content of the food product is lowered. In dried apples it was found that enzymatic browning via PPO was heightened when A_w was between 0.32 and 0.56 (Lavelli & Caronni, 2010). The activity range for PPO is at an A_w between 0.2 and 0.8 (Lavelli & Caronni, 2010). Less than 0.2 A_w there is little water to react with and greater than 0.8 A_w there is so much water that the active components are too diluted (Brennan, 1994). PPO has a direct impact on the color changes occurring in a product and the degradation of polyphenols such as anthocyanins, which results in formation of yellow, brown, and black colors developing over time (Bermejo-Prada & Otero, 2016).

Texture

Aside from the microbiological and chemical reactions that may occur in food products that make food manufacturers provide limitations and ranges for moisture content, there is a

consumer aspect that is also important. Consumers want food products that meet not only their flavor expectations but also maintain their textural expectations. In 2012, 27% of the top launched foods and beverages in the United States carried some sort of texture claim, such as creamy in reference to chocolate characteristics, or crunch in reference to the deep ridges in potato chips, making texture an important characteristic for consumers (Sloan, 2013). Dried fruit is expected to have a low moisture content and A_w because, as the name implies, it has been dried. If a consumer is presented with a dried apple slice and it is soggy, the textural expectation is not met and there is a disconnect with what the product should be and what it actually is. For supplements and other nutraceuticals there is no textural expectation or if there is it may not be inherently positive (a gummy chew may not need to taste good if it is giving the consumer a concentrated health benefit). When foods are outside of that realm not only is it an indicator on an analytical level that something is wrong with the product, but consumer perception of the food product will change to something that is off-putting or unpleasant.

Color

Color is an important aspect of food products, as it contributes to attracting the consumer to purchase the product. This color attraction is true especially with fresh fruits, where color can be an indicator of the quality of the product, such as how a yellow banana indicates good quality because that is the color of ripeness, while a brown or black banana indicates that it has been ripened for too long and is no longer of good quality. In processed foods, however, this association with color is different. Raisins for example do not look as vibrant or full of color as grapes because they have been processed and dried which will result in color change that naturally would be negative. While not all indicators are this obvious in nature, consumers eat

with their eyes so anything that is visually unappealing or out of the ordinary will decrease the consumers appetite for that food product (Marshall, 2017). Fruits such as blueberries and strawberries can undergo enzymatic browning which can deteriorate the color of fruits after food processing (Siddiq & Dolan, 2017) (Bermejo-Prada & Otero, 2016). PPO is a naturally occurring enzyme in plants and causes browning in the presence of oxygen when the PPO reacts with the oxygen causing phenols to convert to melanin (a brown pigment) (Siddiq & Dolan, 2017) (Fang, et al., 2007) (Institute of Food Science and Technology, 2017). Strawberries get their red color from anthocyanins, and PPO can degrade anthocyanins to brown, black, or yellow colors (Bermejo-Prada & Otero, 2016). Processing such as juicing and drying as well as storage conditions can induce PPO reactions causing most color changes and anthocyanin polymerization reactions (Siddiq & Dolan, 2017) (Bermejo-Prada & Otero, 2016) (Jaiswal, et al., 2010). In dried pomegranate arils it was found that oven drying (90^{o}C for 90 minutes, 70^{o}C for 2 hours, and 50^{o}C for 9 hours) versus sun drying (dried in trays over several months in a greenhouse with temperatures between 32-42^{o}C until 76% moisture content was reached) decreased total anthocyanins by 61% and 83%, respectively (Jaiswal, et al., 2010). When PPO activity was evaluated in these same conditions, a 68% and 45% reduction respectively was seen, indicating that oven drying was less destructive to anthocyanins and inhibited PPO activity significantly more than sun drying (Jaiswal, et al., 2010). This less destructive process of oven drying could be due to the longer process of sun-drying (a several month-loing process versus many hours) and that it has been shown that processing above 80^{o}C inhibits PPO reactions – which would be seen in processing such as oven-drying but would not be seen in sun drying (Severini, et al., 2003).

While PPO and its effects on anthocyanins can be quantified, consumers only perceive a change in color. In the food industry, the L*a*b* color matrix is used to systematically assess the color of a product as the consumer would see it (L*representing black/white color scale, a* representing green/red color scale, and b* representing blue/yellow color scale) (Gordillo, et al., 2012). This color matrix can assess change in color over a period of time or in comparison to another product, and this overall change in color is referred to as ΔE (Chong, et al., 2013). This matrix provides more objective data about browning as it relates to consumers perception of products, not just anthocyanins and PPO, although studies have been conducted regarding the L*a*b* color and copigmentation models of anthocyanins to really overlay the chemical and visual components of foods (Gordillo, et al., 2012).

Cranberries (*Vaccinium macrocarpon*)

Information

Cranberries (*Vaccinium macrocarpon)* are a North American fruit that is red in color and astringent in taste. Of the cranberries sold, 95% are in the forms of juices, dried cranberries (snack or baking ingredient), sauces, or cranberry supplements (gummy supplement or spray-dried powder pill), and only 5% are sold as fresh fruit (Grace, et al., 2012). Millennials are seeking healthier food, either on their own accord or through government persuasion like MyPlate initiatives. Fruits with antioxidant properties, like cranberries, have been called “super fruits” (Schaich, 2012), making them a popular ingredient in better-for-you food options. The most common association with cranberries is their role in the maintenance and health of the urinary tract (Howell, 2007). This association is because cranberries are rich in polyphenols, specifically A-type proanthocyanidins which inhibit the adhesion of bacterial cells in the urinary

tract thus aiding in urinary tract health (Krueger, et al., 2013). While many fruits have proanthocyanidins, the A-type proanthocyanidins that cranberries are uniquely rich in help urinary tract health.

While urinary tract health is the cranberry's most well-known health association, cranberries are rich in other compounds that are good for the body and maintaining overall health, such as bioactive polyphenols like anthocyanins. The strength and combination of these compounds in cranberries are suspected to aid in oral health (Feghali, et al., 2012), provide chemoprevention (Caillet, et al., 2012), and aid in the prevention of cardiovascular diseases (Ruel, et al., 2005). There is research quantifying and looking at the polyphenols in fresh cranberries and cranberry juice drinks (Chen & Martynenko, 2016) (Cote, et al., 2011) (Grace, et al., 2012), but many do not cover the stability of these compounds over time, and there is no research looking at these compounds in products such as sweetened dried cranberries, apart from spray-dried powders or freeze-dried cranberries (Leusink, et al., 2010) (Grace, et al., 2012) (Michalska, et al., 2018). While cranberry juice drinks are used as refreshments and beverages, sweetened dried cranberries are used for baking or as a snack, and freeze-dried or spray-dried cranberry powders are a popular option for supplements. The differentiation on the impact of processing on polyphenol stability between the dried cranberry process and the juice making process is an avenue of research that should be further explored. However, there is a lack of research on polyphenols in sweetened dried cranberries and most of the literature on cranberry polyphenols and processing is focused on juice fractionation and processing.

Conclusions

Consumer trends are moving towards bioactive foods, less sugar, and more convenience. The high quantity of polyphenols in fruits such as cranberries make them a good option for millennial consumers, however most research is focused on cranberries as they are used in juice applications. While this form is the most abundant form of cranberries currently, understanding the ability of other cranberry forms to deliver bioactive benefits and viable cranberry polyphenols need to be evaluated as these forms deliver on reduced sugar and more convenience compared to cranberry juice drinks.

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Chapter 2 - Polyphenol Stability and Antioxidant Capacity of Sweetened Dried Cranberries

Abstract

Cranberries are fruits containing phenolic compounds such as anthocyanins and proanthocyanidins. While there is research showing the phenolic composition of cranberry juices and how processing and storage may affect these compounds there is no research on how product matrix and processing affect phenolic compounds in dried cranberries over time. The objectives of this research were to 1) assess the polyphenol content and inherent characteristics of various sweetened dried cranberries, 2) determine the stability of those components and characteristics over time, and 3) assess any relationship between product matrix and polyphenol stability.

This research assessed 5 different commercially available sweetened dried cranberry matrices: 1) sliced soluble corn fiber, glycerin, sucrose, and sucralose infused, 2) sliced apple juice infused, 3) whole apple juice infused, 4) sliced sucrose infused, and 5) whole sucrose infused (3 replicates/treatment). Anthocyanins (HPLC), proanthocyanidins (BL-DMAC and cranberry standard DMAC assay), total phenolic content (Folin-Ciocalteu), antioxidant capacity (DPPH), water activity, moisture content, color, and texture were evaluated over 12 months at 21°C. Data were analyzed by ANOVA ($p<0.05$).

The results demonstrate that sweetened dried cranberry polyphenols are unstable and decline significantly over time regardless of product matrix for anthocyanins, proanthocyanidins, and total phenolic content. As well, antioxidant capacity is negligible compared to fresh cranberries. More research is needed to determine optimal processing parameters for sweetened

dried cranberries to maintain polyphenol stability and antioxidant capacity as healthier food options for consumers.

Introduction

Consumer views on foods have shifted to becoming more health forward and health conscious (Kell, 2016), especially in the United States where chronic diseases such as obesity, diabetes, and heart disease are highly prevalent (Ogden, et al., 2015) (Centers for Disease Control and Prevention, 2011) (Centers for Disease Control and Prevention, 2015). Some of these chronic diseases are a result of under consumption of fruits and vegetables (Centers for Disease Control and Prevention, 2017), with 40% of adults in 2015 admitting to eating fruits and vegetables less than once a day (Centers for Disease Control and Prevention, 2017). These chronic diseases in the United States cause consumers and government bodies to push for healthier food options and move away from heavily processed food products.

One governmental guidance issued was the Center for Nutrition Policy and Promotion (CNPP) created by the USDA in 1994, which aimed to promote a healthy life-style for Americans through diet and nutrition programs (United States Department of Agriculture, 2018). The CNPP started initiatives such as MyPlate aimed to keep obesity and diabetes percentages low in the United States. The MyPlate food guidance system aimed to develop healthy dietary segments by balancing meals with fruits, vegetables, grains, protein, and dairy. The MyPlate initiative recommends that half of a plate should consist of whole fruits and a variety of vegetables (United States Department of Agriculture, 2018).

Even though healthy initiatives such as MyPlate were pushed by the government, obesity and diabetes did not decline as had been the intention of the CNPP (Center for Disease Control

Division of Diabetes Translation, 2018). Healthy options such as fruits are less convenient for consumers than heavily processed, prepackaged foods. Fruit based food manufacturers have not innovated around convenience the same way that vegetable manufactures have. More options are available for prepackaged fresh vegetable options to improve convenience for the consumer, but there are rarely any prepackaged fruits that would be convenient for a consumer. Part of this may be that there is more involved in consuming fresh fruit, and that fresh fruit tends to be more susceptible to chemical reactions than vegetables.

While convenient fresh fruit options may not be avaiable for consumers, dried fruits can provide this convenience while still maintaining the nutrition consumers seek. The dried fruit category is expected to increase at least 5.7% from 2018-2026 due to the consumer need for convenience and healthy food options (Transparency Market Research, 2018). The downside to dried fruits, however, is that when the fruits are dried to remove water, other ingredients are generally added like sugar to increase sweetness in fruits like pineapples and cranberries, which can be seen as negative.

Fruits are rich in bioactive compounds such as flavonoids in berries and resveratrol in grapes (Kris-Etherton, et al., 2002). Cranberries (*Vaccinium macrocarpon*) are fruits composed of bioactive compounds such as anthocyanins and proanthocyanidins, which, in conjunction with other flavan-3-ols, acids and flavonols, make up the overall phenolic content (Blumberg, et al., 2013). Anthocyanins are a class of flavonoid which give pigmentation to fruits and vegetables, usually in the form of reds, blues, purples, and blacks (Steyn, 2009) (Figure 2.1). Proanthocyanidins are a part of the flavonoid group also known as condensed tannins. They are polymers of flavan-3-ols and are commonly consumed parts of the human diet (Gnanavinthan, 2013) (Santos-Buelga & Scalbert, 2000).

The USDA database states that raw cranberries contain 60.42 mg/100 g combined cyanidin and peonidin anthocyanins (Haytowitz, et al., 2018), 30.74 mg/100 g total polyphenols (Haytowitz, et al., 2018), and 354.9 mg/100 g proanthocyanidins (Bhagwat & Haytowitz, 2015). While raw cranberries are rich in bioactive compounds and possess health benefits, consumers do not generally eat cranberries in the raw form, but instead consume them primarily as juices, sweetened dried cranberries (snack or baking ingredient), sauces, or cranberry supplements (Grace, et al., 2012). During production of these products, the cranberries are subjected to high heat which can cause degradation of bioactive compounds. Thermal processing of blueberry purees and blueberry juice (clarified by centrifugation at 6000 *x g* for 10 min) has shown that processing decreases anthocyanin content (Cesa, et al., 2017) (Brownmiller, et al., 2008). In cranberry juice processing, blanching of the fruit resulted in significant reduction of total anthocyanin content (White, et al., 2011). Three different processing conditions were looked at prior to the addition of pectinase and juice clarification, one in which ground fruit was blanched at 95ºC for 3 min, one in which ground fruit was unblanched, and one in which whole fruit was blanched at 95ºC for 3 min, and it was found that anthocyanins were retained at 39%, 53.1%, and 42.4% respectively (compared to the frozen initial fruit) signifying that thermal processing of any kind leave anthocyanins subject to degradation (White, et al., 2011). While there is research which shows processing effects on cranberry juice polyphenols, there is little available information on processing effects in relation to sweetened dried cranberries.

While there is evidence that raw cranberries possess bioactive compounds, research is needed to evaluate how processing affects the bioactive compounds that are abundant in raw cranberries. The objectives of this research were to 1) assess the polyphenol content and inherent characteristics of various sweetened dried cranberries, 2) determine the stability of those

components and characteristics over time, and 3) assess any relationship between product matrix and polyphenol stability.

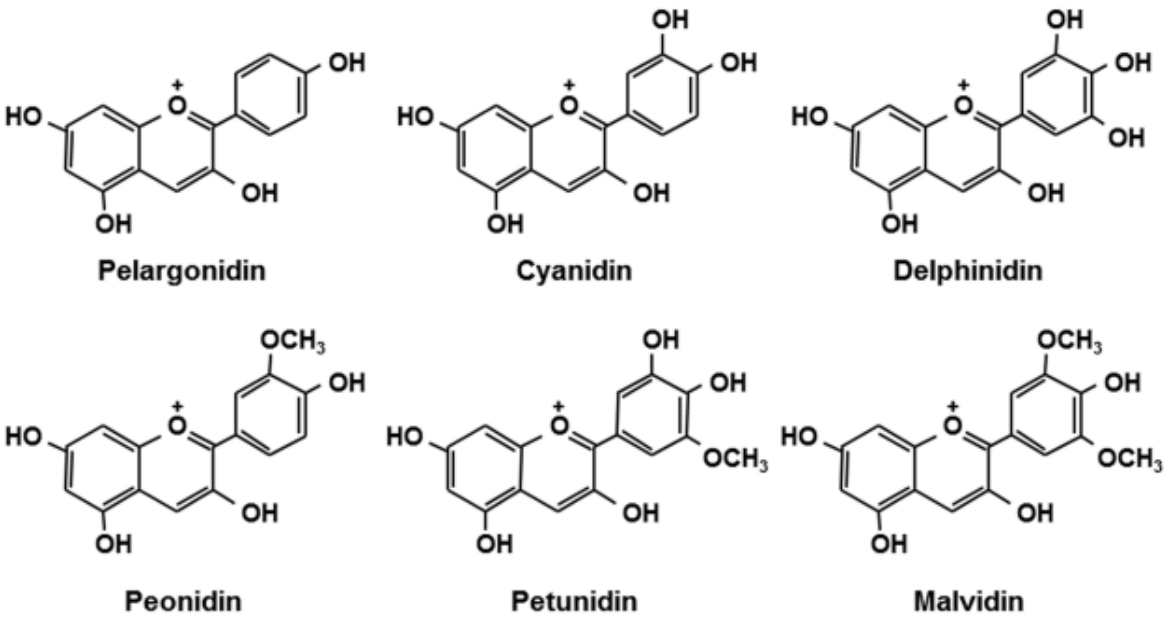

Figure 2.1 Structure of anthocyanidins

Materials and Methods

This study evaluated 2 structures of sweetened dried cranberries across 3 ingredient matrices (Table 2.1). Commercially made 11.34 kg boxes of each treatment were obtained from Ocean Spray Cranberries (Lakeville-Middleboro, MA) within 3 weeks of production from the same plant and repacked into 0.34 oz heat sealed bags with oxygen barrier. Treatments were stored for 360 days at 21^{o}C in a sealed box to reduce light exposure. Analytical measurements were taken on the treatments initially (t=0), then weekly for the first month, and then monthly until 360 days were obtained, except for antioxidant activity which was assessed at 5 time points

due to low antioxidant activity. Analyses were terminated prior to 360 days if two consecutive time points recorded no quantifiable polyphenols. At each time point 3 replicates were analyzed for each treatment. Reagents were analytical grade and were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted.

Table 2.1 Sweetened Dried Cranberries Sample Overview

TREATMENT	STRUCTURE	INGREDIENTS
SAJ	Sliced	Cranberries, Apple Juice Concentrate
WAJ	Whole	Cranberries, Apple Juice Concentrate
SCFG	Sliced	Cranberries, Soluble Corn Fiber, Sugar, Glycerin, Sucralose
SSDC	Sliced	Cranberries, Sugar
WSDC	Whole	Cranberries, Sugar

Proanthocyanidin Content

Proanthocyanidin Extraction

Replicates were introduced to liquid nitrogen and ground into a fine powder. The powdered sample (5 g) was placed into a centrifuge tube along with 15 mL extraction solution (75% acetone, 24.5% deionized water, 0.5% acetic acid (v/v)). The test tube was vortexed for 10 s, sonicated in a Branson ultrasonic water bath (Danbury, CT) for 15 min, and then centrifuged at 7954 *x g* for 20 min. The supernatant was removed from the pellet and placed into a 50 mL Falcon tube. The extraction was repeated two more times with the extraction solution added to the pellet each time and the supernatants combined. After the final extraction each replicate was placed in a glass tube in a Buchi Syncore extraction device (Buchi AG, Flawil, Switzerland) and

placed under vacuum at 45ºC for 5 h. Vacuum settings were based on a five step gradient where 1) 450 mbar – 350 mbar for 10 min 2) 350 mbar – 300 mbar for 5 min 3) 300 mbar – 265 mbar for 105 min 4) 265 mbar – 125 mbar for 80 min 5) 125 mbar – 25 mbar for 100 min. A vacuum pump was connected to a recirculating chiller set to -10ºC.

Proanthocyanidin Content – BL-DMAC

Treatments were assessed for proanthocyanidin content using an A2 dimer procyanidin standard following a modified method of Prior et al (2010). For proanthocyanidin analysis a Precision XS with 96 well plate (Bio-Tek Instruments, Inc., Winooski, VT) was used for serial dilutions. A2 dimer standard was made by taking 5 mg of procyanidin in a 50 mL volumetric flask and bringing up to volume with ethanol. Using a 96-well plate, 140 µL of blank (80% ethanol in deionized water), A2 dimer standard, and the replicate were loaded onto the first column. Precision XS performed serial dilutions by taking 70 µL of blank solution and filling all columns (7 in total). Taken from each well was 70 µL and then mixed with the next well in the series, with the last 70 µL from the final column being discarded so that a final working volume of 70 µL was in each cell. 4-dimethylaminocinnamaldehyde (DMAC) (Sigma-Aldrich, St. Louis, MO) (210 µL) (0.1 DMAC powder in 100 mL 75% ethanol, 12.5% HCl, and 12.5% deionized water (v/v)). The well plate was loaded into Synergy 2 microplate reader with GEN5 software (Bio-Tek Instruments, Inc., Winooski, VT) and analyzed at 25ºC and 640 nm every min for 30 min. A calibration curve was generated from the A2 standard used. The concentration of proanthocyanidin in each replicate was determined using a calibration curve (A2 dimer standard absorbance versus A2 dimer concentration) regression line and the below equation where *c* is the concentration of proanthocyanidin in the extraction (g/L), *d* is the dilution factor, *v* is the volume

of the extraction fluid after vacuum (mL), and *w* is the weight of the replicate used for extraction (g).

$$PAC = \frac{c\ x\ d\ x\ v}{(1000\ x\ w)}$$

Proanthocyanidin Content – Cranberry Standard Assay

Treatments were assessed for proanthocyanidin content using a modified method of Krueger et. al (2016). Bio-Rad Poly-prep® columns (Hercules, CA) (2 per replicate) were set up in column racks and pre-hydrated lipophilic Sephadex® LH20 (22%) (Sigma-Aldrich, St. Louis, MO) was added to the column to a volume of 1.2 mL. Deionized water was then added to the column to 7.5 mL. Once the column was drained, 1 g of replicate was added to the column. Deionized water was then added to the top of the column. Once the column was drained, 10 mL 25% reagent alcohol in deionized water was added to the column and allowed to drain. The columns were then transferred to 15 mL Falcon tubes and washed twice with 2.5 mL 70% acetone in deionized water. The liquid collected in the Falcon tube was then vortexed for 5 s. The liquid (1 mL) was added to glass test tubes by auto-pipette using 70% acetone as the blank. DMAC solution (3 mL) (0.1% DMAC) (Sigma-Aldrich, St. Louis, MO) in HCl:Methanol (30:70) was transferred by autopipette to a test tube and vortexed for 5 s. After 3 min the liquid was vortexed for 3 s. After 5 min the absorbance of the sample was read spectrophotometrically (640 nm). Proanthocyanidin content was calculated using the below equation where ABS is the absorbance, RF is the response factor, *w* is the weight of the replicate loaded onto the column, *d* is the dilution factor if used (Replicate Weight/Water + Replicate Weight), and %S is the percent solids (100-Moisture Content). Proanthocyanidin content was then converted to mg/g. Replicates

were diluted prior to analysis so that an absorbance reading between 0.2 and 0.8 was obtained when read spectrophotometrically.

$$\frac{ug\ PAC}{g\ DWB} = \frac{\frac{ABS\ x\ 5}{RF\ x\ w\ x\ d\ x\ \%S}}{0.95}$$

Anthocyanin Content

An Agilent 1260 HPLC (Agilent Technologies, Santa Clara, CA) was used for HPLC analysis of anthocyanins (Brown and Shipley 2011). Replicates were added to liquid nitrogen and ground until a fine powder was obtained. The powder (1.5 g) was added to 20 mL of 2% HCl:Methanol (2:98). The solution was sonicated in a Branson ultrasonic water bath (Danbury, CT) for 15 min, shaken for 30 min, and centrifuged at 2324 *x g* for 5 min. The supernatant was removed from the pellet and used in HPLC analysis.

The HPLC was run with 2 mobile phases (v/v) consisting of A) deionized water and o-phosphoric acid (99.5:0.5) and B) deionized water, acetonitrile, acetic acid, and o-phosphoric acid (50:48.5:1.0:0.5). Each replicate was analyzed via HPLC for 35 min with a 10 µL injection volume, 1.0 mL/min flow rate, and the absorbance was read at 520 nm and 25ºC, using a Water X-Select HSS T3 5µm, 4.6 x 150 mm reversed-phase C18 column. Replicates were analyzed for cyanidin-3-galactoside (cy-3-gal), cyanidin-3-glucoside (cy-3-glu), cyanidin-3-arabinoside (cy-3-arab), peonidin-3-galactoside (peo-3-gal), peonidin-3-glucoside (peo-3-glu), and peonidin-3-arabinoside (peo-3-arab). Standards for cy-3-gal, cy-3-glu, cy-3-arab, and peo-3-glu were obtained via Phytolab (Vestenbergsgreuth, Germany). Peo-3-glu and peo-3-arab were reported as peo-3-glu equivalents due to limited commercial manufacturing of the standards and the retention times were based on those validated by Brown & Shipley (2011). The minimum

detection limit for cyanidins was 0.02 µg/mL and for peonidins 0.01 µg/mL. Flow rate was kept consistent throughout the run (1.0 mL/min) and ratio of mobile phase A to mobile phase B was decreased for the first 32 min and then increased to the initial settings for the remaining 3 min. The anthocyanin content of each sample was calculated using Agilent Open lab software (Agilent Technologies, Santa Clara, CA) based on the extraction dilution and reported as ppm.

Total Phenolic Content

Total phenolic content was measured using the Folin-Ciocalteu colorimetric assay (Singleton & Rossi, 1965) (Asami, et al., 2003). Each replicate (10 g) was placed in a Warren blender with 90 g deionized water and blended on the low setting for 3 min. Each replicate was analyzed in duplicate and deionized water was used as the blank. Diluted replicates and blank (100 µL) were pipetted into a glass test tube. Deionized water (3.9 mL) was added to each test tube and vortexed for 5 s. Folin-Ciocalteu reagent (5 mL 2N Folin-Ciocalteu Phenol Reagent in 50 mL deionized water) (250 µL) was added to the test tube and vortexed for 5 s. Sodium carbonate solution (7.5% sodium carbonate anhydrous [Sigma-Aldrich, St. Louis, MO] in deionized water) (750 µL) was added to each test tube and vortexed for 5 s. The replicates were stored in the dark for 30 min and absorbance was read spectrophotometrically (765 nm). Gallic acid (Sigma-Aldrich, St. Loius, MO) was used to create a standard calibration curve where 0.5% gallic acid solution was prepared and diluted to 0, 10, 50, 100, 150, and 200 mg/L of gallic acid and the standard curve was made by plotting absorbance versus concentration. Total phenolic content of the replicates was determined by using the gallic acid calibration curve, dilution factor of the replicate, and the moisture content of the replicate to report total phenolic content as mg/g gallic acid equivalent (GAE).

Antioxidant Activity

Antioxidant activity was assessed using a reaction with 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) (AOAC 2012.04). Each replicate was introduced to liquid nitrogen and ground in a to a fine powder. The powder (15 g) was added to a 100 mL volumetric flask with 50 mL 95% Ethanol. The solution was shaken on a shaker table for 2 h then filtered through Whatman #4 filter paper. Solutions were diluted to 0.1, 0.5, 1.0, 2.0, 5.0, and 7.0 g/mL in 95% ethanol and vortexed. Each solution (0.1 mL) was placed in a glass test tube with 0.1 mL 95% ethanol as a control. DPPH (Sigma-Aldrich, St. Louis, MO) solution (0.0394 g DPPH in 1000 mL methanol) was added to the test tube (2.9 mL) and vortexed for 5 s. Dilutions were stored in the dark for 30 min and read spectrophotometrically (517 nm). Deionized water was used as the blank. Percentage radical scavenging activity was calculated with the below equation.

$$DPPH\ Scavenging\ Activity\ (\%) = \frac{ABS(control) - ABS(sample)}{ABS(control)}\ x\ 100$$

The concentration of antioxidant required to inhibit 50% of the oxidation reaction (IC_{50}) was calculated by plotting % DPPH scavenging activity against the concentration and using the logarithmic trendline. Equations for these calculations are below where *g replicate* (g) is the weight of the fine powder used in the extraction and *mg dilution* (mg) is the weight of the serial dilutions. For the IC_{50} calculation *a* is the slope of the natural logarithmic tread line based on the plot of % scavenging versus concentration, and *b* is the y-intercept of the natural logarithmic tread line based on the plot of % scavenging versus concentration.

$$Concentration\frac{mg}{mL} = \left(\frac{g\ replicate}{50\ mL}\ x\ 1000\right) x\ (mg\ Dilution)$$

$$IC50{:}\ 50 = aln(x) + b$$

Water Activity and Moisture Content

Water Activity

Water activity (A_w) was measured using a calibrated Aqua Lab 4TE (Meter Group Inc., Pullman, WA). Each replicate was measured in triplicate.

Moisture Content

Moisture content was assessed via Karl-Fischer titration using a calibrated Metrohm KF 901 Titrando auto-titrator (Metrohm, Herisau, Switzerland). Each replicate (5 g) was added to a stainless-steel homogenization flask and 100 g Karl-Fischer Grade low water methanol was added. The replicate was then homogenized for 5 min using an Omni Mixer (Omni International, Kennesaw, GA) set to speed 4. After homogenization, the flask was disconnected and covered with parafilm. The liquid sat undisturbed for 5 min before being analyzed for moisture content. Each replicate was then taken into a 3 mL syringe and was run through the auto-titrator. Each replicate was run in triplicate.

Colorimetric and Texture Analyses

Color

Color was analyzed using a calibrated handheld Konica Minolta CR-410 Colorimeter (Tokyo, Japan). Each replicate was read 5 times by the handheld colorimeter, which was rotated

90° between readings and the average of the readings was reported for L*, a*, and b* values which were used to calculate ΔE according to the below calculation. Each replicate was read in triplicate.

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

Texture

Texture was analyzed using a calibrated TA.XT.Plus from Texture Technologies Corporation (Hamilton, MA). The TA.XT.Plus was configured with a TA-30 cylinder probe (3" diameter aluminum cylinder, 10 mm height), 0.50 mm/s pre-test speed, 0.5 mm/s test speed, 10 mm/s post-test speed, 500 g applied force, 10 mm return distance, and 10 s contact time. Each replicate (25 g) was loaded onto the rounded base plate and the test was run. Adhesion force (g) was recorded by the texture analysis software (Exponent 32, version 6, Texture Technologies Cop, Scarsdale, NY).

Statistical Analyses

Data were analyzed using Minitab 16 (State College, PA) for analysis of variance (ANOVA) for all treatments. Shapiro-Wilk test for normality was assessed. Tukey's Honest Significant Difference Test (HSD) was used for post-hoc analyses. Prior to ANOVA significance level of $p<0.05$ was chosen.

Results and Discussion

Proanthocyanidin Content

All treatments had a significant ($p<0.05$) decrease in proanthocyanidin content by both BL-DMAC and DMAC with cranberry standard analyses indicating that proanthocyanidins are unstable in sweetened dried cranberries over time regardless of product matrix (Table 2.3) (Table 2.4) (Figure 2.2) (Figure 2.3). In all treatments, cranberry standard proanthocyanidin values were significantly higher and degraded less than BL-DMAC values (both initially and finally). While both DMAC assays show the same pattern of degradation across treatments, BL-DMAC assay values are significantly lower in all treatments than those seen using cranberry standard DMAC assay. Other studies in which both assays were used (Martin, et al., 2015) (Krueger, et al., 2016) showed similar results in which BL-DMAC assay yielded proanthocyanidin values significantly lower than cranberry standard DMAC. Cause for the lower proanthocyanidin values via BL-DMAC was hypothesized to be because the cranberry standard assay utilized a standard derived from the cranberry fruit as opposed to BL-DMAC which uses an A2 dimer standard. Cranberries have proanthocyanidins that are more complex than those seen with procyanidin A2 dimer, so using A2 dimer standard leads to underestimation of proanthocyanidin content, as cranberries not only contain simple dimer proanthocyanidins, but also more complex oligomers that would not be quantified using A2 dimer standard (Krueger, et al., 2016). Despite BL-DMAC being the industry standard for proanthocyanidin analysis, products containing complex oligomer proanthocyanidins such as cranberry should be analyzed using cranberry standard DMAC assay so that all proanthocyanidins in the sample are quantified.

When analyzed using BL-DMAC, proanthocyanidin content in sweetened dried cranberries was comparable to values seen by Blumberg et., al (2013). SCFG had the

significantly highest initial proanthocyanidin content (1.04 mg/g) while WAJ had significantly the lowest (0.30 mg/g). Sliced treatments resulted in significantly higher proanthocyanidin content (initial and final) than whole treatments. Slicing of the fruit prior to thermal processing allows for higher infusion rate due to larger infusion surface area which introduces more polyphenols. SCFG had significantly higher initial and final proanthocyanidin content compared to other sliced treatments indicating that bulking agents in SCFG such as soluble corn fiber and glycerin may have insulating effects compared to sucrose and apple juice and thus reduce degradation of polyphenols during processing. A study on sour cherry puree found that natural sweeteners such as palm sugar, erythritol, xylitol, and other agents such as inulin, inhibited polyphenol degradation, which could be the case in the SCFG treatment (Nowicka & Wojdylo, 2016). Overall degradation of proanthocyanidin (88.3% in SAJ, 87.8% in WAJ, 92.8% in SSDC, 100% in WSDC, and 87.8% in SCFG) indicate that proanthocyanidins are extremely unstable in sweetened dried cranberries over time. The most significant decrease in proanthocyanidins occurred during the first 90 days (57.1% decrease in SAJ, 56.2% decrease in WAJ, 24.9% decrease in SSDC, 91.4% decrease in WSDC, and 76.9% decrease in SCFG) possibly due to oxidation reactions and polymerization of proanthocyanidins which would be steepest early on in shelf life when higher proanthocyanidin content is viable.

When analyzed using cranberry standard DMAC assay, SCFG had the significantly highest initial proanthocyanidin (3.74 mg/g) while WSDC had the significantly lowest (1.10 mg/g). As previously discussed, slicing resulted in significantly higher proanthocyanidin content (initial and final) than whole treatments. As previously discussed, SCFG had the significantly highest initial and final proanthocyanidin content due to insulation effects during processing from the bulking agents (soluble corn fiber and glycerin) compared to other sliced treatments

(Nowicka & Wojdylo, 2016). Overall degradation of proanthocyanidins using cranberry standard DMAC assay (67.8% in SAJ, 74.5% in WAJ, 82.3% in SSDC, 91.6% in WSDC, and 70.2% in SCFG) indicate that proanthocyanidins are extremely unstable in sweetened dried cranberries over time.

Despite higher proanthocyanidin values using cranberry standard, compared to raw cranberries (3.59 mg/g proanthocyanidins via BL-DMAC) none of the treatments had a final proanthocyanidin content comparable to raw cranberries, and therefore do not contribute a significant amount of proanthocyanidins (Bhagwat & Haytowitz, 2015). Treatments with the largest initial proanthocyanidin content (despite analytical method used to determine content) had the highest rates of degradation during storage (Table 2.2). The continued degradation of proanthocyanidins during storage indicates that while proanthocyanidins do degrade during processing, oxidation reactions and polymerization of proanthocyanidins during storage leads to further degradation, with increases in rates of degradation dependent on content of proanthocyanidins in the food.

Table 2.2 Rate of change of sweetened dried cranberries

TREATMENT	SLICED APPLE JUICE INFUSED (SAJ)	WHOLE APPLE JUICE INFUSED (WAJ)	SLICED SUCROSE INFUSED (SSDC)	WHOLE SUCROSE INFUSED (WSDC)	SLICED SOLUBLE CORN FIBER, GLYCERIN, SUCROSE, AND SUCRALOSE INFUSED (SCFG)
Proanthocyanidin Content (BL-DMAC Assay)	$-0.0014^{ab} \pm 0.0002$	$-0.0007^{a} \pm 0.0002$	$-0.0016^{b} \pm 0.0004$	$-0.0023^{c} \pm 0.0002$	$-0.0025^{c} \pm 0.0003$
Proanthocyanidin Content (Cranberry Standard Assay)	$-0.0036^{ab} \pm 0.0007$	$-0.0029^{a} \pm 0.0004$	$-0.0058^{bc} \pm 0.0003$	$-0.0028^{a} \pm 0.0000$	$-0.0073^{c} \pm 0.0018$
Anthocyanin Content	$-0.0590^{c} \pm 0.0012$	$-0.0345^{b} \pm 0.0010$	$-0.1089^{d} \pm 0.0041$	$-0.0087^{a} \pm 0.0003$	$-0.1977^{e} \pm 0.0080$
Total Phenolic Content	$-0.0052^{b} \pm 0.0002$	$-0.0093^{d} \pm 0.0012$	$-0.0010^{a} \pm 0.0002$	$-0.0008^{a} \pm 0.0007$	$-0.0072^{c} \pm 0.0000$
A_w	$-0.0000^{b} \pm 0.0000$	$-0.0001^{c} \pm 0.0000$	$0.0001^{a} \pm 0.0000$	$0.0001^{a} \pm 0.0000$	$-0.0000^{b} \pm 0.0000$
Moisture Content	$-0.0044^{b} \pm 0.0014$	$0.0052^{b} \pm 0.0013$	$0.0009^{ab} \pm 0.0048$	$0.0063^{a} \pm 0.0040$	$-0.0061^{b} \pm 0.0024$
ΔE	$0.0094^{bc} \pm 0.0009$	$0.0119^{b} \pm 0.0016$	$0.0067^{c} \pm 0.0012$	$0.0092^{c} \pm 0.0019$	$0.0188^{a} \pm 0.0003$
Adhesion Force	$0.0423^{a} \pm 0.1240$	$-0.0091^{a} \pm 0.1101$	$0.0608^{a} \pm 0.0287$	$0.0985^{a} \pm 0.0372$	$-0.2986^{b} \pm 0.0925$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 2.3 Proanthocyanidin content (BL-DMAC Assay) of sweetened dried cranberries over time

TIME (DAYS)	SLICED APPLE JUICE INFUSED (SAJ)	WHOLE APPLE JUICE INFUSED (WAJ)	SLICED SUCROSE INFUSED (SSDC)	WHOLE SUCROSE INFUSED (WSDC)	SLICED SOLUBLE CORN FIBER, GLYCERIN, SUCROSE, AND SUCRALOSE INFUSED (SCFG)
0	$0.56^{aB} \pm 0.07$	$0.30^{bC} \pm 0.06$	$0.61^{cB} \pm 0.12$	$0.84^{aA} \pm 0.08$	$1.04^{aA} \pm 0.12$
7	$0.48^{aC} \pm 0.06$	$0.41^{aC} \pm 0.07$	$0.89^{bA} \pm 0.03$	$0.62^{bB} \pm 0.04$	$0.59^{bB} \pm 0.08$
14	$0.51^{aB} \pm 0.08$	$0.29^{bC} \pm 0.04$	$0.90^{bA} \pm 0.03$	$0.53^{bB} \pm 0.05$	$0.53^{bcB} \pm 0.05$
21	$0.44^{abB} \pm 0.12$	$0.25^{bcC} \pm 0.01$	$1.19^{aA} \pm 0.09$	$0.33^{cC} \pm 0.04$	$0.55^{bcB} \pm 0.04$
28	$0.41^{abB} \pm 0.10$	$0.26^{bcC} \pm 0.04$	$0.96^{bA} \pm 0.04$	$0.22^{dC} \pm 0.03$	$0.44^{cdB} \pm 0.01$
60	$0.31^{bcB} \pm 0.05$	$0.18^{cdC} \pm 0.03$	$0.50^{cdA} \pm 0.05$	$0.11^{eC} \pm 0.03$	$0.31^{deB} \pm 0.01$
90	$0.24^{cdB} \pm 0.03$	$0.13^{deC} \pm 0.02$	$0.44^{deA} \pm 0.04$	$0.07^{efC} \pm 0.02$	$0.24^{efB} \pm 0.02$
120	$0.28^{bcdA} \pm 0.03$	$0.14^{deB} \pm 0.02$	$0.33^{efA} \pm 0.03$	$0.03^{efC} \pm 0.01$	$0.27^{eA} \pm 0.02$
150	$0.24^{cdA} \pm 0.02$	$0.09^{defB} \pm 0.01$	$0.22^{fgA} \pm 0.01$	$0.05^{efB} \pm 0.03$	$0.24^{efA} \pm 0.01$
180	$0.21^{cdeA} \pm 0.01$	$0.13^{deB} \pm 0.03$	$0.19^{fghA} \pm 0.02$	$0.05^{efC} \pm 0.01$	$0.21^{efA} \pm 0.01$
210	$0.22^{cdeA} \pm 0.04$	$0.13^{deB} \pm 0.00$	$0.20^{fghA} \pm 0.02$	$0.03^{efC} \pm 0.00$	$0.23^{efA} \pm 0.05$
240	$0.21^{cdeA} \pm 0.00$	$0.09^{efB} \pm 0.00$	$0.18^{ghiA} \pm 0.02$	$0.02^{fB} \pm 0.00$	$0.21^{efA} \pm 0.01$
270	$0.20^{cdeA} \pm 0.02$	$0.06^{efB} \pm 0.01$	$0.09^{ghiB} \pm 0.01$	$0.00^{fB} \pm 0.00$	$0.21^{efA} \pm 0.02$
300	$0.20^{cdeA} \pm 0.02$	$0.03^{fB} \pm 0.01$	$0.09^{ghiB} \pm 0.01$	$0.00^{fC} \pm 0.00$	$0.23^{efA} \pm 0.02$
330	$0.11^{deA} \pm 0.02$	$0.02^{fC} \pm 0.00$	$0.08^{hiAB} \pm 0.01$	$0.00^{fC} \pm 0.00$	$0.12^{fA} \pm 0.01$
360	$0.07^{eB} \pm 0.01$	$0.04^{fC} \pm 0.00$	$0.04^{iC} \pm 0.01$	$0.00^{fD} \pm 0.00$	$0.13^{fA} \pm 0.01$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

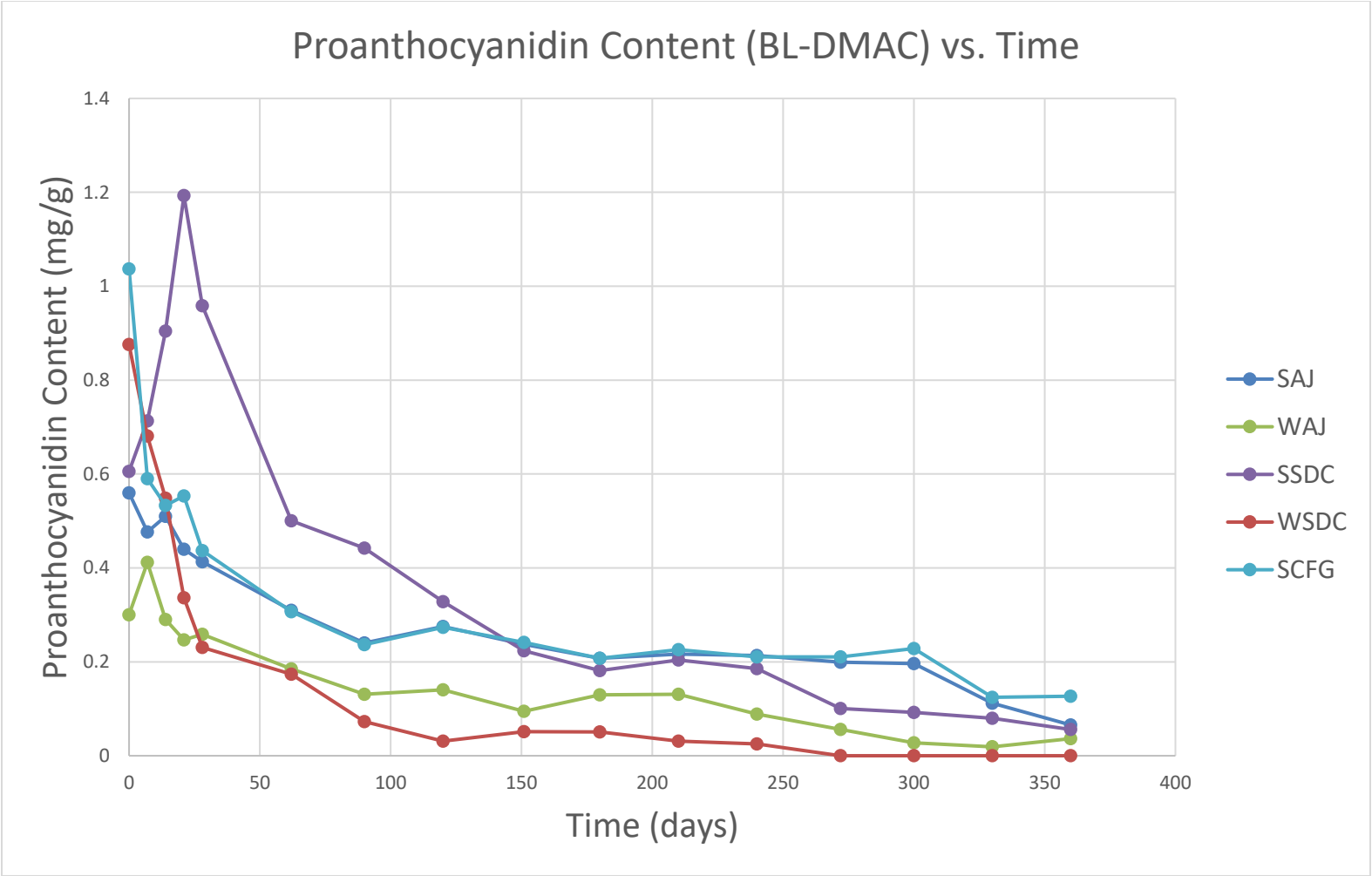

Figure 2.2 Proanthocyanidin content (BL-DMAC Assay) versus time of sweetened dried cranberries

Table 2.4 Proanthocyanidin content (Cranberry Standard) of sweetened dried cranberries over time

TIME (DAYS)	SLICED APPLE JUICE INFUSED (SAJ)	WHOLE APPLE JUICE INFUSED (WAJ)	SLICED SUCROSE INFUSED (SSDC)	WHOLE SUCROSE INFUSED (WSDC)	SLICED SOLUBLE CORN FIBER, GLYCERIN, SUCROSE, AND SUCRALOSE INFUSED (SCFG)
0	$1.89^{aBC} \pm 0.16$	$1.42^{bC} \pm 0.13$	$2.54^{cdB} \pm 0.14$	$1.10^{aC} \pm 0.05$	$3.74^{aA} \pm 0.63$
7	$1.85^{aB} \pm 0.24$	$1.83^{aB} \pm 0.19$	$2.67^{cA} \pm 0.014$	$1.01^{aC} \pm 0.04$	$2.86^{bA} \pm 0.35$
14	$2.13^{aB} \pm 0.34$	$1.36^{bC} \pm 0.23$	$2.96^{cA} \pm 0.13$	$0.87^{bD} \pm 0.02$	$2.71^{bcA} \pm 0.42$
21	$1.80^{abC} \pm 0.41$	$1.13^{bcdD} \pm 0.03$	$3.57^{abA} \pm 0.45$	$0.76^{bcD} \pm 0.01$	$2.83^{bB} \pm 0.29$
28	$1.60^{abcC} \pm 0.26$	$1.15^{bcdD} \pm 0.19$	$3.90^{aA} \pm 0.19$	$0.73^{cE} \pm 0.02$	$2.10^{cdB} \pm 0.08$
60	$1.20^{cdB} \pm 0.14$	$1.33^{bcB} \pm 0.06$	$3.08^{bcA} \pm 0.26$	$0.68^{cC} \pm 0.02$	$1.60^{deB} \pm 0.06$
90	$1.01^{deC} \pm 0.09$	$1.09^{bcdeC} \pm 0.08$	$2.06^{deA} \pm 0.20$	$0.65^{cdD} \pm 0.05$	$1.30^{efB} \pm 0.05$
120	$1.12^{cdC} \pm 0.02$	$0.90^{deC} \pm 0.11$	$1.91^{efA} \pm 0.30$	$0.68^{cD} \pm 0.06$	$1.44^{defB} \pm 0.09$
150	$1.29^{bcdA} \pm 0.05$	$0.73^{efB} \pm 0.04$	$1.24^{ghA} \pm 0.05$	$0.36^{fgC} \pm 0.08$	$1.41^{defA} \pm 0.05$
180	$1.12^{cdB} \pm 0.07$	$0.96^{cdeB} \pm 0.08$	$1.11^{ghB} \pm 0.10$	$0.34^{gC} \pm 0.05$	$1.44^{defA} \pm 0.04$
210	$1.12^{cdC} \pm 0.12$	$1.06^{deC} \pm 0.08$	$1.47^{fgA} \pm 0.10$	$0.44^{efgD} \pm 0.05$	$1.26^{efB} \pm 0.08$
240	$0.93^{deB} \pm 0.05$	$0.46^{fgC} \pm 0.04$	$1.22^{ghA} \pm 0.06$	$0.51^{eC} \pm 0.01$	$1.15^{efA} \pm 0.04$
270	$0.83^{deB} \pm 0.07$	$0.40^{fgC} \pm 0.02$	$1.07^{ghA} \pm 0.08$	$0.55^{deC} \pm 0.02$	$1.12^{efA} \pm 0.12$
300	$0.62^{eC} \pm 0.04$	$0.32^{gD} \pm 0.03$	$1.01^{ghA} \pm 0.04$	$0.48^{eD} \pm 0.02$	$0.88^{fB} \pm 0.06$
330	$0.61^{eB} \pm 0.06$	$0.36^{gC} \pm 0.01$	$0.85^{hiA} \pm 0.01$	$0.46^{efC} \pm 0.02$	$0.82^{fA} \pm 0.04$
360	$0.61^{eB} \pm 0.10$	$0.37^{fgC} \pm 0.00$	$0.45^{iBC} \pm 0.06$	$0.08^{hD} \pm 0.02$	$1.12^{efA} \pm 0.11$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

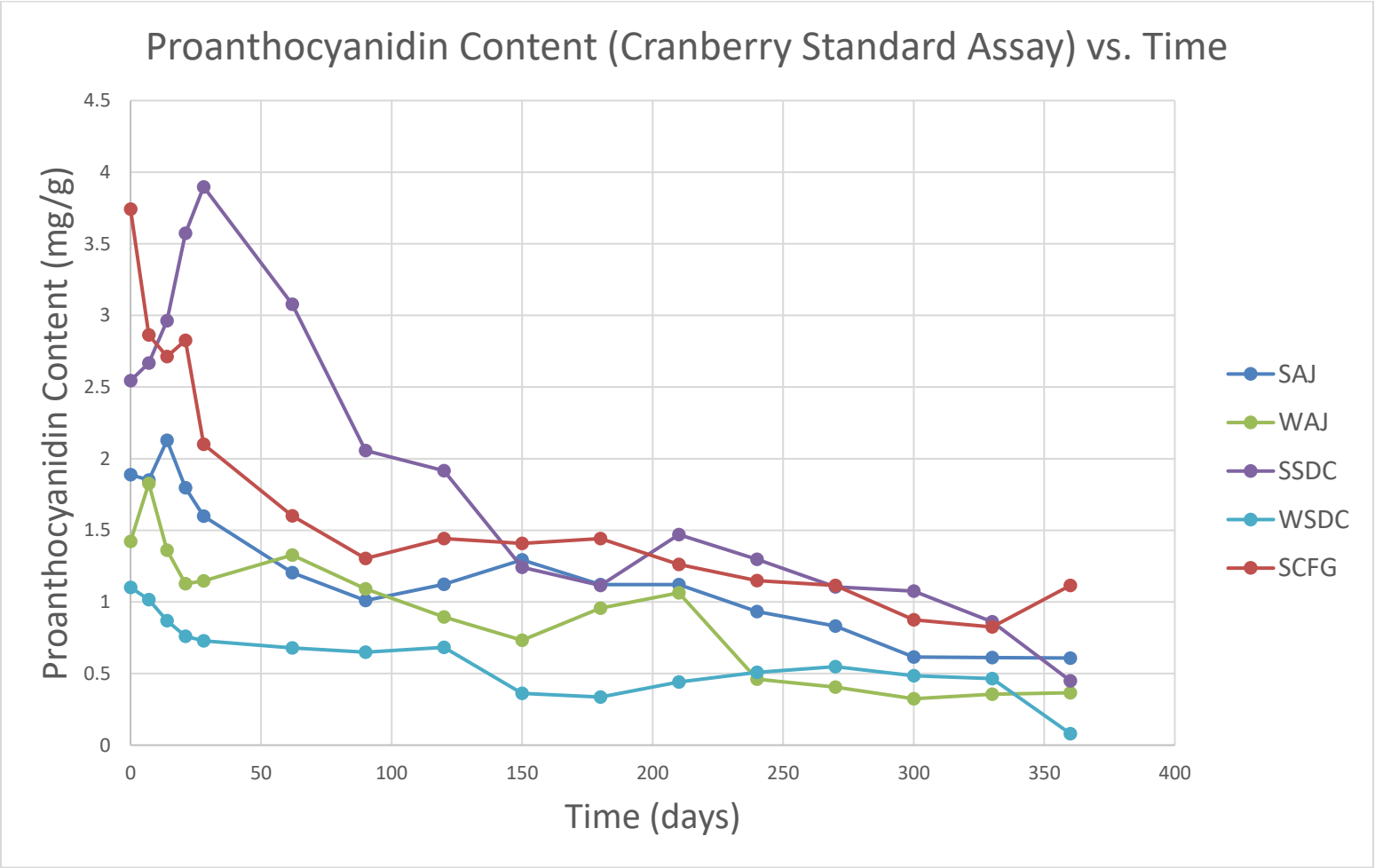

Figure 2.3 Proanthocyanidin Content (Cranberry Standard Assay) versus time of sweetened dried cranberries

Anthocyanin Content

For all treatments there was a significant ($p<0.05$) decrease in anthocyanins indicating that anthocyanins significantly degrade over time regardless of product matrix (Table 2.5) (Figure 2.4). Initial anthocyanin content in sweetened dried cranberries was comparable to values reported in Blumberg et., al (2013). As previously discussed with proanthocyanidins, slicing resulted in a significantly higher anthocyanin content (both initial and final) compared to whole treatments. Both whole treatments at the end of the study had no quantifiable anthocyanin content. Whole cranberries are larger individual pieces than sliced cranberries, so it is possible that a combination of higher heat and longer drying time to achieve the same A_w caused the significant reduction in anthocyanins. Anthocyanins are susceptible to heat degradation so increased time in the dryer or increased drying temperature in the whole treatments could significantly lower the anthocyanin content compared to the sliced treatments. In a study comparing the anthocyanin content of raw plums to prunes, high drying temperatures led to degradation of anthocyanins (Piga, et al., 2003) which was mirrored in this study. SCFG had the highest initial anthocyanin content (72.61 ppm) while WSDC had the lowest initial anthocyanin content (3.14 ppm). As previously discussed, SCFG bulking agents such as soluble corn fiber and glycerin may have insulated some of the anthocyanin compounds during processing, resulting in significantly higher initial and final anthocyanin values compared to other sliced treatments (Nowicka & Wojdylo, 2016).

Overall degradation of anthocyanins (93.5% SAJ, 100% WAJ, 97.8% SSDC, 100% WSDC, and 98% SCFG) indicate that anthocyanins in sweetened dried cranberries are unstable and decrease more than proanthocyanidins. Flavonol monomer polymerization induced by oxidation via processing produces proanthocyanidins (Beecher, 2003), so as monomers are

polymerized proanthocyanidins may appear more stable than anthocyanins, which are not formed during this polymerization. While degradation of anthocyanins is a result of drying conditions such as time and temperature, further degradation occurs during storage indicating that enzymatic oxidation reactions occur and degrade anthocyanins further during storage. The rates of degradation during storage indicate that treatments with lower initial anthocyanin content degraded at a significantly slower rate than those with a higher anthocyanin content (Table 2.2). Treatments with higher anthocyanin content had a higher chance of reactions between anthocyanin molecules than those with a lower initial anthocyanin content, resulting in the difference in rates. Despite the difference in rate of degradation between treatments, copigmentation in sweetened dried cranberries does not provide stability in the lower quantities of anthocyanins displayed in this study. Copigmentation occurs when anthocyanin molecules stack on top of each other providing insulation of compounds from oxidation reactions which may degrade anthocyanins (Gomez-Miguez, et al., 2006) (Rustioni, et al., 2012). At low levels of anthocyanins seen in the treatments, there is not a large enough quantity of anthocyanins for stacking and copigmentation to prevent degradation of the anthocyanins.

Compared to raw cranberries (604.2 ppm anthocyanins), sweetened dried cranberries are not a source of anthocyanins (Haytowitz, et al., 2018). HPLC spectra indicate that across treatments, after 360 days cyanidin-3-glucoside and peonidin-3-glucoside were not present, indicating that these anthocyanin compounds are the most susceptible to degradation (Figures 2.5-2.14). SSDC was the only treatment with cyanidin-3-glucoside present in the initial sample. In soybean crackers it was reported that cyanidin-3-glucoside was degraded at an average of 70.3% during high temperature with short baking time (Slavin, et al., 2013). Thermal processing of sweetened dried cranberries could have also resulted in this loss of cyanidin-3-glucoside,

which is why it was not present in most treatments. As previously stated sliced treatments like SSDC undergo shorter thermal processing due to the structure of the cranberry, making the anthocyanin content higher in sliced treatments than whole. Since SSDC is both sliced and has a larger anthocyanin content than other treatments, cyanidin-3-glucoside could be detectable initially, compared to other treatments with less anthocyanin content. All treatments however did not have any cyanidin-3-glucoside after 360 days, further validating the instability of the compound during storage, not just during processing. Peonidin-3-glucoside and cyanidin-3-glucoside were present in no treatments after 360 days, indicating that glucoside substituents are more susceptible to degradation than galactoside and arabinoside, possibly due to being more susceptible to hydrolysis.

Table 2.5 Anthocyanin content of sweetened dried cranberries over time

TIME (DAYS)	SLICED APPLE JUICE INFUSED (SAJ)	WHOLE APPLE JUICE INFUSED (WAJ)	SLICED SUCROSE INFUSED (SSDC)	WHOLE SUCROSE INFUSED (WSDC)	SLICED SOLUBLE CORN FIBER, GLYCERIN, SUCROSE, AND SUCRALOSE INFUSED (SCFG)
0	$22.72^{aC} \pm 0.20$	$12.4^{aD} \pm 0.36$	$40.10^{aB} \pm 1.00$	$3.14^{aE} \pm 0.10$	$72.61^{aA} \pm 2.50$
7	$16.75^{bB} \pm 1.86$	$8.19^{bC} \pm 0.94$	$41.35^{aA} \pm 2.54$	$3.02^{aD} \pm 0.03$	$40.25^{bA} \pm 1.43$
14	$16.58^{bB} \pm 0.90$	$6.53^{cC} \pm 0.71$	$37.98^{abA} \pm 3.07$	$2.38^{bD} \pm 0.26$	$23.07^{cB} \pm 7.61$
21	$12.21^{cB} \pm 1.84$	$6.31^{cBC} \pm 0.66$	$33.56^{bA} \pm 1.01$	$2.09^{bC} \pm 0.06$	$22.31^{cA} \pm 1.00$
28	$13.61^{cB} \pm 0.09$	$4.77^{dC} \pm 0.95$	$28.73^{cA} \pm 2.98$	$1.55^{cD} \pm 0.07$	$16.88^{cB} \pm 1.94$
60	$8.70^{dB} \pm 0.69$	$3.14^{eC} \pm 0.15$	$27.33^{cA} \pm 2.62$	$1.06^{dD} \pm 0.05$	$8.33^{dB} \pm 1.44$
90	$6.03^{eB} \pm 0.47$	$2.26^{efC} \pm 0.16$	$10.68^{dA} \pm 0.75$	$0.67^{eD} \pm 0.07$	$6.20^{deB} \pm 0.63$
120	$4.70^{efC} \pm 0.64$	$1.44^{fgD} \pm 0.09$	$9.31^{deA} \pm 0.97$	$0.30^{fE} \pm 0.31$	$6.71^{deB} \pm 0.21$
150	$3.90^{efgB} \pm 0.16$	$1.10^{fghC} \pm 0.04$	$5.32^{efA} \pm 0.73$	$0.08^{fD} \pm 0.14$	$3.48^{deB} \pm 0.47$
180	$3.22^{fghB} \pm 0.13$	$0.87^{ghC} \pm 0.38$	$4.93^{efA} \pm 0.13$	$0.00^{fD} \pm 0.00$	$3.17^{deB} \pm 0.33$
210	$3.51^{fghB} \pm 0.29$	$0.78^{ghD} \pm 0.02$	$4.45^{fA} \pm 0.12$	$0.00^{fE} \pm 0.00$	$2.30^{deC} \pm 0.43$
240	$2.70^{fghB} \pm 0.17$	$0.36^{ghC} \pm 0.15$	$4.57^{fA} \pm 0.18$	$0.00^{fD} \pm 0.00$	$1.97^{deB} \pm 0.33$
270	$2.59^{fghB} \pm 0.11$	$0.08^{hD} \pm 0.14$	$3.59^{fA} \pm 0.31$	$0.00^{fD} \pm 0.00$	$1.83^{deC} \pm 0.17$
300	$1.93^{ghA} \pm 0.22$	$0.08^{hB} \pm 0.14$	$2.07^{fA} \pm 0.08$	$0.00^{fB} \pm 0.00$	$1.57^{eA} \pm 0.10$
330	$1.52^{hA} \pm 0.14$	$0.00^{hB} \pm 0.00$	$1.61^{fA} \pm 0.27$	$0.00^{fB} \pm 0.00$	$1.46^{eA} \pm 0.56$
360	$1.47^{hA} \pm 0.28$	$0.00^{hB} \pm 0.00$	$0.89^{fAB} \pm 0.23$	$0.00^{fB} \pm 0.00$	$1.45^{eA} \pm 0.61$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

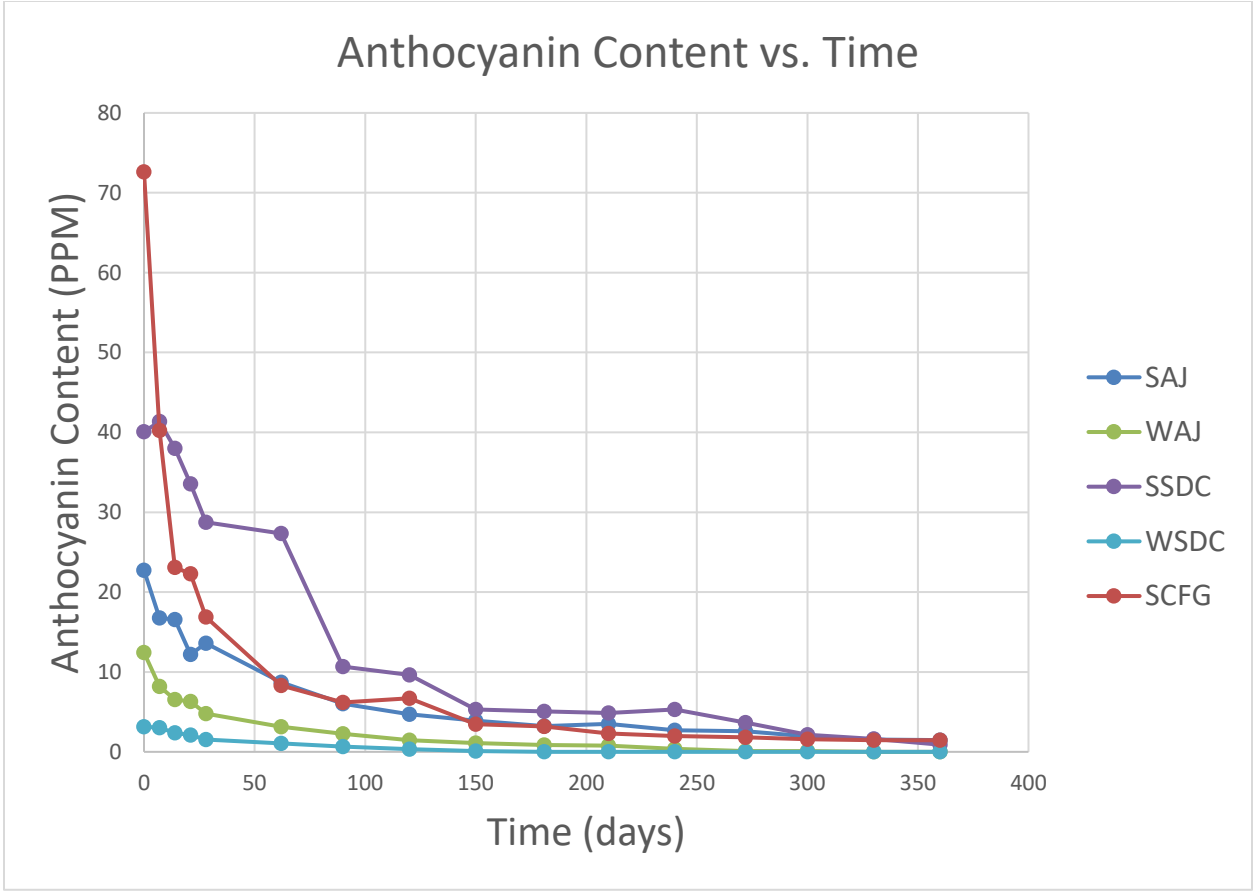

Figure 2.4 Anthocyanin content versus time of sweetened dried cranberries

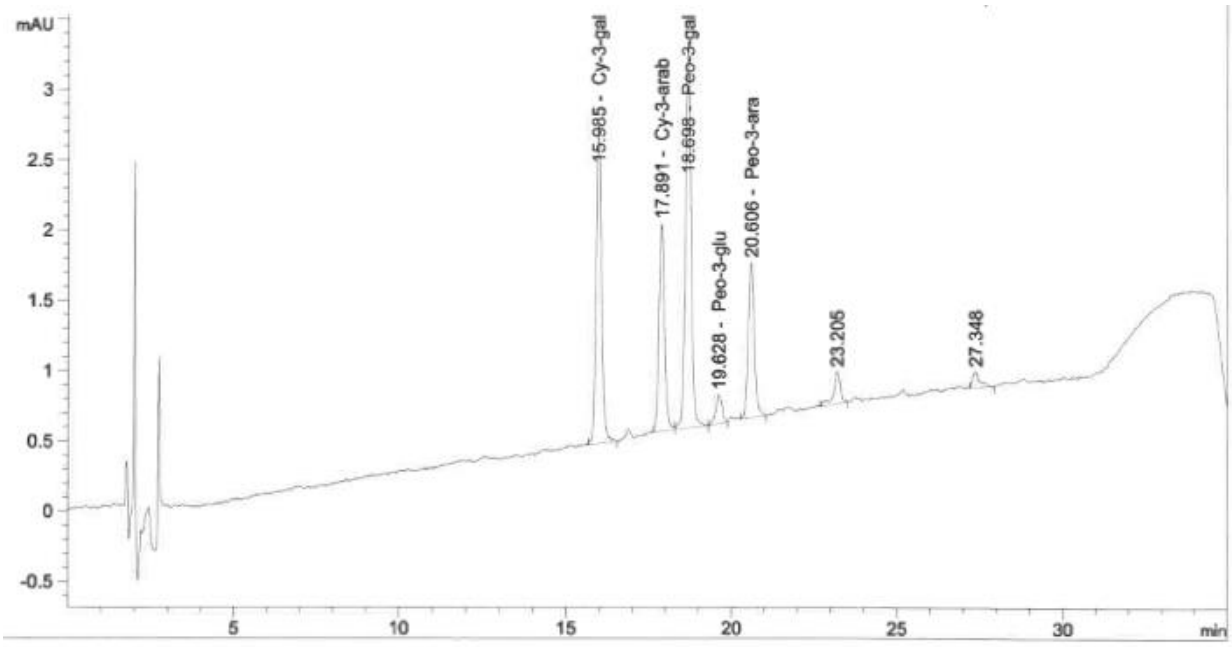

Figure 2.5 Initial anthocyanin profile of SAJ

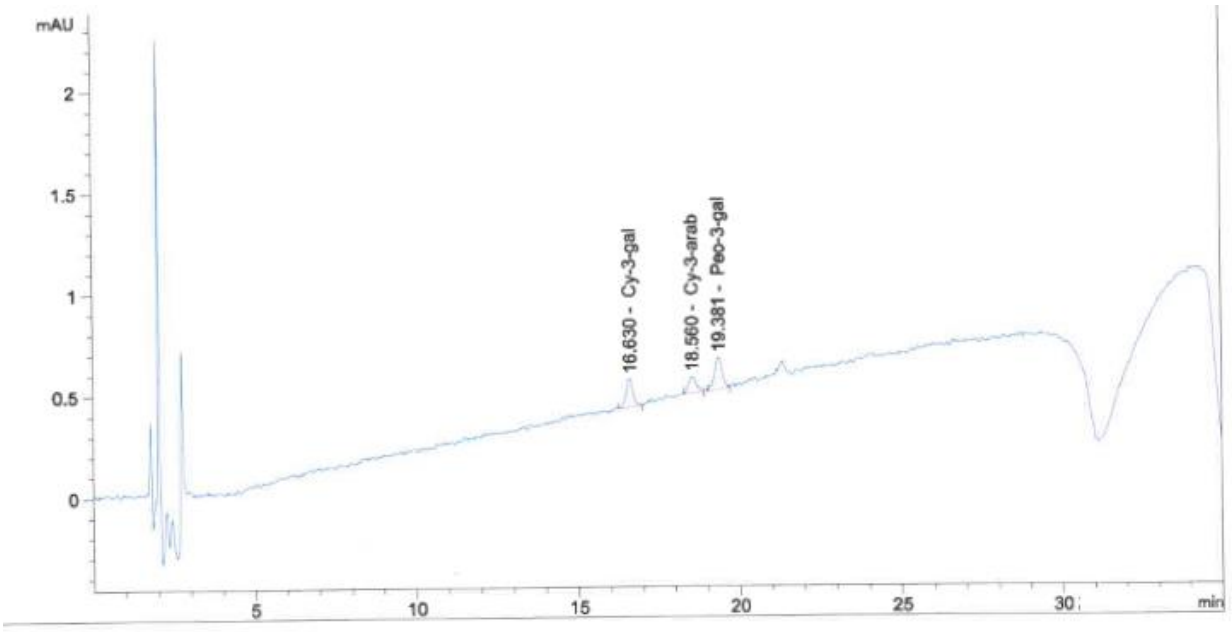

Figure 2.6 Final anthocyanin profile of SAJ

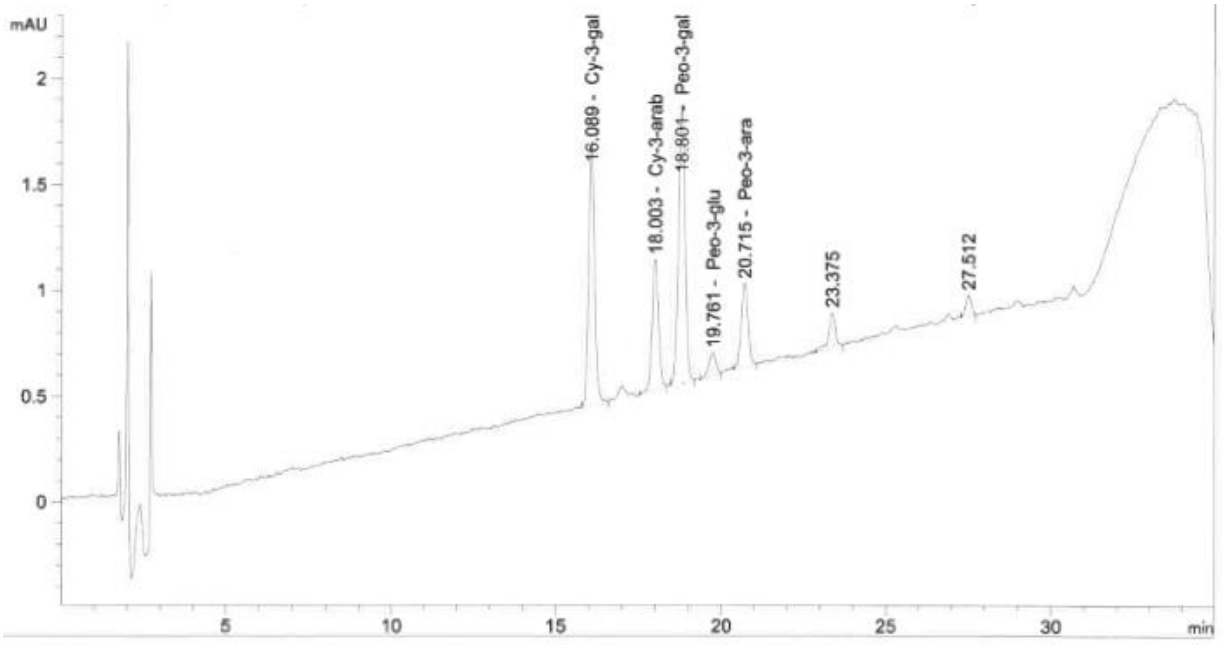

Figure 2.7 Initial anthocyanin profile of WAJ

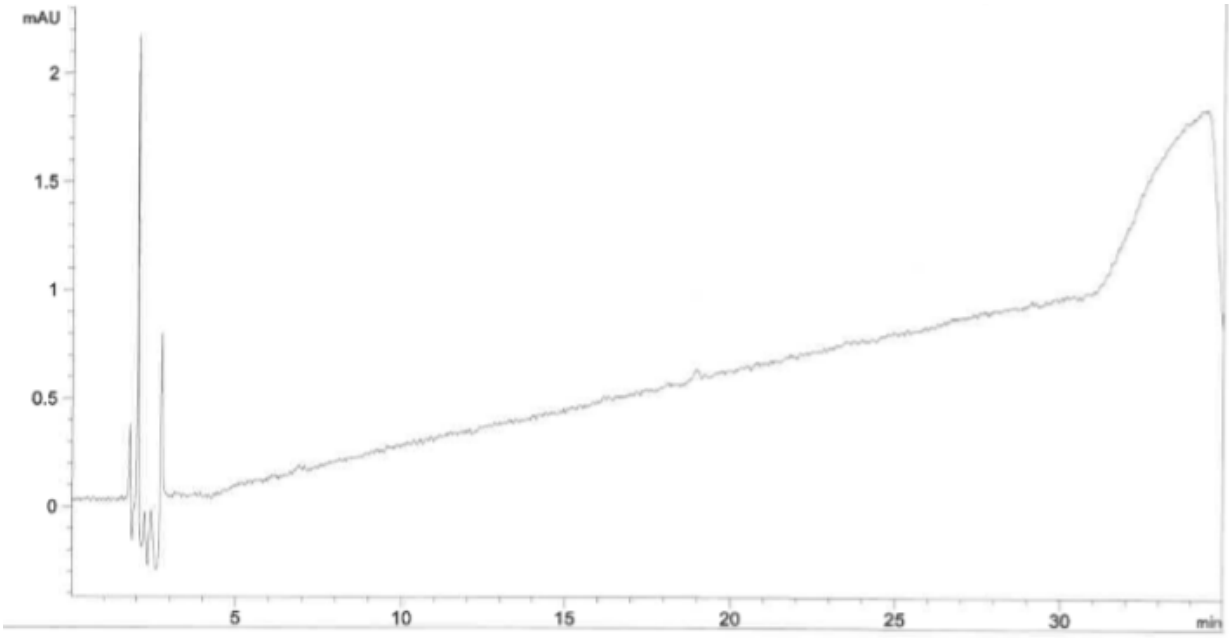

Figure 2.8 Final anthocyanin profile of WAJ

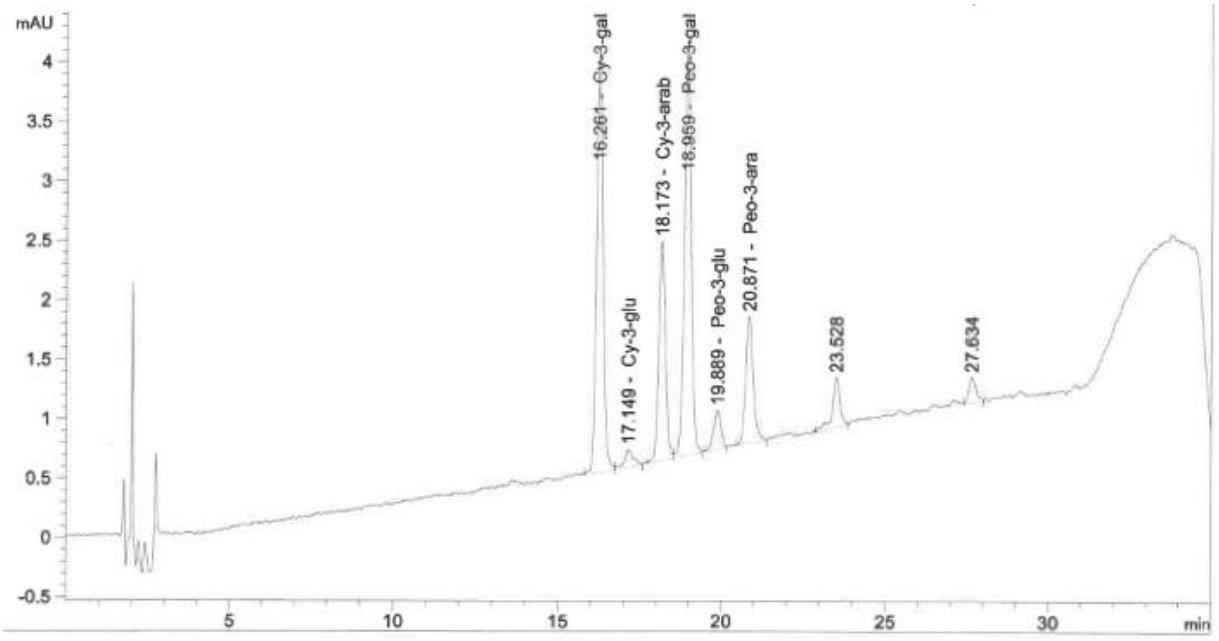

Figure 2.9 Initial anthocyanin profile of SSDC

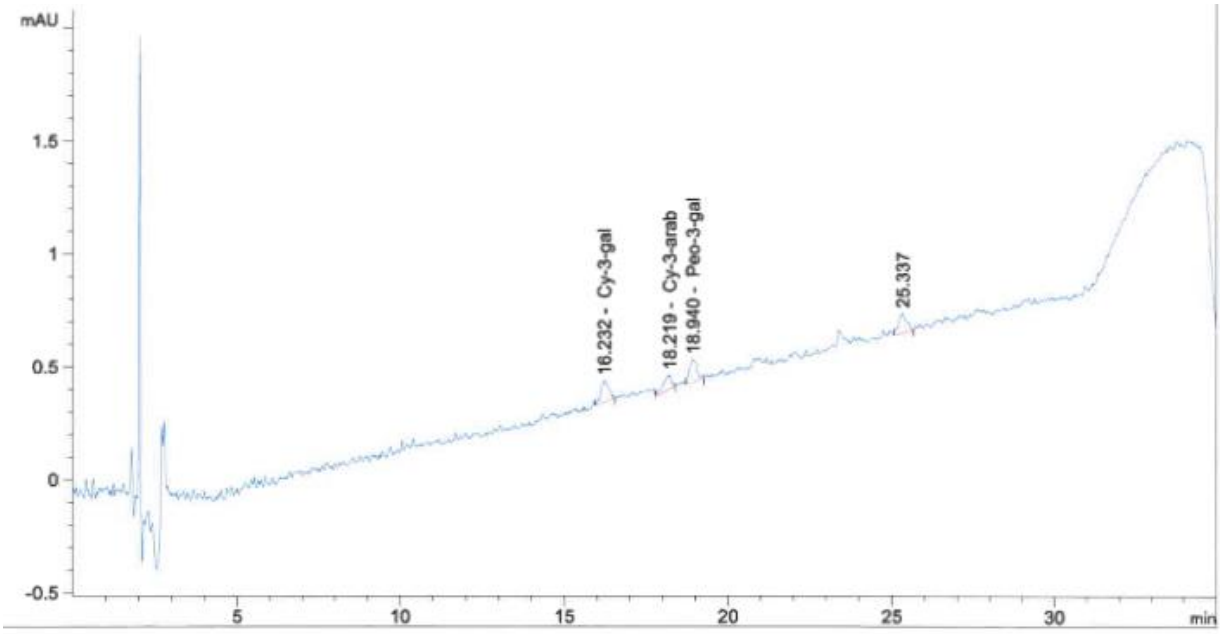

Figure 2.10 Final anthocyanin profile of SSDC

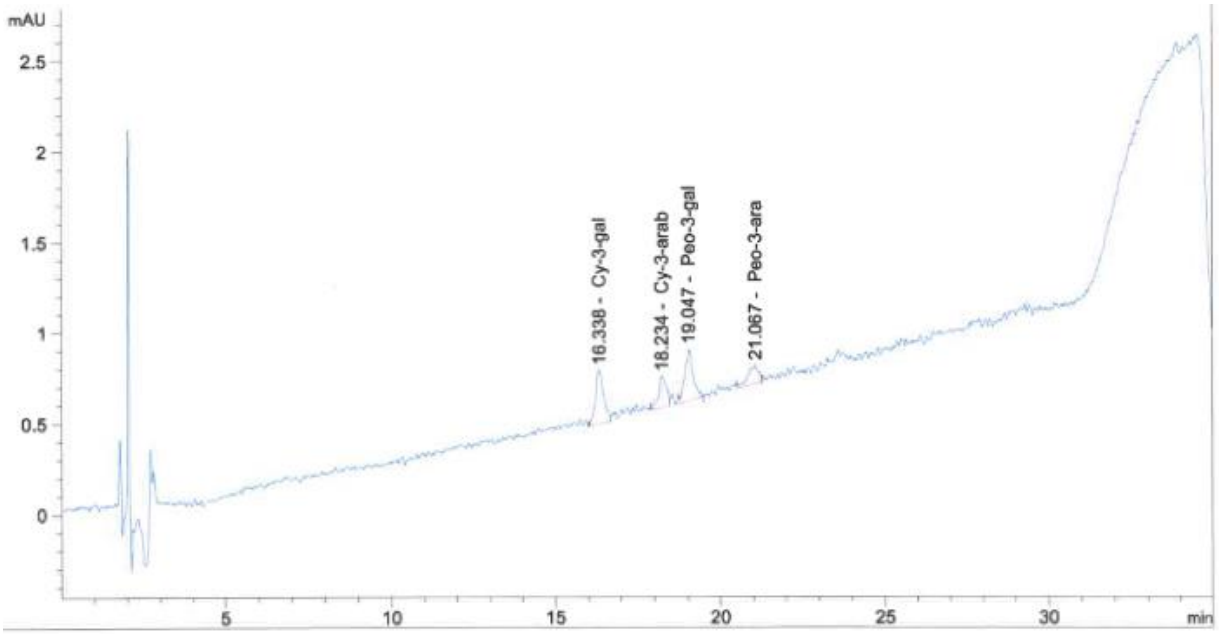

Figure 2.11 Initial anthocyanin profile of WSDC

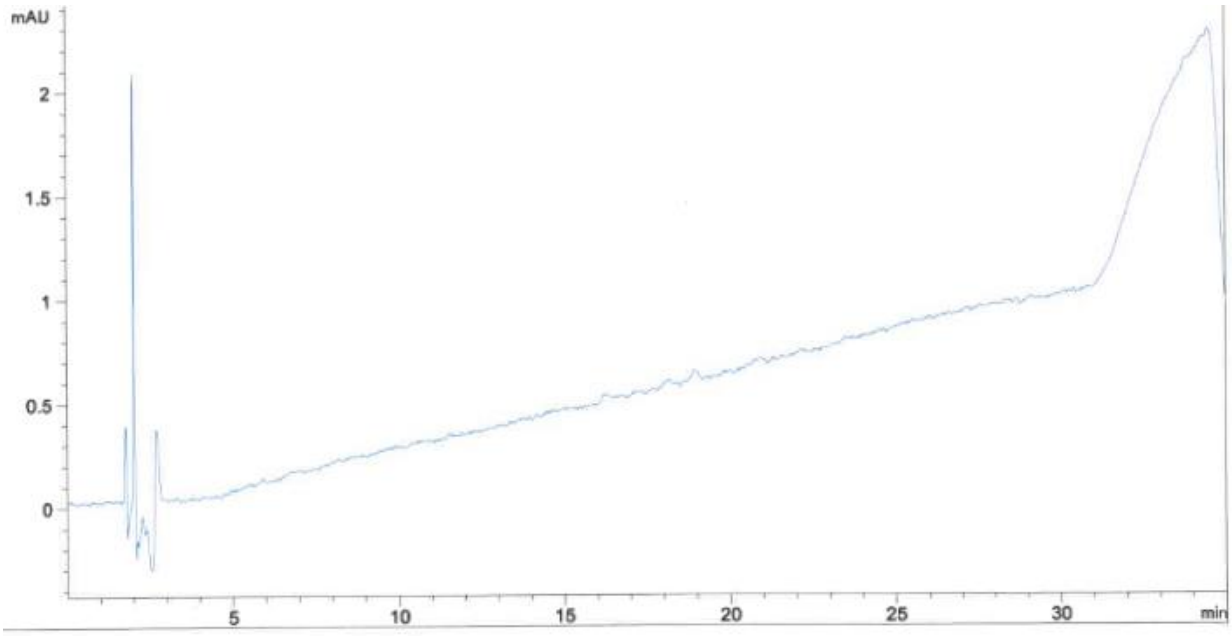

Figure 2.12 Final anthocyanin profile of WSDC

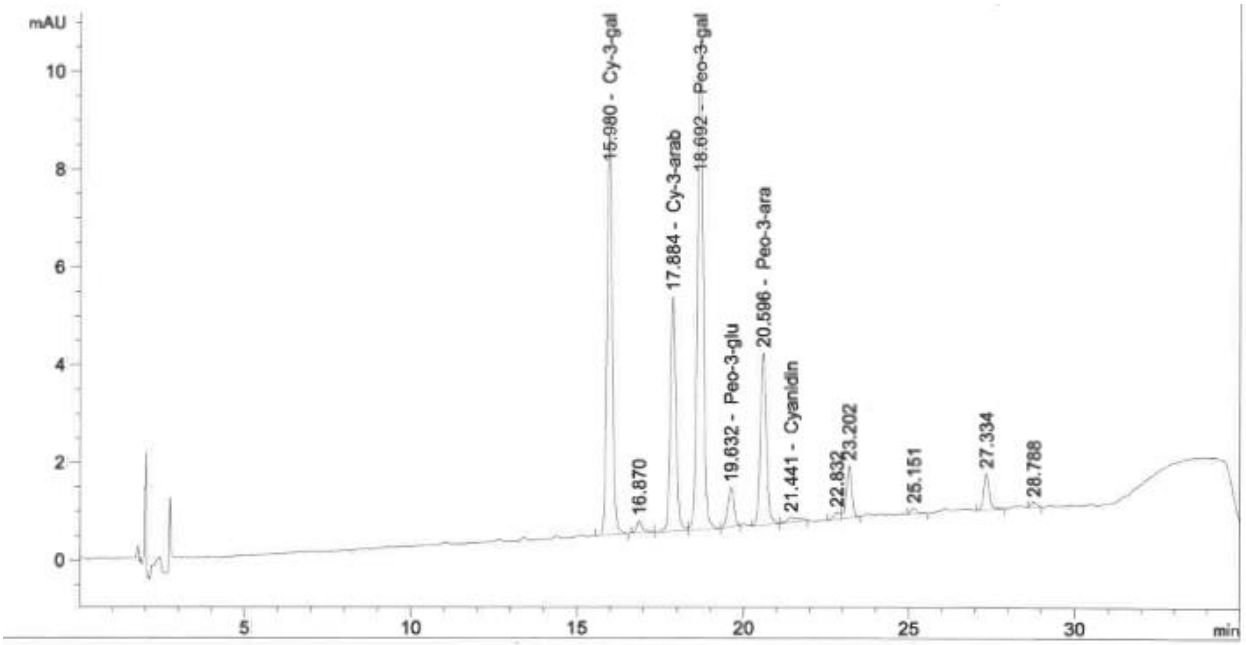

Figure 2.13 Initial anthocyanin profile of SCFG

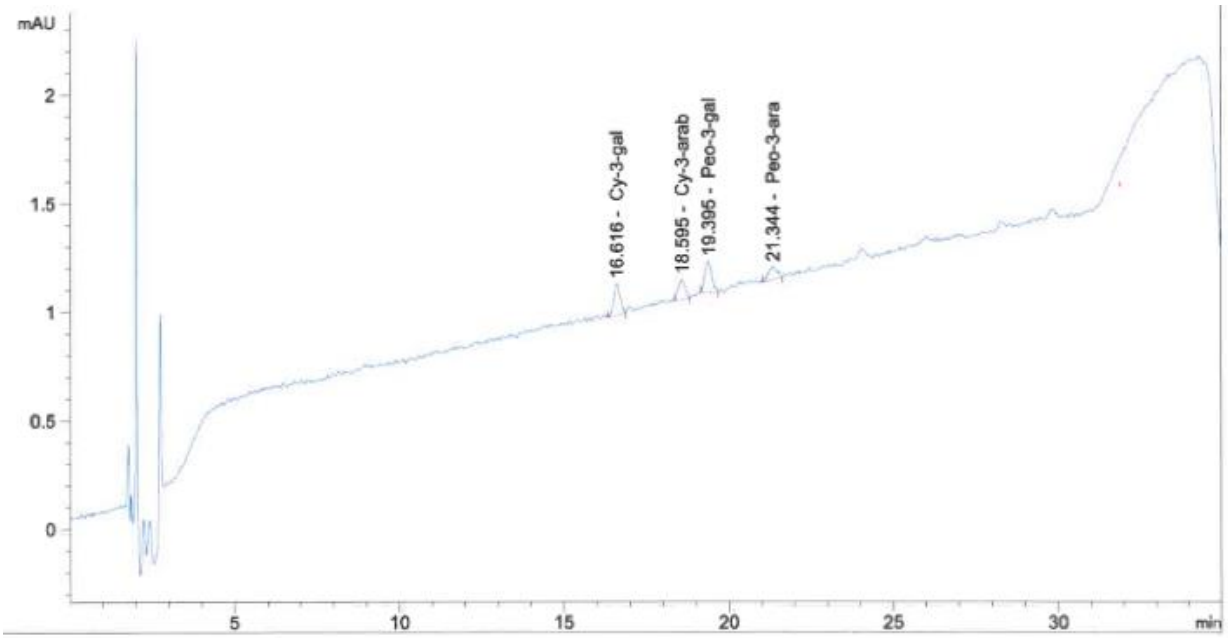

Figure 2.14 Final anthocyanin profile of SCFG

Total Phenolic Content

For all treatments, there was a significant ($p<0.05$) difference in the initial total phenolic content, however, no significant difference in the final total phenolic content, indicating that total phenolic content stabilizes over time regardless of initial value (Table 2.6) (Figure 2.15). Total phenolic content values were comparable to values reported in Grace et al., 2012 for sweetened dried cranberries. Unlike previously discussed with proanthocyanidins and anthocyanins, structure had no impact on the overall total phenolic content in the samples, with WAJ having the highest initial total phenolic content (5.87 mg/g) while WSDC had the lowest initial total phenolic content (2.64 mg/g). WAJ and SAJ contain added apple juice which provides additional polyphenols resulting in higher total phenolic content than SSDC and WSDC. This increase is not mirrored in anthocyanins and proanthocyanidins because the major polyphenols in apple juice are in the form of chlorogenic acid, as well as some quercetin (Gliszczynska-Swiglo & Tyrakowska, 2003) which would not be read as anthocyanins or proanthocyanidins. Compared to raw cranberries (1.6 mg/g total phenolic content), all matrices of sweetened dried cranberries had significantly higher initial total phenolic content as well as final total phenolic content. Treatments with the highest total phenolic content compared to the raw cranberries were WAJ, SCFG, and SAJ. WAJ and SAJ both are composed of apple juice concentrate containing quercetin, which in studies have been showed to retain total phenolic content and can be regenerated upon heated to various quercetin glycosides which may result in the higher total phenolic content (Michalska, et al., 2018) (Aherne & O'Brien, 2002) (White, et al., 2011). Compared to proanthocyanidins and anthocyanins, total phenolic content is significantly more stable, only decreasing by 42.7% in SAJ, 56.9% in WAJ, 13.9% in SSDC, 10.9% in WSDC, and 51.6% in SCFG. Proanthocyanidins and anthocyanins are only two compounds that make up the

overall total phenolic content in cranberries, so while proanthocyanidins and anthocyanins saw more degradation throughout shelf-life, the rest of the total phenolic content such as acids and other flavonols were more stable, leading to more stability in overall total phenolic content. SSDC and WSDC had the lowest initial proanthocyanidin content (2.69 mg/g for SSDC and 2.64 mg/g for WSDC, respectively), but there was no significant decrease in total phenolic content for these treatments throughout shelf life. SAJ, WAJ, and SCFG saw significant decreases in their total phenolic content due to higher initial content which eventually decreased to the content initially seen in SSDC and WSDC. This is confirmed in the rates of degradation for the treatments, with the treatments having the highest initial content having the largest rate of total phenolic content degradation (Table 2.2).

Table 2.6 Total phenolic content of sweetened dried cranberries over time

TIME (DAYS)	SLICED APPLE JUICE INFUSED (SAJ)	WHOLE APPLE JUICE INFUSED (WAJ)	SLICED SUCROSE INFUSED (SSDC)	WHOLE SUCROSE INFUSED (WSDC)	SLICED SOLUBLE CORN FIBER, GLYCERIN, SUCROSE, AND SUCRALOSE INFUSED (SCFG)
0	$4.37^{aC} \pm 0.20$	$5.87^{aA} \pm 0.46$	$2.69^{aD} \pm 0.06$	$2.64^{aD} \pm 0.08$	$5.04^{aB} \pm 0.03$
7	$4.51^{abcB} \pm 0.25$	$5.34^{abA} \pm 0.09$	$2.60^{abcC} \pm 0.02$	$2.62^{aC} \pm 0.11$	$4.35^{bcdB} \pm 0.54$
14	$4.59^{abC} \pm 0.08$	$5.42^{abA} \pm 0.15$	$2.50^{abcDde} \pm 0.02$	$2.59^{aD} \pm 0.07$	$4.95^{Ba} \pm 0.03$
21	$4.28^{abcC} \pm 0.11$	$5.27^{bA} \pm 0.06$	$2.61^{abD} \pm 0.04$	$2.49^{abD} \pm 0.02$	$4.67^{Babc} \pm 0.17$
28	$4.51^{abcB} \pm 0.08$	$5.58^{abA} \pm 0.20$	$2.54^{abcCd} \pm 0.11$	$2.44^{abcC} \pm 0.04$	$4.85^{abB} \pm 0.12$
60	$3.84^{cC} \pm 0.11$	$5.36^{abA} \pm 0.24$	$2.53^{abcdD} \pm 0.09$	$2.56^{aD} \pm 0.10$	$4.04^{dB} \pm 0.08$
90	$3.99^{bc} \pm 0.07$	$3.29^{c} \pm 0.32$	$2.66^{ab} \pm 0.13$	$2.55^{ab} \pm 0.03$	$4.28^{cd} \pm 0.11$
120	$2.85^{dA} \pm 0.33$	$2.75^{cdA} \pm 0.08$	$2.28^{deA} \pm 0.09$	$2.40^{abcAd} \pm 0.09$	$2.87^{eA} \pm 0.43$
150	$2.57^{dA} \pm 0.02$	$2.65^{dA} \pm 0.08$	$2.22^{eA} \pm 0.08$	$2.27^{bcdAe} \pm 0.06$	$2.33^{eA} \pm 0.05$
180	$2.58^{dA} \pm 0.04$	$2.43^{dA} \pm 0.05$	$2.26^{deA} \pm 0.09$	$2.09^{deA} \pm 0.05$	$2.37^{eA} \pm 0.01$
210	$2.56^{dA} \pm 0.07$	$2.80^{cdA} \pm 0.11$	$2.24^{eA} \pm 0.07$	$2.23^{bcdAe} \pm 0.20$	$2.46^{eA} \pm 0.02$
240	$2.56^{dA} \pm 0.05$	$2.79^{cdA} \pm 0.07$	$2.29^{deA} \pm 0.08$	$2.40^{abcAd} \pm 0.18$	$2.47^{eA} \pm 0.03$
270	$2.51^{dA} \pm 0.06$	$2.94^{cdA} \pm 0.22$	$2.38^{bcdeA} \pm 0.03$	$2.43^{abcA} \pm 0.08$	$2.46^{eA} \pm 0.03$
300	$2.55^{dA} \pm 0.09$	$2.94^{cdA} \pm 0.05$	$2.24^{eA} \pm 0.11$	$2.16^{cdeA} \pm 0.08$	$2.41^{eA} \pm 0.05$
330	$2.61^{dA} \pm 0.17$	$2.64^{dA} \pm 0.10$	$2.26^{deA} \pm 0.23$	$2.05^{eA} \pm 0.08$	$2.58^{eA} \pm 0.15$
360	$2.51^{dA} \pm 0.18$	$2.53^{dA} \pm 0.04$	$2.32^{cdeA} \pm 0.05$	$2.35^{abcdeA} \pm 0.17$	$2.44^{eA} \pm 0.02$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

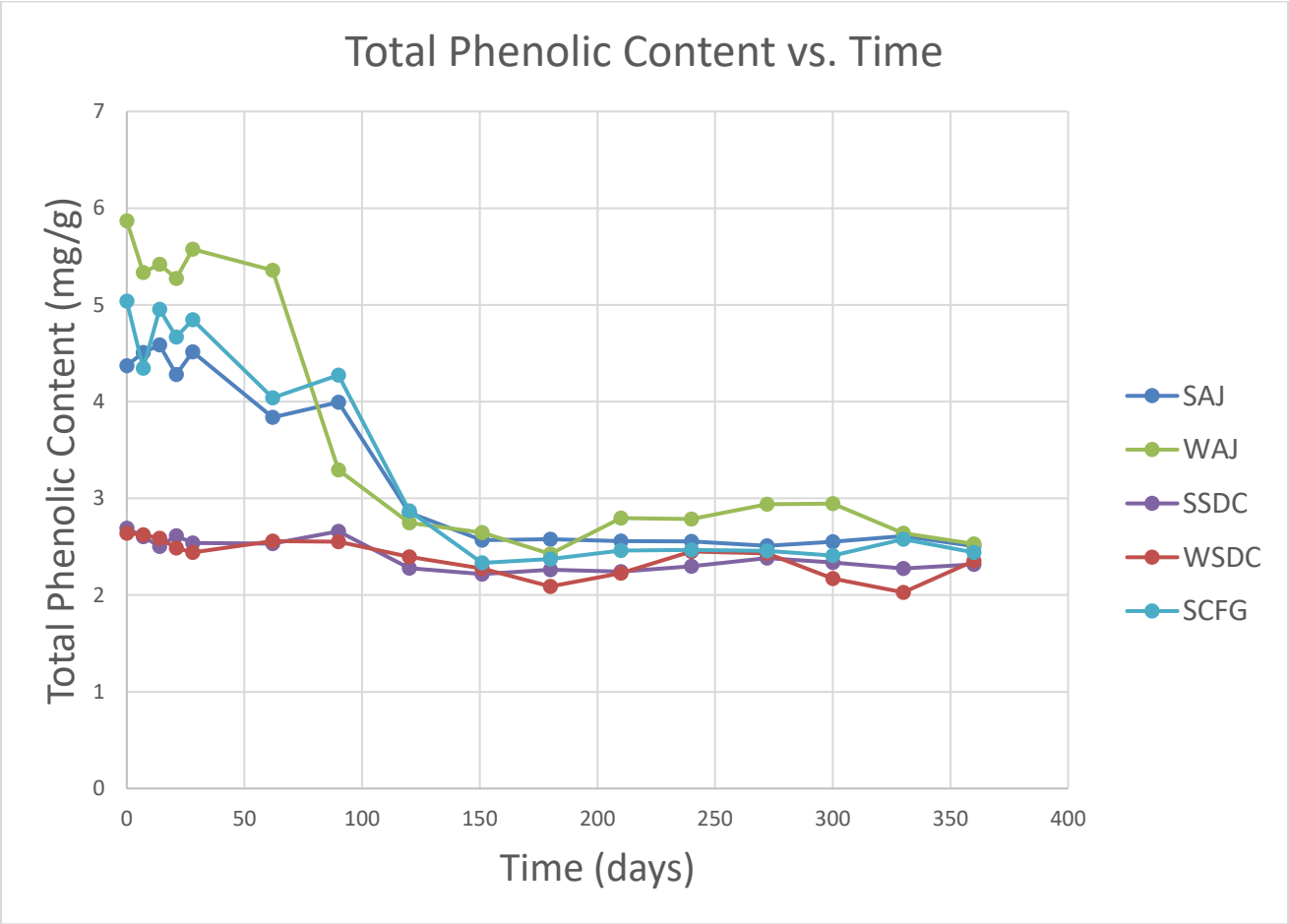

Figure 2.15 Total phenolic content versus time of sweetened dried cranberries

Antioxidant Activity

While sweetened dried cranberries contained no viable antioxidant activity when analyzed by DPPH assay (Table 2.7), similar results seen for analysis of raw cranberries indicate that other methods of antioxidant analysis may be better suited to identify cranberry polyphenol antioxidant activity. Significantly higher IC_{50} values for all treatments compared to ascorbic acid standard (79.84 mg/ml) confirms that the significant processing of sweetened dried cranberries depletes antioxidant activity. The DPPH method however did show that the sweetened dried cranberries are comparable to raw cranberries which scavenged 36.71% of DPPH free radicals with an IC_{50} of 2.91×10^6 mg/mL. While this is contrary to what was seen with proanthocyanidins, anthocyanins, and total phenolic content (where degradation from processing degraded polyphenols compared to raw cranberries) there have been studies which show that thermal processing may increase antioxidant activity (Turkmen, et al., 2005) (Nicoli, et al., 1999) (Gazzani, et al., 1998) (Manzocco, et al., 1998). Various studies have found that in fruits, vegetables, and teas, thermal processing may increase antioxidant activity by production of antioxidant forming compounds during thermal treatment or inactivation of pro-oxidant compounds already in the fruits and vegetables (Turkmen, et al., 2005) (Nicoli, et al., 1999) (Gazzani, et al., 1998) (Manzocco, et al., 1998). These findings would support what was seen in this study, where sweetened dried cranberries showed comparable antioxidant scavenging activity compared to raw cranberries. SSDC had the most antioxidant activity and was able to scavenge 51.85% of the DPPH free radicals, compared to ascorbic acid standard which had a maximum DPPH scavenging of 95.30%. Despite agreement with previous research which validates the findings in this study, there are studies which show that compared to other fruits such as apples, plums, and oranges, raw cranberries possess higher antioxidant capacity (Vinson,

et al., 2001). Since different fruits may not have the same antioxidant mechanism, other methods should be evaluated on raw cranberries compared to dried cranberries to determine the best method for cranberry antioxidant capacity analysis as there are indications that different antioxidant assays can yield different results based on the antioxidant mechanism of the food product (Roy, et al., 2010)

Table 2.7 Antioxidant activity of sweetened dried cranberries

TREATMENT	DPPH SCAVENGING (%)	IC_{50} (MG/ML)
Sliced Apple Juice Infused (SAJ)	$30.18^{b} \pm 1.83$	$5.38 \times 10^{7a} \pm 3.04 \times 10^{7}$
Whole Apple Juice Infused (WAJ)	$35.15^{b} \pm 1.05$	$2.14 \times 10^{7a} \pm 2.53 \times 10^{6}$
Sliced Sucrose Infused (SSDC)	$51.85^{a} \pm 2.55$	$2.54 \times 10^{6a} \pm 3.50 \times 10^{6}$
Whole Sucrose Infused (WSDC)	$36.71^{b} \pm 0.76$	$1.33 \times 10^{7a} \pm 3.02 \times 10^{6}$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$11.01^{c} \pm 0.58$	$3.49 \times 10^{13b} \pm 3.32 \times 10^{13}$

Each value is the average ± standard deviation (n=5). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Water Activity and Moisture Content

Water Activity

For all treatments A_w did not significantly ($p>0.05$) change from initial to final indicating that A_w is stable over time regardless of product matrix (Table 2.8) (Figure 2.16). Despite no significant changes in A_w over time within treatments, there were significant ($p<0.05$) differences in the A_w between treatments and in the rates of degradation between treatments (Table 2.2). According to Sillick and Gregson (2010), A_w is impacted by the type of sugar in the matrix, with sucrose having a lower impact on A_w and a lower impact on the glass transition

temperature (Sillick & Gregson, 2010). Glass transition temperature is the temperature at which a material goes from the glassy state to rubbery (Bell & Hageman, 1994). Both A_w and glass transition temperature can have effects on the reactions that occur in a system (Bell & Hageman, 1994). This finding would corroborate that SSDC and WSDC have the lowest A_w and have a positive rate of change, as they have the largest amount of sucrose, followed by SAJ and WAJ treatments which have fructose from apple juice concentrate, and then SCFG which contains soluble corn fiber and glycerin in addition to sucrose which would increase the glass transition temperature and cause the A_w to be larger. SAJ and WAJ, as well as SSDC and WSDC, did not have significantly different A_w among them indicating that slicing of the cranberries does not impact A_w despite the higher surface area. Sweetened dried cranberries are not significantly impacted by A_w throughout shelf life, therefore the A_w levels seen in these sweetened dried cranberry treatments are stable and would not pose a risk to product quality or food safety.

Table 2.8 Comparison of initial and final A_w of sweetened dried cranberries

TREATMENT	INITIAL	FINAL
Sliced Apple Juice Infused (SAJ)	$0.536^{bA} \pm 0.011$	$0.523^{bcA} \pm 0.004$
Whole Apple Juice Infused (WAJ)	$0.542^{bA} \pm 0.005$	$0.510^{cA} \pm 0.003$
Sliced Sucrose Infused (SSDC)	$0.483^{cA} \pm 0.002$	$0.504^{cA} \pm 0.005$
Whole Sucrose Infused (WSDC)	$0.495^{cA} \pm 0.007$	$0.541^{bA} \pm 0.017$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$0.597^{aA} \pm 0.005$	$0.590^{aA} \pm 0.007$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

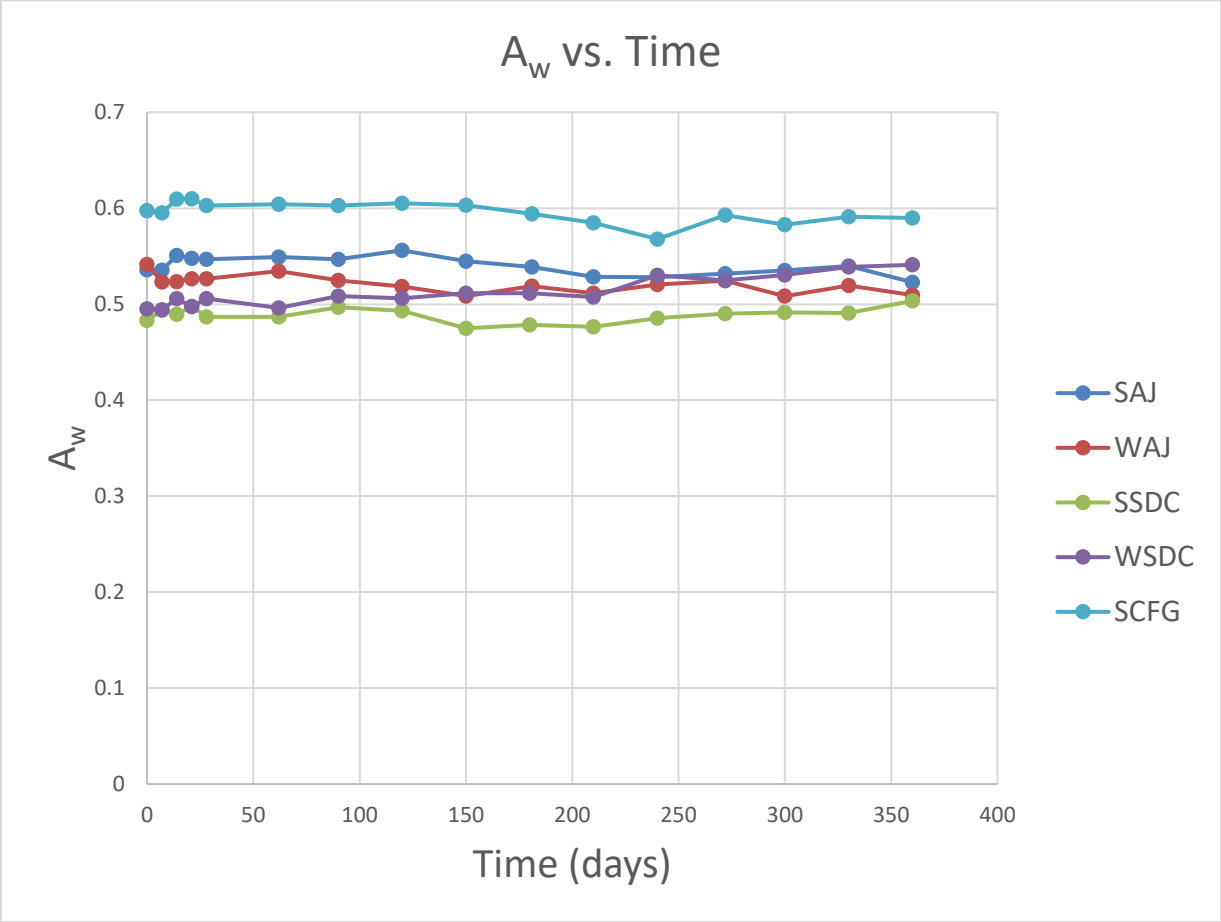

Figure 2.16 A_w versus time of sweetened dried cranberries

Moisture Content

SSDC was the only treatment without a significant ($p>0.05$) increase in moisture content between initial (14.37%) and final (14.70%), indicating that while A_w does not significantly change, sweetened dried cranberries are more susceptible to changes in the total amount of water in the matrix (Table 2.9) (Figure 2.17). Structure does not appear to significantly impact moisture content over time, as WAJ decreased in moisture content (16.70% to 14.82%) while WSDC increased in moisture content (13.40% to 15.65%) and SAJ decreased in moisture content (16.52% to 15.28%) while there was no significant change in SSDC. SCFG had the highest initial moisture content (18.42%), mirroring the largest A_w. Glycerin has been shown to

have moisture absorption properties which would help in the drying process and would cause the A_w and moisture content to be higher in SCFG (Yan, et al., 2012). SSDC and WSDC had a significantly lower initial moisture content (14.37% and 13.40% respectively). As previously seen with A_w sucrose has a lower effect on glass transition temperature, so sucrose infused treatments (SSDC and WSDC) also have a lower moisture content and positive rate of change, compared to other treatments with negative rates of change (Sillick & Gregson, 2010). This effect is mirrored in A_w, indicating that sucrose infusion in the cranberries pre-drying also resulted in a lower amount of free water in SSDC and WSDC treatments. While final A_w between treatments differed, differences in initial moisture content did not cause significant differences in final moisture content between treatments. A study with dried apricots demonstrated that diffusivity (the ability of water to move through the material) in dried apricots during storage was affected by the glass transition (as was previously seen with A_w) resulting in a decrease in moisture content over time (Miranda, et al., 2014). This effect is seen in sweetened dried cranberries except for WSDC which increased in moisture possibly due to the fructose from the apple juice, resulting in more free moisture compared to treatments with sucrose.

Table 2.9 Comparison of initial and final moisture content of sweetened dried cranberries

TREATMENT	INITIAL (%)	FINAL (%)
Sliced Apple Juice Infused (SAJ)	16.52 ± 1.24^{aA}	15.28 ± 0.25^{aB}
Whole Apple Juice Infused (WAJ)	16.70 ± 0.38^{aA}	14.82 ± 0.36^{aB}
Sliced Sucrose Infused (SSDC)	14.37 ± 0.11^{bA}	14.70 ± 1.66^{aA}
Whole Sucrose Infused (WSDC)	13.40 ± 0.26^{bA}	15.65 ± 1.45^{aB}
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	18.42 ± 0.89^{aA}	16.30 ± 0.15^{aB}

Each value is the average ± standard deviation (n=3). Treatments were stored at 21°C in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

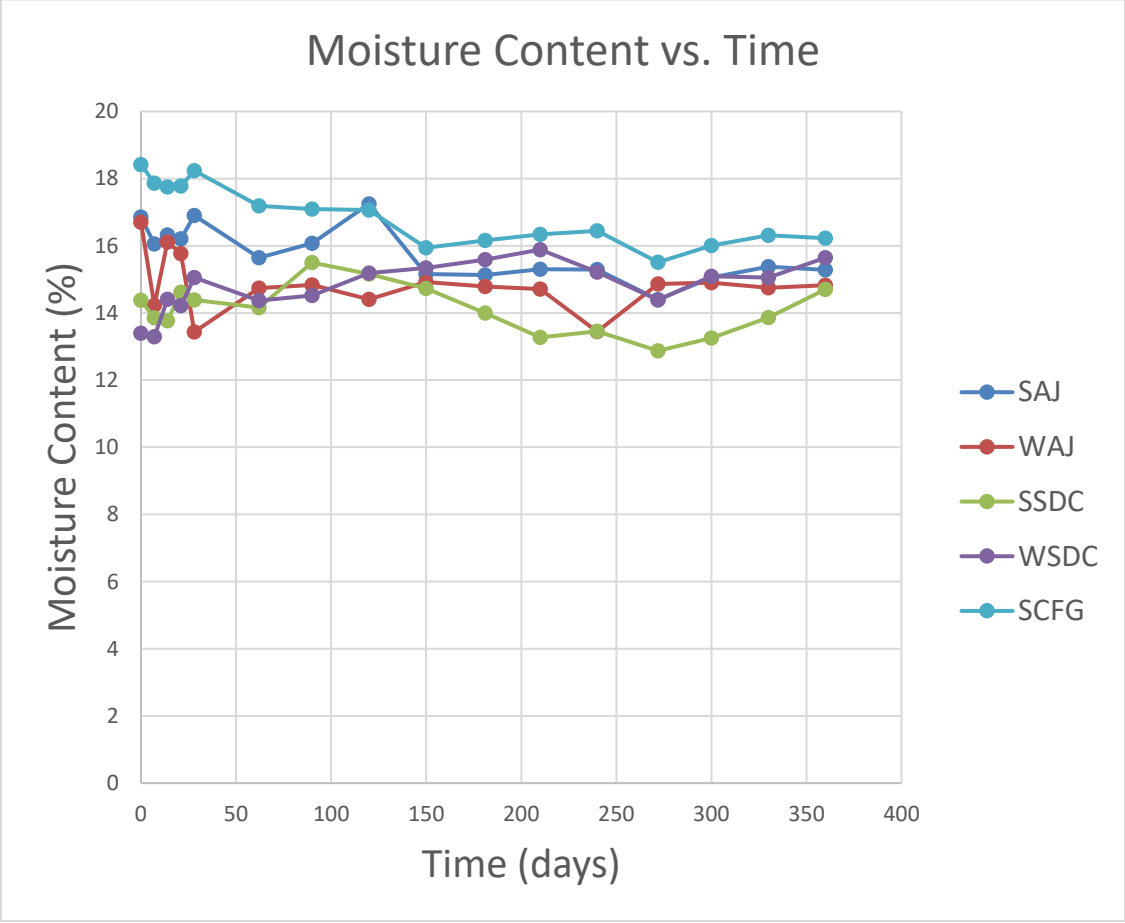

Figure 2.17 Moisture content versus time of sweetened dried cranberries

Colorimetric and Texture Analyses

Color

SAJ and SCFG were the only treatments with a significant ($p<0.05$) decrease in the initial (11.51 for SAJ and 12.07 for SCFG, respectively) and final (8.84 for SAJ and 6.25 for SCFG, respectively) a* values, indicating that most sweetened dried cranberry samples do not significantly change in redness over time (Table 2.11). WSDC had the lowest initial a* value (4.64) and final a* value (5.47), so while this treatment is significantly less red than the other treatments, there was not a significant change in the redness of the treatment over time. The possible reason for WSDC being significantly lower in a* is because it is infused with apple juice concentrate which is brown in color and may lower a* value, compared to others with no apple juice concentrate. The apple juice concentrate, in conjunction with longer drying times for whole treatments compared to sliced treatments, could lower a*. While there was a strong positive correlation ($p=0.003$) between initial a* and initial anthocyanins, there was no correlation between final a* and final anthocyanins ($p=0.153$), due to overall high degradation in anthocyanins over time, compared to a* which decreased 23.3% for SAJ, increased 5.2% for WAJ, decreased 25.3% for SSDC, increased 6.9% for WSDC, and decreased 48.2% for SCFG.

There was a significant increase in ΔE over time, indicating color change in all treatments over time (Table 2.13). L*, a*, and b* values used for ΔE calculations can be seen in Tables 2.9-2.11. Studies have reported that blueberries and strawberries undergo enzymatic browning after food processing which deteriorates color (Siddiq & Dolan, 2017) (Bermejo-Prada & Otero, 2016). Polyphenol oxidase (PPO) is a naturally occurring enzyme that causes browning in the presence of oxygen when the PPO reacts with oxygen causing phenols to convert to melanin (brown pigment) (Siddiq & Dolan, 2017) (Fang, et al., 2007) (Institute of Food Science

and Technology, 2017). PPO can degrade anthocyanins to brown, black, or yellow colors from the natural red, purple, and blue pigments (Bermejo-Prada & Otero, 2016). Processing, such as juicing and drying, as well as storage conditions, can promote PPO reactions causing most color changes and anthocyanin polymerization reactions (Siddiq & Dolan, 2017) (Bermejo-Prada & Otero, 2016) (Jaiswal, et al., 2010). Color change was not influenced by slicing, as SCFG had the largest ΔE (6.75) but WAJ had the second largest ∆E (4.28). SCFG had the largest rate of change in color, which could be due to the significantly higher amount of proanthocyanidins and anthocyanins in that treatment available for PPO reactions to occur (Table 2.2). WAJ also had the largest total phenolic content which could explain why it was higher in ∆E and second largest rate of change. SCFG had the highest rate of change also due to having the largest proanthocyanidin and anthocyanin content for reactions to occur, which coincides with the largest overall change in color. There was no significant difference in the overall rate of change between SAJ and WAJ or SSDC and WSDC, indicating that rate of change for ∆E is dependent on product matrix. Consumer testing may need to be done to indicate whether the change in color seen in the treatments impact acceptability.

Table 2.10 Comparison of initial and final L* in sweetened dried cranberries

TREATMENT	L* INITIAL	L* FINAL
Sliced Apple Juice Infused (SAJ)	$21.15^{aA} \pm 0.67$	$19.73^{aA} \pm 0.75$
Whole Apple Juice Infused (WAJ)	$16.01^{dB} \pm 0.53$	$20.08^{aA} \pm 0.56$
Sliced Sucrose Infused (SSDC)	$18.91^{bA} \pm 0.24$	$18.77^{aA} \pm 0.27$
Whole Sucrose Infused (WSDC)	$17.50^{cB} \pm 0.21$	$19.80^{aA} \pm 0.40$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$18.78^{bA} \pm 0.53$	$20.38^{aA} \pm 0.75$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 2.11 Comparison of initial and final a* in sweetened dried cranberries

TREATMENT	A* INITIAL	A* FINAL
Sliced Apple Juice Infused (SAJ)	$11.51^{aA} \pm 0.74$	$8.84^{aB} \pm 0.25$
Whole Apple Juice Infused (WAJ)	$6.78^{bA} \pm 0.70$	$7.15^{bA} \pm 0.14$
Sliced Sucrose Infused (SSDC)	$7.32^{bA} \pm 0.97$	$5.47^{cA} \pm 0.42$
Whole Sucrose Infused (WSDC)	$4.64^{cA} \pm 0.37$	$4.98^{cA} \pm 1.09$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$12.07^{aA} \pm 0.91$	$6.25^{bcB} \pm 0.28$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 2.12 Comparison of initial and final b* in sweetened dried cranberries

TREATMENT	B* INITIAL	B* FINAL
Sliced Apple Juice Infused (SAJ)	$3.62^{aA} \pm 0.66$	$2.30^{aA} \pm 0.40$
Whole Apple Juice Infused (WAJ)	$1.26^{bA} \pm 0.25$	$0.17^{bB} \pm 0.03$
Sliced Sucrose Infused (SSDC)	$-0.30^{cA} \pm 0.27$	$-0.32^{bA} \pm 0.11$
Whole Sucrose Infused (WSDC)	$-0.30^{cA} \pm 0.11$	$0.07^{bA} \pm 0.51$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$0.52^{bcA} \pm 0.27$	$-0.49^{bB} \pm 0.05$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 2.13 ΔE in sweetened dried cranberries

TREATMENT	ΔE
Sliced Apple Juice Infused (SAJ)	$3.39^{bc} \pm 0.33$
Whole Apple Juice Infused (WAJ)	$4.28^{b} \pm 0.56$
Sliced Sucrose Infused (SSDC)	$2.43^{c} \pm 0.43$
Whole Sucrose Infused (WSDC)	$2.50^{c} \pm 0.70$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$6.75^{a} \pm 0.12$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

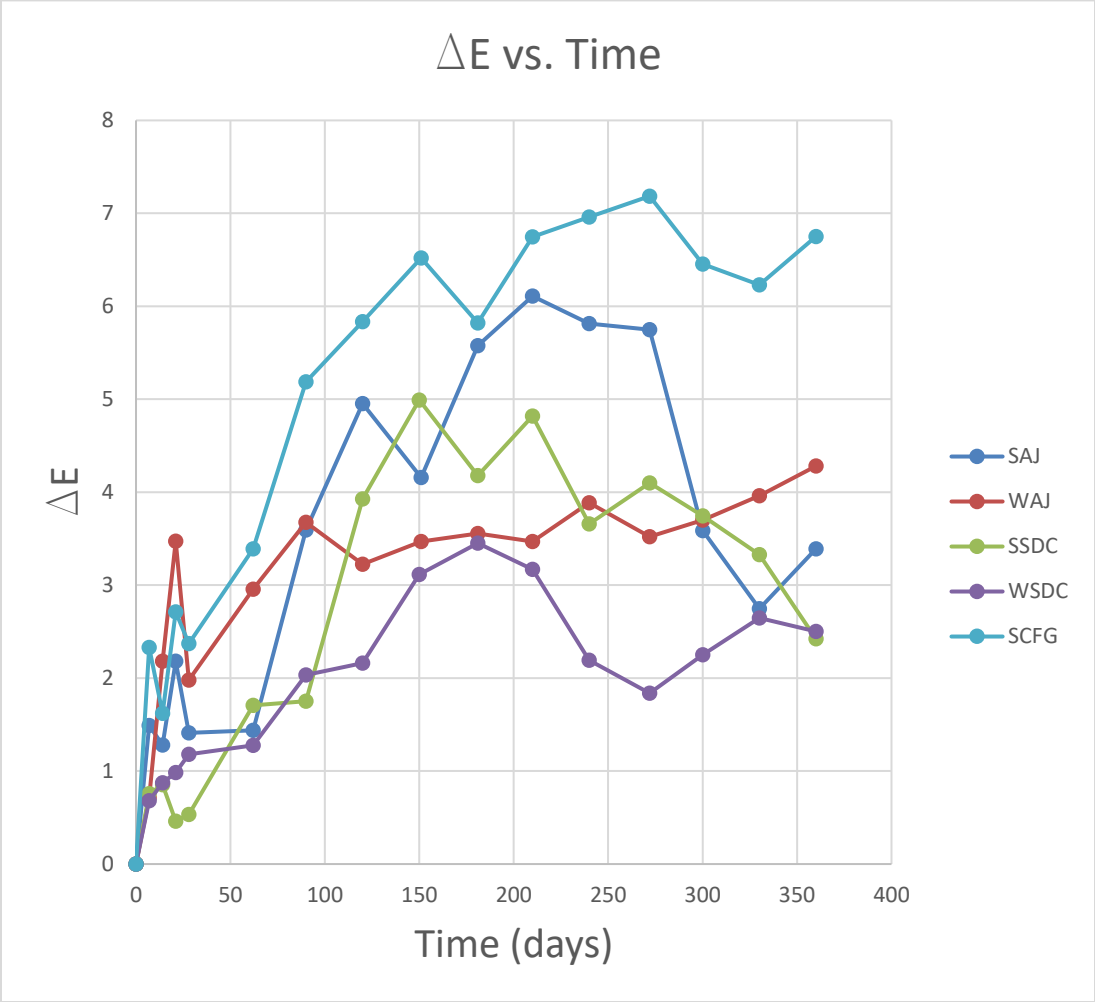

Figure 2.18 ΔE versus time of sweetened dried cranberries

Texture

Treatments of similar product matrix (SAJ/WAJ and SSDC/WSDC) did not have significantly ($p>0.05$) different texture indicating that slicing does not have an impact on texture compared to whole (Table 2.14) (Figure 2.19). SCFG had significantly higher adhesion force initially, indicating that SCFG was significantly more sticky than other treatments. SCFG also was the only treatment with a significantly higher rate of change in texture. SCFG had the highest initial moisture content and A_w among the treatments leading to more adhesion and a

more significant rate of change of the adhesion force. Studies of pear fruit leather reported similarly that additional water (moisture content or A_w) increased cohesion and surface moisture or adhesion (Huang & Hsieh, 2005). The same pear fruit leather study also reported that both corn syrup and pectin in pear fruit leather increased adhesion, which could be seen similarly in SCFG with the added bulking ingredients (Huang & Hsieh, 2005). Adhesion force decreasing is an indication of the treatments drying out during shelf life. SAJ, WAJ, and SSDC retained their initial adhesion properties while WSDC became more adhesive, and SCFG became less adhesive and drier. For WSDC and SCFG this change in adhesion was mirrored with the increase (WSDC) and decrease (SCFG) in moisture content. WSDC increased significantly in moisture content throughout shelf life, becoming stickier and thus increasing in adhesion properties. SCFG conversely decreased significantly in moisture content, leading to decreased adhesion properties and becoming drier. A_w properties also were mirrored in adhesion properties, with SSDC and WSDC having the lowest initial A_w and moisture content and therefore having the lowest adhesion force. Across treatments, apple juice concentrate infused treatments were significantly more adhesive than sucrose infused treatments, but less adhesive than SCFG, mirroring the results found in moisture content and A_w. SCFG was the only treatment with a significantly lower rate of change indicating that the additional bulking ingredients that led to higher A_w may also lead to a reduced rate of change compared to other treatments. Consumer testing would be needed to indicate whether these textural changes would impact consumer acceptability.

Table 2.14 Comparison of initial and final adhesion force of sweetened dried cranberries

TREATMENT	INITIAL (G)	FINAL (G)
Sliced Apple Juice Infused (SAJ)	$199.70^{bA} \pm 58.03$	$214.88^{aA} \pm 21.06$
Whole Apple Juice Infused (WAJ)	$180.11^{bA} \pm 27.03$	$176.83^{aA} \pm 13.89$
Sliced Sucrose Infused (SSDC)	$90.00^{cA} \pm 15.54$	$111.91^{bA} \pm 19.97$
Whole Sucrose Infused (WSDC)	$61.25^{cA} \pm 7.87$	$96.70^{bB} \pm 16.03$
Sliced Soluble Corn Fiber, Glycerin, Sucrose, and Sucralose Infused (SCFG)	$292.52^{aA} \pm 29.88$	$182.02^{aB} \pm 13.89$

Each value is the average ± standard deviation (n=3). Treatments were stored at 21ºC in individually sealed bags in boxes to limit light exposure for 360 days. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$). Treatments were analyzed by ANOVA (Tukey's HSD).

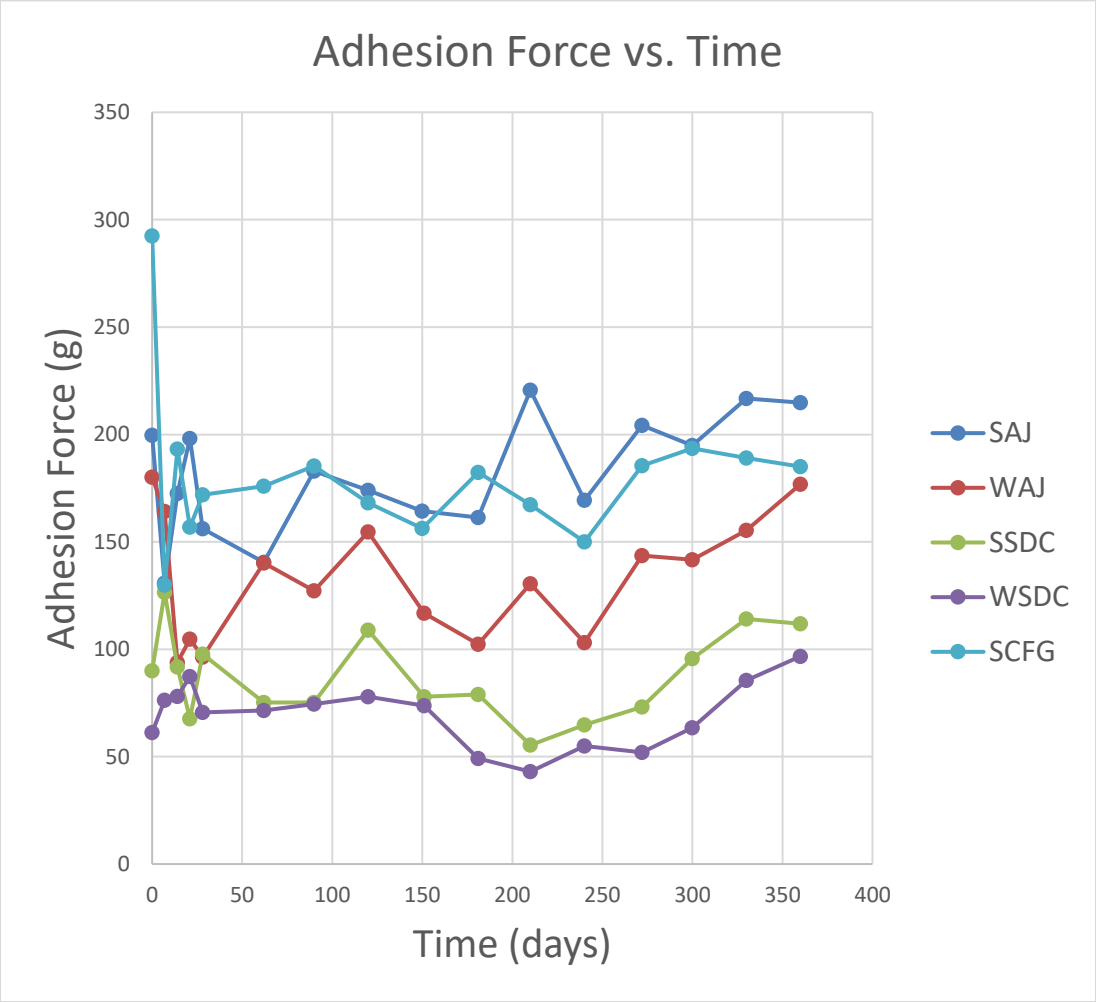

Figure 2.19 Adhesion force versus time of sweetened dried cranberries

Conclusions

Regardless of product matrix, sweetened dried cranberry polyphenols are unstable and undergo significant degradation over time, with anthocyanins and proanthocyanidins being more unstable than total phenolic content. Sliced sweetened dried cranberries provide a better product matrix for polyphenol stability and content due to increased infusion and decreased thermal processing compared to whole sweetened dried cranberries. Stability of polyphenols during processing is also increased by bulking ingredients, such as soluble corn fiber and glycerin, which provide protection from degradation of polyphenols. Rates of degradation were increased

for treatments with higher initial polyphenol content, and the rates followed the first order kinetic model.

Despite insulation, increased stability of polyphenols, and increased polyphenol content, antioxidant activity when measured using DPPH assay showed negligible antioxidant capacity, most likely due to the processing conditions that sweetened dried cranberries undergo, as well as the mechanism of antioxidant scavenging which may not be fully represented using DPPH assay. While treatments did not show a significant change in A_w, change in moisture content varied between treatments.

While sweetened dried cranberries are a convenient option for a snack or ingredient, compared to raw cranberries, the bioactive compounds are negatively impacted by processing over time limiting application as a bioactive food, except for antioxidant activity, which was comparable to raw cranberries when measured using DPPH assay. Processing and storage parameters could be optimized to minimize polyphenol degradation and better position sweetened dried cranberries as bioactive foods.

Examples include 1) reducing the time and temperature of the drying process to better retain the bioactive compounds found in raw cranberries, 2) introducing a nitrogen flush to the packaging to limit oxygen exposure and oxidation reactions during storage, and 3) utilizing different packaging materials which may be better inhibitors of oxidation.

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Chapter 3 - Polyphenol Stability and Antioxidant Capacity of Cranberry Supplements

Abstract

Cranberries are fruits containing phenolic compounds, such as anthocyanins and proanthocyanidins, which can be concentrated in extract form and used as supplements for health-conscious consumers. Supplements may be consumed in two common product matrices, a spray-dried capsule or a chewable gummy with filler ingredients, however while there is research showing the phenolic composition of cranberry supplements, there is little research on how product matrix and processing affect phenolic compounds in supplements over time. The objectives of this research were to 1) assess the polyphenol content of various cranberry supplements, 2) determine the stability of those components over time, and 3) assess any relationship between product matrix and polyphenol stability.

This research assessed 3 different commercially available cranberry supplements: 1) Theracran® One Cranberry Supplement (spray-dried treatment), 2) Azo Cranberry® Gummy (high filler ingredient bulk treatment), and 3) Ocean Spray® Cranberry + health™ Gummy (low filler ingredient wrapped treatment) (3 replicates/treatment). Anthocyanins (HPLC), proanthocyanidins (BL-DMAC and cranberry standard DMAC assay), total phenolic content (Folin-Ciocalteu), antioxidant capacity (DPPH), water activity, moisture, color, and texture were evaluated over 12 months at 21°C. Data were analyzed by ANOVA ($p<0.05$).

The results demonstrate that the spray-drying and encapsulation of cranberry extracts provide greater polyphenol stability and content than gummy supplements due to the protective

properties that are a result of encapsulation compared to wrapped or bulk gummies, the absence of filler ingredients, higher quality and quantity of extracts, and the impacts of lower moisture content and A_w from optimized time and temperature conditions of spray-drying compared to gummy manufacturing. Within gummy treatments it was seen that product matrix, as well as packaging type impacted polyphenol content and stability, with the individually wrapped treatment with fewer filler ingredients having increased initial polyphenols compared to the bulk-packed high filler ingredient treatment.

These results were also seen with regards to antioxidant capacity, indicating not only that the same conclusions can be drawn regarding product matrix between spray-dried supplements and gummy supplements, but also that there is a strong correlation between phenolic content and antioxidant activity in cranberry supplements across treatments. More research is needed to optimize gummy supplement processing, product matrix, and packaging attributes to increase polyphenolic content and stability of polyphenols over time, as well as to better understand health-conscious consumer attitudes and preferences to consume raw fruit, spray-dried supplements, or gummy supplements.

Introduction

Nutraceuticals harness the biologically active compounds in whole fruits, for example, in a nonconventional form of a food such as a pill or gummy. Ginger shots and extracts are used to relieve symptoms of nausea (Semwal, et al., 2015), and cranberry extracts can aid in urinary tract health via bacterial anti-adhesion effects (Singh, et al., 2016). Nutraceuticals are aimed at health-conscious consumers who may want to self-diagnose the need for select bioactives, however, the FDA requires companies to place disclaimers on packaging to alert the public that the claims that

the products are making have not been evaluated by the FDA and are not intended to replace pharmaceutical medications. An example is a claim suggesting that a product may aid in urinary tract health is allowed, but a claim stating that a product cures UTI is not allowed. The statement required is the following: "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease" (21CFR101.93). Nutraceuticals are an easy option for consumers compared to the rigor that comes with preparing meals or the amount of a whole fruit needed to be consumed to get the same benefits as an extract, which removes the water in the fruit to concentrate bioactive compounds in a more convenient form. The extract, which is then more concentrated in bioactive compounds, can be added to different forms of products such as a spray-dried capsule or a gummy supplement. While the different product forms may be more appealing to different consumers, the way they are processed are also different, leading to potential differences in the content and stability of the bioactive compounds in the nutraceutical.

Millennials make up the largest age group in the United States and thus drive a large majority of the eating trends in the United States (Rosenbloom, 2018). Eating healthy by preparing meals at home is expensive and time consuming. A 2017 study found that 55% of Millennials are driven by the convenience of a food over the taste of food (Rosenbloom, 2018). In addition to convenience, sugar is an ingredient that many Millennials are looking to avoid. As many processed fruit products, such as juices and dried fruits, contain high amounts of sugar, supplements may appeal more to these health-conscious sugar-avoidant consumers. Within the supplement category, consumers have the choice between a non-caloric spray-dried extract pill or a chewy gummy which mimics fruit more than a traditional pill supplement.

Cranberries (*Vaccinium macrocarpon*) are fruits composed of bioactive compounds such as anthocyanins and proanthocyanidins which are polyphenols that make up some of the overall phenolic content of cranberries. By extracting the bioactive compounds in cranberries and putting them in an easy to consume supplement form, consumers can add the benefit of cranberries to their diet without the inconvenience and sugar of whole fruits or processed fruit products. Polyphenols are abundant in fruits and vegetables, however the amount needed to provide health benefits tend to be larger than a person might consume on a daily basis. Consumers would need to eat large quantities of fruits and vegetables to get these polyphenol benefits, however consumers are now eating on-the-go and live a fast-paced lifestyle, which makes eating whole fruits and vegetables less conducive to their busy lifestyle. The convenient option of supplements allows consumers to get the health benefits of polyphenols in fruits in a smaller and more manageable form.

Anthocyanins are a class of flavonoid which give pigmentation to fruits and vegetables, usually in the form of reds, blues, purples, and blacks (Steyn, 2009). Cranberries are made up primarily of cyanidin and peonidin, two out of six classes of anthocyanidins (Figure 3.1) (Kato, et al., 2015). The metabolites of cyanidin and peonidin have been reported to be antioxidants possessing anti-inflammatory properties (Fang, 2015) (Kakkar & Bais, 2014). Studies with mulberry extracts fed to mice and rats, as well as introduced to human cancer cells, report that anthocyanins (cyanidin-3-glucose, cyanidin-3-rutinoside, and peonidin-3-glucoside) possess other beneficial health attributes, especially in relation to cancer prevention (Huang, Chang, Wu, Hung, & Wang, 2011) (Long, et al., 2018) (Li, et al., 2016). A 2011 study reported anthocyanin extracts from mulberries (cyanidin-3-glucoside and cyanidin-3-rutinoside) were effective in inhibiting gastric cancer cells in mice by inducing apoptosis in the cancerous glandular cells,

thus reducing the amount of cancer cells that could grow via p38 caspase 8 signaling and p38 Bac signaling pathways (Huang, Chang, Wu, Hung, & Wang, 2011). Anthocyanin-induced apoptosis of cancer cells was reported again with mulberry anthocyanins in relation to thyroid cancer where mulberry anthocyanins acted as thyroid tumor suppressors by deactivating Akt/mTOR signaling in cancer cells (Long, et al., 2018). Anthocyanins induce cell death in cancer cells independent of where the cells are derived from. In patients with trastuzumab-resistant breast cancer, anthyocyanin extracts of mulberry (cyanidin-3-glucoside and peonidin-3-glucoside) were once again reported to induce apoptosis in the cancerous cells which limited cancer cell migration and overall growth, both *in vitro* and *in vivo* (Li, et al., 2016). Cyanidin-3-glucoside and peonidin-3-glucoside found in mulberry extracts are also found in cranberries, supporting the important role fruit bioactive consumption can have on human health.

Proanthocyanidins are a part of the flavonoid group also known as condensed tannins; they are polymers of flavan-3-ols and are commonly consumed in the human diet (Gnanavinthan, 2013) (Santos-Buelga & Scalbert, 2000). Proanthocyanidins are produced when flavanol monomers are polymerized, which can occur naturally within plants or from oxidation during processing (Beecher, 2003). Proanthocyanidins are found as either B-type carbon-carbon linkages or A-type carbon-oxygen linkages. Figure 3.2 shows a comparison of proanthocyanidin dimers between a B-type linkage and an A-type linkage (Xu, et al., 2015). The main difference between A-type and B-type linkages, as illustrated, is the addition of an ether bond within the A-type linkages. Foods such as grapes and blueberries contain the widely appearing B-type linkages, while cranberries and plums contain A-type linkages (Xu, et al., 2015) (Howell, 2007) (Gu, et al., 2004). A-type linkages are associated with anti-adhesion activity (Howell, 2007) (Gu, et al., 2004). Cranberries have A-type proanthocyanidins and the anti-adhesion properties

associated with A-type proanthocyanidins make cranberries good promotors of urinary tract health (Howell, 2007). Anti-adhesion properties make it so that harmful bacteria such as P-fimbriated uropathogenic *E. coli* cannot adhere within the body and cause infections (Howell, 2007). This effect occurs because the A-type proanthocyanidins bind to *E. coli* fimbrial tips which inhibits the bacteria from adhering (Hisano, et al., 2012).

A 2003 study reported that B-type proanthocyanidins from apple juice, green tea, and dark chocolate provided no anti-adhesion *in vitro* while A-type proanthocyanidins from cranberry juice cocktail showed *in vitro* anti-adhesion (Howell, et al., 2005). While A-type proanthocyanidins are more well-known for their anti-adhesion properties in urinary tract health, a 2010 study reported that A-type proanthocyanidins are responsible for the antioxidant capacity of lychee seeds *in vivo*, and proanthocyandin litchitannin A2 exhibited anti-coxsackie virus B3 activity *in vitro*, indicating that A-type proanthocyanidins are beneficial to health as an antioxidant, an anti-viral agent, as well as for anti-adhesion (Xu, et al., 2010).

The USDA database states that raw cranberries contain 60.42 mg/100 g cyanidin and peonidin anthocyanin (Haytowitz, et al., 2018), 30.74 mg/100 g total polyphenols (Haytowitz, et al., 2018), and 354.9 mg/100 g proanthocyanidins (Bhagwat & Haytowitz, 2015). While raw cranberries are rich in bioactive compounds which may possess health benefits, cranberries are generally not consumed raw. Only 5% of cranberries are sold as raw fruit, with the rest being sold as juices, dried cranberries, sauces, or supplements (Grace, et al., 2012). Cranberry supplements harness the bioactive components of cranberries in an easier to consume form such as a spray-dried capsule or a chewable gummy. The gummies are available in two formats, bulk packaging with many filler ingredients or individually wrapped with fewer filler ingredients. The quality and quantity of cranberry extract may also differ.

While information on the processing effects on content and stability of bioactive compounds in juices is well established, there is a need for research on the polyphenol content and stability of different cranberry supplements to ensure that supplements are meeting claims and providing viable bioactive compounds not just initially, but over time.

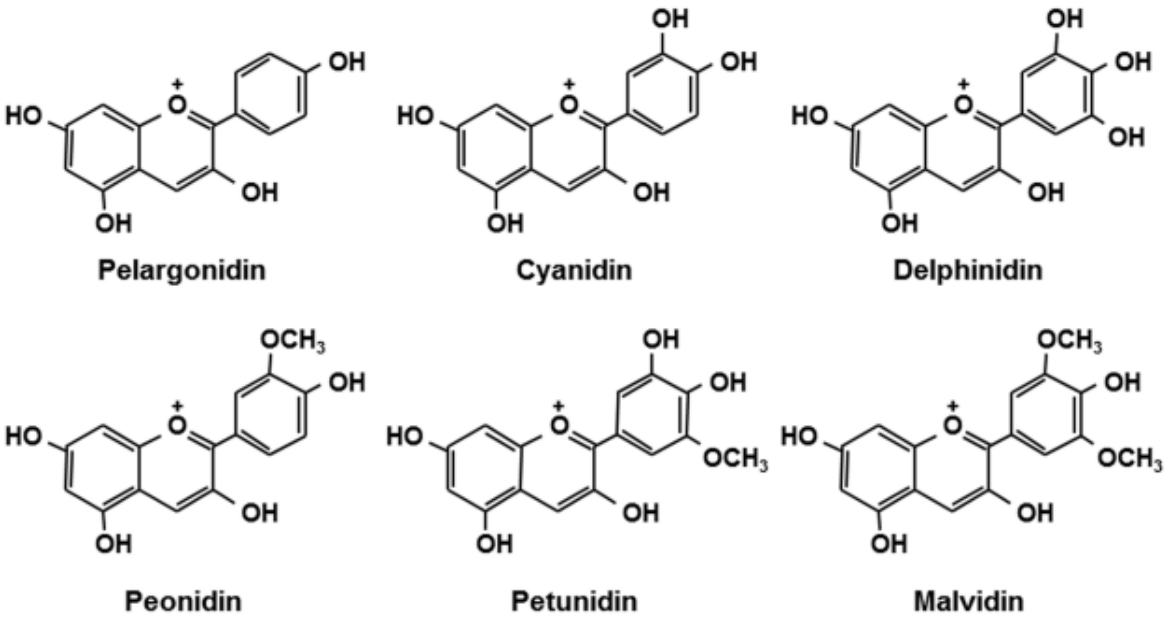

Figure 3.1 Structure of anthocyanidins

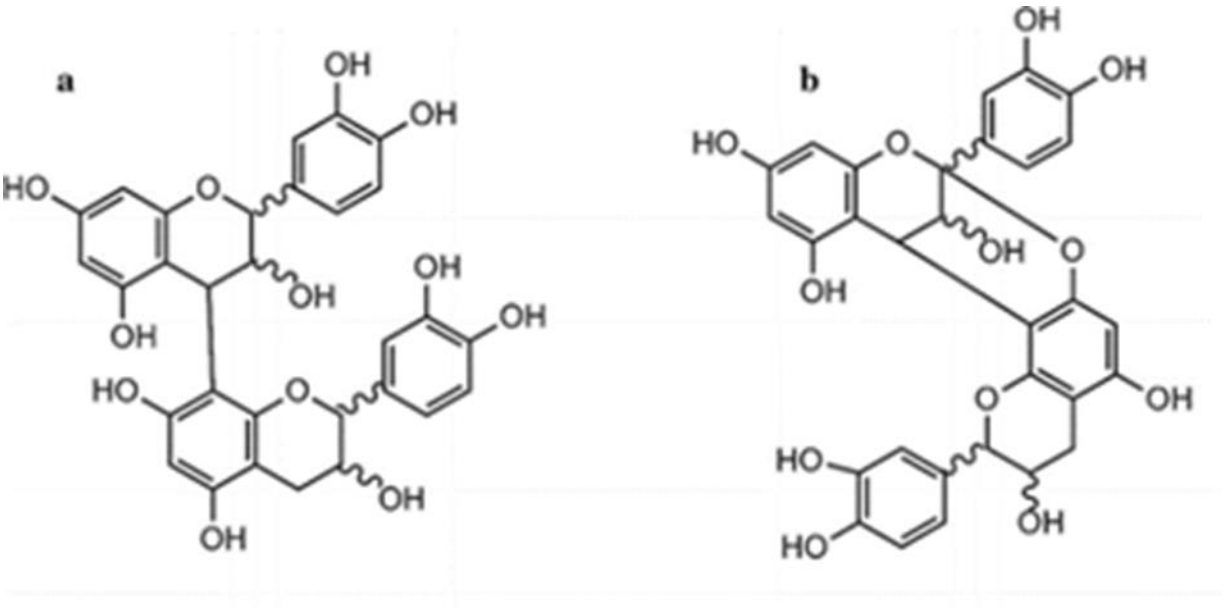

Figure 3.2 a) B-type epicatechin dimer from granny smith apples and b) A-type epicatechin dimer from red peanut skin (Xu et al., 2015)

Materials and Methods

This research assessed 3 types of commercially available cranberry supplements 1) individually wrapped low filler ingredient gummy supplement (OSC), 2) bulk high filler ingredient gummy supplement (AZO), and 3) spray dried encapsulated supplement (TC) (Table 3.1). Treatments were stored for 360 days at 21^{o}C in original packaging within a sealed box to reduce light exposure. Bulk plastic containers were opened for each time point. Analytical measurements were taken on the treatments initially (t=0), weekly for the first month, then monthly until 360 days were obtained. Analyses were terminated prior to 360 days if two consecutive analyses contained no quantifiable polyphenols. At each time point 3 replicates were analyzed for each treatment. Reagents were analytical grade and were purchased from Fischer Scientific (Waltham, MA) unless otherwise noted.

Table 3.1 Cranberry Supplements Sample Overview

TREATMENT	STRUCTURE	PACKAGING	INGREDIENT
OSC	Gummy Supplement	Individually wrapped	Cranberry Fruit Extract, Apple Juice Concentrate, Apple Puree, Pectin
AZO	Gummy Supplement	Bulk plastic container	Cranberry Whole Fruit Powder, Glucose Syrup, Sugar, Water, Natural Flavors, Pectin, Sodium Citrate, Colors (Grape Juice Concentrate, Titanium Dioxide), Citric Acid, Coconut Oil, Carnauba Wax
TC	Spray-Dried Capsule Supplement	Bulk plastic container	Cranberry Extract, Tricalcium Phosphate, Gelatin (bovine), Calcium Silicate, Magnesium Stearate, Silicon Dioxide, Rice Flour

Proanthocyanidin Content

Extraction of OSC and AZO

Replicates were added to a Warren blender with deionized (DI) water (1:11) and blended for 3 min on the low setting. The liquid replicate (5 g) was placed into a centrifuge tube along with 15 mL extraction solution (75% acetone, 24.5% DI water, 0.5% acetic acid (v/v)). The centrifuge tube was vortexed for 10 s, sonicated using a Branson ultrasonic water bath (Danbury, CT) for 15 min, and then centrifuged at 7954 *x g* for 20 min. The supernatant was removed from the pellet and placed into a 50 mL Falcon tube. The extraction was repeated two more times with

the extraction solution added to the pellet each time and the supernatants combined. After the final extraction each replicate was placed in a glass tube in a Buchi Syncore extraction device (Flawil, Switzerland) and placed under vacuum at 45ºC for 5 h. Vacuum settings were based on a five step gradient where 1) 450 mbar – 350 mbar for 10 min 2) 350 mbar – 300 mbar for 5 min 3) 300 mbar – 265 mbar for 105 min 4) 265 mbar – 125 mbar for 80 min 5) 125 mbar – 25 mbar for 100 min. A vacuum pump was connected to a recirculating chiller set to -10ºC.

Extraction of TC

Replicates (2.5 g) were placed into a centrifuge tube along with 7.5 mL of extraction solution (75% acetone, 24.5% DI water, 0.5% acetic acid (v/v)). The test tube was vortexed for 10 s, sonicated using a Branson ultrasonic water bath (Danbury, CT) for 15 min, and then centrifuged at 7954 *x g* for 20 min. The supernatant was removed from the pellet and placed into a 50 mL Falcon tube. The extraction was repeated two more times with the extraction solution added to the pellet each time and the supernatants combined. After the final extraction each replicate was placed in a glass tube in a Buchi Syncore extraction device (Flawil, Switzerland) and placed under vacuum at 45ºC for 5 h. Vacuum settings were based on a five step gradient where 1) 450 mbar – 350 mbar for 10 min 2) 350 mbar – 300 mbar for 5 min 3) 300 mbar – 265 mbar for 105 min 4) 265 mbar – 125 mbar for 80 min 5) 125 mbar – 25 mbar for 100 min. A vacuum pump was connected to a recirculating chiller set to -10ºC.

Proanthocyanidin Content – BL-DMAC Assay

Treatments were assessed for proanthocyanidin content using an A2 dimer procyanidin standard following a modified method of Prior et al (2010). For proanthocyanidin analysis a

Precision XS with 96 well plate (Bio-Tek Instruments, Inc., Winooski, VT) was used for serial dilutions. A2 dimer standard was made by taking 5 mg of procyanidin in a 50 mL volumetric flask and bringing up to volume with ethanol. Using a 96-well plate, 140 µL of blank (80% ethanol in DI water), A2 dimer standard, and the replicates were loaded onto the first column. Precision XS performed serial dilutions by taking 70 µL of blank solution and filling all 7 columns. Taken from each well was 70 µL and then mixed with the next well in the series, with the last 70 µL from the final column being discarded so that a final working volume of 70 µL was in each cell. 4-dimethylaminocinnamaldehyde (DMAC) (Sigma-Aldrich, St. Louis, MO) (210 µL) (0.1 DMAC powder in 100 mL 75% ethanol, 12.5% HCl, and 12.5% deionized water (v/v)). The well plate was loaded into Synergy 2 microplate reader with GEN5 software (Bio-Tek Instruments, Inc., Winooski, VT) and analyzed at 25ºC and 640 nm every min for 30 min. A calibration curve was generated from the A2 standard used. The concentration of proanthocyanidins in each replicate was determined using a calibration curve (A2 dimer standard absorbance versus A2 dimer concentration) regression line and the below equation where *c* is the concentration of proanthocyanidins in the extraction (g/L), *d* is the dilution factor, *v* is the volume of the extraction fluid after vacuum (mL), and *w* is the weight of the replicate used for extraction (g).

$$PACs = \frac{c\ x\ d\ x\ v}{(1000\ x\ w)}$$

Proanthocyanidin Content – Cranberry Standard Assay

Treatments were assessed for proanthocyanidin content using a modified method of Krueger et. al (2016). Bio-Rad Poly-prep® columns (Hercules, CA) (2 per replicate) were set up in column racks and pre-hydrated lipophilic Sephadex® LH20 (22%) (Sigma-Aldrich, St. Louis,

MO) was added to the column to a volume of 1.2 mL. Each replicate was read in duplicate. DI water was then added to the column to 7.5 mL. Once the column was drained 1 g of replicate was added to the column. DI water was then added to the top of the column. Once the column was drained 10 mL of 25% reagent alcohol in DI water was added to the column and allowed to drain. The columns were then transferred to 15 mL Falcon tubes and washed twice with 2.5 mL 70% acetone in DI water. The liquid collected in the Falcon tube was then vortexed for 5 s. The liquid (1 mL) was added to glass test tubes by auto-pipette using 70% acetone as the blank. DMAC solution (0.1% DMAC) (Sigma Aldrich, St. Louis, MO) in HCl:Methanol (30:70). DMAC solution (3 mL) was transferred by autopipette to a test tube and vortexed for 5 seconds. After 3 min the liquid was vortexed for 3 s. After 5 min the absorbance of the sample was read spectrophotometrically (640 nm). Proanthocyanidin content was calculated using the below equation where ABS is the absorbance, RF is the response factor, *w* is the weight of the replicate loaded onto the column, *d* is the dilution factor if used (Replicate Weight/Water + Replicate Weight), and %S is the percent solids (100-Moisture Content). Proanthocyanidin content was then converted to mg/g. Replicates were diluted prior to analysis so that the absorbance was between 0.2 and 0.8 when read spectrophotometrically.

$$\frac{ug\ PACs}{g\ DWB} = \frac{\frac{ABS\ x\ 5}{RF\ x\ w\ x\ d\ x\ \%S}}{0.95}$$

Anthocyanin Content

An Agilent 1260 HPLC (Agilent Technologies, Santa Clara, CA) was used for HPLC analysis of anthocyanins (Brown and Shipley 2011). Replicates (1.5 g) were added to 20 mL of

2% HCl:Methanol (2:98) (OSC and AZO treatments used 1:11 DI water dilution). The solution was sonicated for 15 min, shaken for 30 min, and centrifuged at 2324 *x g* for 5 min. The supernatant was removed from the pellet and used in HPLC analysis.

The HPLC was run with 2 mobile phases (v/v) consisting of A) DI water (99.5%) and o-phosphoric acid (0.5%) and B) deionized water (50%), acetonitrile (48.5%), acetic acid (1%), and o-phosphoric acid (0.5%). Each replicate was analyzed via HPLC for 35 min with a 10 µL injection volume, 1.0 mL/min flow rate, and the absorbance at 520 nm and 25ºC, using a Water X-Select HSS T3 5µm, 4.6 x 150 mm reversed-phase C18 column. Replicates were analyzed for cyanidin-3-galactoside (cy-3-gal), cyanidin-3-glucoside (cy-3-glu), cyanidin-3-arabinoside (cy-3-arab), peonidin-3-galactoside (peo-3-gal), peonidin-3-glucoside (peo-3-glu), and peonidin-3-arabinoside (peo-3-arab). Standards for cy-3-gal, cy-3-glu, cy-3-arab, and peo-3-glu were obtained via Phytolab (Vestenbergsgreuth, Germany). Peo-3-glu and peo-3-arab were reported as peo-3-glu equivalents due to limited commercial manufacturing of the standards and the retention times were based on those validated by Brown & Shipley (2011). The minimum detection limit for cyanidins is 0.02 µg/mL and for peonidins 0.01 µg/mL. Flow rate was kept consistent throughout the run (1.0 mL/min) and ratio of mobile phase A to mobile phase B was decreased for the first 32 min and then increased to the initial settings for the remaining 3 min. The anthocyanin content of each sample was calculated using Agilent Open lab software (Agilent Technologies, Santa Clara, CA) based on the extraction dilution and reported as ppm.

Total Phenolic Content

Total phenolic content was measured using the Folin-Ciocalteu colorimetric assay (Singleton & Rossi, 1965) (Asami, et al., 2003). For gummy treatments the 1:11 water dilution

was used, and replicates were further diluted 1:20 in DI water for OSC and 1:2 for AZO. TC (1.2 g) were dissolved in 45 mL of DI water then further diluted 1:45. Each replicate was analyzed in duplicate and DI water was used as the blank. Diluted sample and blank (100 µL) were pipetted into a glass test tube. DI water (3.9 mL) was added to each test tube and vortexed for 5 s. Folin-Ciocalteu reagent (1:10 2N Folin-Ciocalteu Phenol Reagent in DI water) (250 µL) was added to the test tube and vortexed for 5 s. Sodium carbonate solution (7.5%) (750 µL) was added to each test tube and vortexed for 5 s. The replicates were stored in the dark for 30 min and absorbance was read spectrophotometrically (765 nm). Gallic acid (Sigma-Aldrich, St. Loius, MO) was used to create a standard calibration curve where 0.5% gallic acid solution was prepared and diluted to 0, 10, 50, 100, 150, and 200 mg/L of gallic acid and the standard curve was made by plotting absorbance versus concentration. Total phenolic content in the replicates was determined by using the gallic acid calibration curve, dilution factor of the replicate, and the moisture content of the replicate to report TPC as mg/g gallic acid equivalent (GAE).

Antioxidant Activity

Antioxidant activity was assessed using a reaction with 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO) (AOAC 2012.04). For OSC and AZO treatments a 1:11 water dilution was used and 15 g of each replicate was added to a 100 mL volumetric flask with 50 mL 95% ethanol. TC (0.2 g) were added to a 100 mL volumetric flask with 50 mL 95% ethanol. The solution was shaken on a shaker table for 2 h then filtered through Whatman #4 filter paper. Solutions were diluted to 0.1, 0.5, 1.0, 2.0, 5.0, and 7.0 g/mL in 95% ethanol and vortexed. Each solution (0.1 mL) was placed in a glass test tube with 0.1 mL of 95% ethanol as a control. 2.9 mL of DPPH solution (2.9 mL of 0.0394 g in 1000 mL methanol) was added to the

test tube and vortexed for 5 s. Dilutions were stored in the dark for 30 min and read spectrophotometrically (517 nm). DI water was used as the blank. Percentage radical scavenging activity was calculated with the below equation.

$$DPPH\ Scavenging\ Activity\ (\%) = \frac{ABS(control) - ABS(sample)}{ABS(control)}\ x\ 100$$

The concentration of antioxidant required to inhibit 50% of the oxidation reaction (IC_{50}) was calculated by plotting % DPPH scavenging activity against the concentration and using the logarithmic trendline. Equations for these calculations are below where *g replicate* (g) is the weight of the powder used in the extraction and *mg dilution* (mg) is the weight of the serial dilutions. For the IC50 calculation, *a* is the slope of the natural logarithmic tread line of the plot of % scavenging versus concentration, and *b* is the y-intercept of the natural logarithmic tread line of the plot of % scavenging versus concentration.

$$Concentration\frac{mg}{mL} = \left(\frac{g\ replicate}{50\ mL}\ x\ 1000\right) x\ (mg\ Dilution)$$

$$IC50{:}\ 50 = aln(x) + b$$

Water Activity and Moisture Content

Water Activity

Water activity (A_w) was measured using a calibrated Aqua Lab 4TE (Meter Group Inc., Pullman, WA). Each replicate was measured in triplicate.

Moisture Content

Moisture content was assessed via Karl-Fischer titration using a calibrated Metrohm KF 901 Titrando auto-titrator (Metrohm, Herisau, Switzerland). For OSC and AZO treatments each replicate (5 g) was added to a stainless-steel homogenization flask and 50g of 50:50 Karl-Fischer Grade low water methanol and Formamide was added. The replicate was then homogenized for 5 min using an Omni Mixer (Omni International, Kennesaw, GA) set to speed 4. After homogenization, the flask was disconnected and covered with parafilm. The liquid sat undisturbed for 5 min before being analyzed for moisture content. Each replicate was then taken into a 3 mL syringe and was run through the auto-titrator. Each replicate was run in triplicate. TC (1 g) was added to a 50 mL volumetric flask with 20 g of Karl-Fischer Grade low water methanol. The replicate was shaken for 5 min and then the flask sat undistributed for 5 min before being analyzed for moisture content. Each replicate was then taken into a 3 mL syringe and was run through the auto-titrator. Each replicate was run in triplicate.

Colorimetric and Texture Analyses

Color

Color was analyzed for OSC and AZO using a calibrated Digieye color analyzer (VeriVide Ltd., Leicester, UK). Each replicate was read in triplicate and measured for L*, a*, and b* values which were used to calculate ΔE according to the below calculation.

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

Texture

Texture was analyzed for AZO and OSC treatments only using a calibrated TA.XT.Plus from Texture Technologies Corporation (Hamilton, MA). The TA.XT.Plus was configured with a TA-52 2 mm probe, 1.0 mm/s pre-test speed, 2.0 mm/s test speed, 5.0 mm/s post-test speed, 20.0 mm target distance, 5.0 g auto trigger, and return to start in compression test setting. Each replicate was loaded onto the base plate and the test was run. Hardness (g/sec), toughness (g/sec), and adhesion force (g) was recorded by the texture analysis software (Exponent 32, version 6, Texture Technologies Corp, Scarsdale, NY).

Statistical Analysis

Data were analyzed using Minitab 16 Software (State College, PA) for analysis of variance (ANOVA) for all treatments. Shapiro-Wilk test for normality was assessed. Tukey's Honest Significant Difference Test (HSD) was used for post-hoc analyses. Significance level of $p<0.05$ was used for all analyses.

Results and Discussion

Proanthocyanidin Content

While analysis using BL-DMAC assay indicated a significant ($p<0.05$) decrease in proanthocyanidin content regardless of product matrix, analysis using cranberry standard DMAC assay did not yield the same results, indicating that method of analysis for proanthocyanidin analysis should be taken into consideration when determining proanthocyanidin content in cranberry supplements as well as comparing results between tests (Table 3.3) (Table 3.4) (Figure 3.3) (Figure 3.4) (Figure 3.5) (Figure 3.6). In all treatments, BL-DMAC yielded

proanthocyanidin content significantly lower than when assessed using cranberry standard DMAC assay. Significantly lower proanthocyanidin values from BL-DMAC assay compared to cranberry standard DMAC assay were also seen in previous studies (Martin, et al., 2015) (Krueger, et al., 2016). Lower proanthocyanidin values from BL-DMAC assay could be due to the standard utilized (A2 dimer standard in BL-DMAC compared to cranberry derived standard used in the cranberry standard assay). Proanthocyanidins in cranberries are more complex than other fruits which may only have procyanidin A2 dimer, so underestimation occurs as the more complex oligomers are not quantified from the A2 dimer standard (Krueger, et al., 2016). Although BL-DMAC assay is the industry standard for proanthocyanidin quantification, cranberry supplements containing complex proanthocyanidin oligomers should be analyzed using cranberry standard DMAC assay so that more complex proanthocyanidins are quantified as well.

When analyzed using BL-DMAC assay there was a significant decrease in proanthocyanidin content from initial (t=0) to final (t=360) regardless of product matrix. Across treatments initially there was a significant difference in proanthocyanidin content, with TC having the highest (77.17 mg/g), OSC next (3.22 mg/g), and AZO having the lowest (0.32 mg/g). Final proanthocyanidin content across treatments indicated that TC was still significantly higher (48.81 mg/g) while there was no difference in final proanthocyanidin content between OSC and AZO (0.33 mg/g and 0.11 mg/g, respectively). TC had a significantly higher rate of change compared to OSC and AZO due to the higher initial content (Figure 3.2). A reason for the difference in proanthocyanidin content across treatments is likely due to product matrix, which is linked to processing conditions. Studies have reported that spray-drying preserves heat sensitive compounds such as polyphenols compared to gummy manufacturing which relies on longer heat

treatments, slower water removal, and incorporation of filler ingredients (Fang & Bhandari, 2011) (Garcia-Ruiz, et al., 2017). Spray-drying and encapsulation can provide stability of the phenolic compounds by protecting them from reactivity during procesing (heat, oxidation, moisture, light) (Desai & Park, 2005). Both OSC and AZO are gummy supplements which are subject to longer exposure to heat and slower water removal compared to spray drying and also contain other filler ingredients. The added filler ingredients change the composition resulting in less proanthocyanidin content by weight compared to TC. Within the gummy treatments, AZO has more ingredients (12) compared to OSC (4), so having lower proanthocyanidin content could be attributed to the type and amount of cranberry extract added as well as more ingredients reducing the concentration of proanthocyanidins in the treatment. Both AZO and OSC are also produced via depositing technology, which undergoes heat treatment to remove water through evaporation. Polyphenols, including proanthocyanidins, are heat sensitive and water soluble so the combination of heat and water evaporation during processing could also cause the lower initial proanthocyanidin content compared to spray-dried TC. There were also differences in the polyphenol content of OSC compared to AZO despite both of them being gummy supplements, indicating that even within a product matrix (gummy supplements), content can be significantly impacted by other factors such as other ingredients and type and quantity of extract used in the formulation. OSC had significantly higher initial proanthocyanidin content compared to AZO despite DMAC assay used. The overall decrease in proanthocyanidins was significantly larger in OSC and AZO, amounting to 36.8% decrease in TC compared to 89.7% decrease in OSC and 64.5% decrease in AZO. The larger overall degradation of proanthocyanidins in AZO and OSC compared to TC further validates that spray-drying and encapsulation aids in stabilization of

proanthocyanidins (Desai & Park, 2005), while also validating that even within gummy treatments, packaging and product matrix can impact proanthocyanidin content and stability.

When analyzed by cranberry standard DMAC assay, OSC was the only treatment that showed a significant decrease in proanthocyanidin content (15.14 mg/g to 3.13 mg/g), indicating that complex proanthocyanidin oligomers may be in larger quantities and have more stability. TC had a significantly higher initial proanthocyanidin content (306.6 mg/g) as well as a significantly higher final proanthocyanidin content (278.0 mg/g) than both OSC and AZO (15.14 mg/g to 3.13 mg/g for OSC and 2.50 mg/g to 1.73 mg/g for AZO, respectively). Overall degradation between treatments were significantly less in all treatments compared to BL-DMAC assay (79.3% decrease for OSC, 30.8% decrease for AZO, and 8.70% decrease for TC), once again confirming that the complex proanthocyanidin oligomers are more stable than the dimers that are quantified using BL-DMAC. Compared to BL-DMAC, there was no significant difference in the rates of degradation between treatments.

Compared to the proanthocyanidin content of raw cranberries (3.59 mg/g), OSC is comparable initially, AZO has significantly less, and TC has significantly more, making TC the best option for proanthocyanidins as a supplement for cranberry proanthocyanidins (Bhagwat & Haytowitz, 2015). Even though TC may be the best option for consumers wanting to add in proanthocyanidins to their diet, the appeal of taking a very medicinal looking supplement as opposed to whole fruit or gummies may deter purchase intent of the TC, even though it may provide more benefits. The ability of future research to increase quantity of proanthocyanidins in gummy supplements and increase gummy supplement proanthocyanidins stability to equal level of spray-dried supplements may increase consumer purchase.

Table 3.2 Rates of Change of Cranberry Supplements

TREATMENT	OSC	AZO	TC
Proanthocyanidin Content (BL-DMAC Assay)	-0.0080^{a} ± 0.0002	-0.0006^{a} ± 0.0002	-0.0788^{b} ± 0.0067
Proanthocyanidin Content (Cranberry DMAC Assay	-0.0334^{a} ± 0.0026	-0.0021^{a} ± 0.0008	-0.0740^{a} ± 0.0505
Anthocyanin Content	-0.7269^{b} ± 0.0605	-0.0454^{a} ± 0.0404	0.2112^{a} ± 0.2067
Total Phenolic Content	-0.0044^{a} ± 0.0113	-0.0021^{a} ± 0.0009	-0.0997^{b} ± 0.0167
A_w	-0.0000^{a} ± 0.0000	-0.0005^{a} ± 0.0008	-0002^{a} ± 0.0000
Moisture Content	-0.0139^{c} ± 0.0015	-0.0059^{b} ± 0.0016	0.0000^{a} ± 0.0000
ΔE	0.0092^{a}± 0.0005	0.0054^{b} ± 0.0009	-
Hardness	0.4264^{a} ± 0.2623	0.5628^{a} ± 0.1245	-
Toughness	1.659^{a} ± 0.8770	1.047^{a} ± 1.455	-
Adhesion	0.2532^{b} ± 0.0980	0.5726^{a} ± 0.1262	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21^{o}C. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 3.3 Proanthocyanidin content of cranberry supplements by BL-DMAC assay over time

TIME (DAYS)	OSC	AZO	TC
0	3.22^{bA} ± 0.05	0.32^{cA} ± 0.06	77.17^{aBCDE} ± 1.98
7	2.92^{bAB} ± 0.42	0.20^{cB} ± 0.02	65.39^{aCDEF} ± 5.35
14	3.17^{bAB} ± 0.24	0.20^{cB} ± 0.04	105.42^{aA} ± 5.29
21	2.80^{bB} ± 0.09	0.16^{cBC} ± 0.01	69.45^{aBCDE} ± 3.68
28	3.07^{bAB} ± 0.18	0.12^{cBCD} ± 0.02	85.90^{aAB} ± 9.68
60	2.06^{bC} ± 0.08	0.10^{cBCD} ± 0.01	80.11^{aBC} ± 3.85
90	1.71^{bCD} ± 0.03	0.18^{cBC} ± 0.02	87.39^{aAB} ± 2.55
120	1.54^{bDE} ± 0.05	0.12^{cBCD} ± 0.01	88.84^{aAB} ± 3.81
150	1.19^{bEF} ± 0.08	0.16^{cBC} ± 0.05	77.67^{aBCD} ± 3.41
180	0.98^{bFG} ± 0.05	0.13^{cBC} ± 0.01	60.16^{aDEF} ± 3.10
210	0.84^{bFG} ± 0.03	0.10^{cCD} ± 0.03	64.50^{aCDEF} ± 0.76
240	0.59^{bGHI} ± 0.04	0.11^{cBCD} ± 0.01	58.13^{aCDEF} ± 12.74
270	0.74^{bGH} ± 0.00	0.02^{cD} ± 0.04	57.49^{aEF} ± 3.02
300	0.61^{bGHI} ± 0.04	0.10^{cCD} ± 0.01	69.36^{aBCDE} ± 9.22
330	0.28^{bI} ± 0.02	0.08^{bCD} ± 0.06	79.17^{aBCD} ± 15.26
360	0.33^{bHI} ± 0.04	0.11^{bBCD} ± 0.01	48.81^{aF} ± 0.97

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing an uppercase letter are significantly ($p<0.05$) different. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

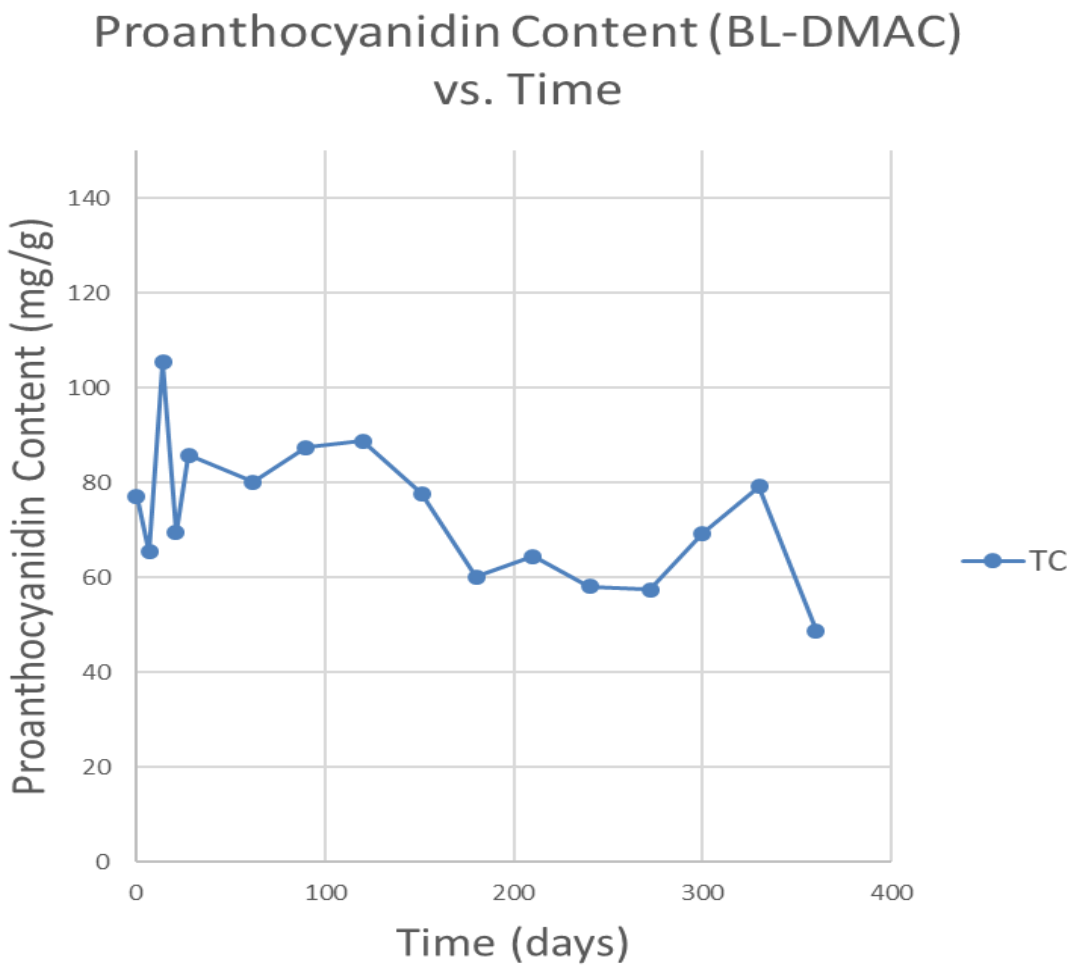

Figure 3.3 Proanthocyanidin content (BL-DMAC Assay) versus time of TC

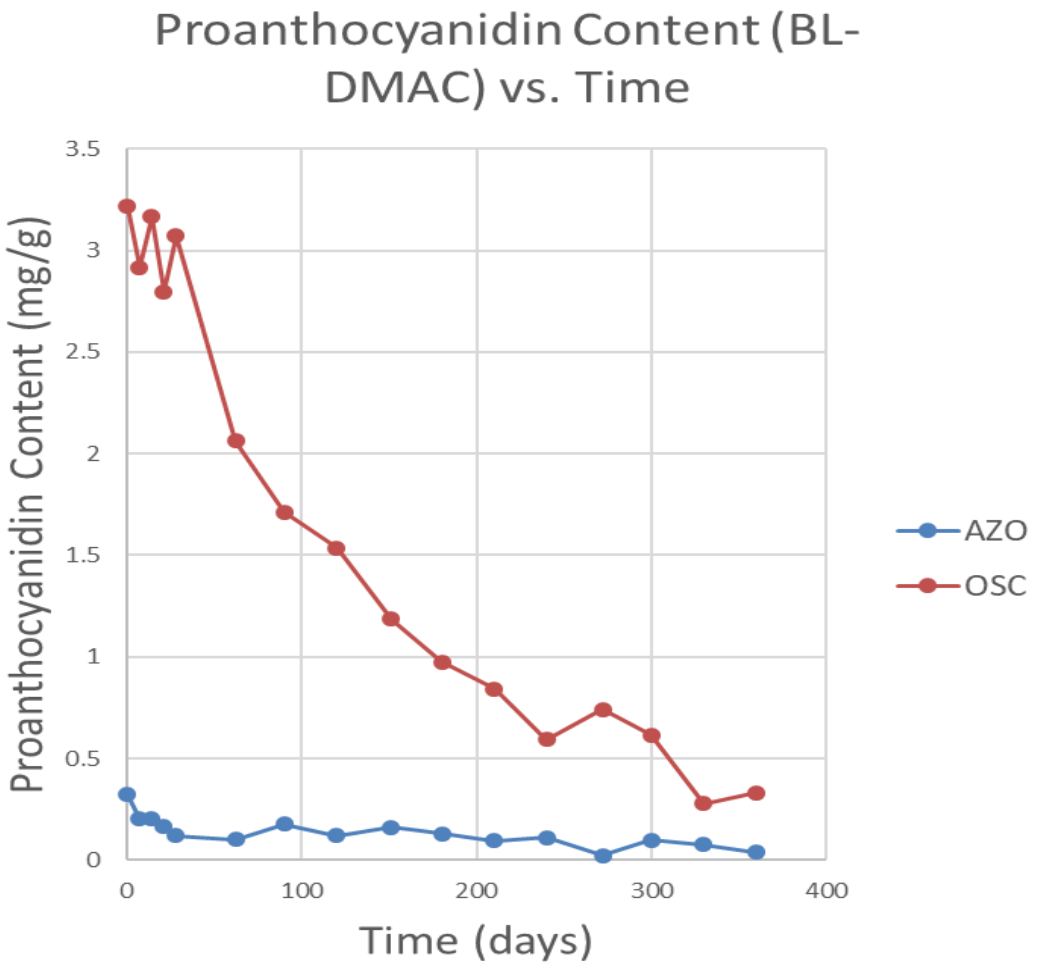

Figure 3.4 Proanthocyanidin content (BL-DMAC Assay) versus time of OSC and AZO

Table 3.4 Proanthocyanidin content of cranberry supplements by DMAC assay utilizing cranberry standard over time

TIME (DAYS)	OSC	AZO	TC
0	$15.14^{bA} \pm 0.67$	$2.50^{bAB} \pm 0.04$	$306.60^{aABC} \pm 11.27$
7	$14.83^{bAB} \pm 0.19$	$2.43^{bABC} \pm 0.29$	$286.47^{aBC} \pm 13.47$
14	$13.67^{bABC} \pm 0.97$	$2.75^{bA} \pm 0.17$	$272.17^{aBCDE} \pm 5.63$
21	$12.82^{bC} \pm 0.11$	$1.97^{bABCDE} \pm 0.19$	$248.27^{aCDEF} \pm 8.36$
28	$13.15^{bBC} \pm 0.44$	$2.07^{bABCD} \pm 0.39$	$356.23^{aA} \pm 29.30$
60	$10.77^{bD} \pm 1.09$	$2.36^{bABCD} \pm 0.41$	$263.71^{aBCDEF} \pm 40.76$
90	$9.03^{bDE} \pm 0.26$	$2.01^{bABCDE} \pm 0.63$	$198.78^{aF} \pm 12.33$
120	$7.56^{bEF} \pm 1.26$	$2.02^{bABCD} \pm 0.15$	$216.16^{aDEF} \pm 14.35$
150	$7.37^{bEF} \pm 0.50$	$1.88^{bABCDE} \pm 0.49$	$257.32^{aBCDEF} \pm 21.65$
180	$6.28^{bFG} \pm 0.37$	$1.53^{bCDE} \pm 0.28$	$304.74^{aABC} \pm 6.68$
210	$5.41^{bGH} \pm 0.49$	$1.45^{bDE} \pm 0.38$	$297.52^{aABC} \pm 24.60$
240	$3.23^{bI} \pm 0.47$	$1.64^{bBCDE} \pm 0.41$	$287.08^{aBC} \pm 28.19$
270	$3.11^{bI} \pm 0.38$	$1.50^{bCDE} \pm 0.17$	$208.44^{aEF} \pm 9.11$
300	$3.75^{bHI} \pm 0.29$	$1.56^{bCDE} \pm 0.11$	$306.56^{aABC} \pm 22.17$
330	$3.04^{bI} \pm 0.12$	$1.01^{bE} \pm 0.04$	$317.39^{aAB} \pm 44.42$
360	$3.13^{bI} \pm 0.25$	$1.73^{bABCDE} \pm 0.28$	$280.00^{aBCD} \pm 23.73$

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Values within columns not sharing an uppercase letter are significantly ($p<0.05$) different excluding rate of degradation. Treatments were analyzed by ANOVA (Tukey's HSD).

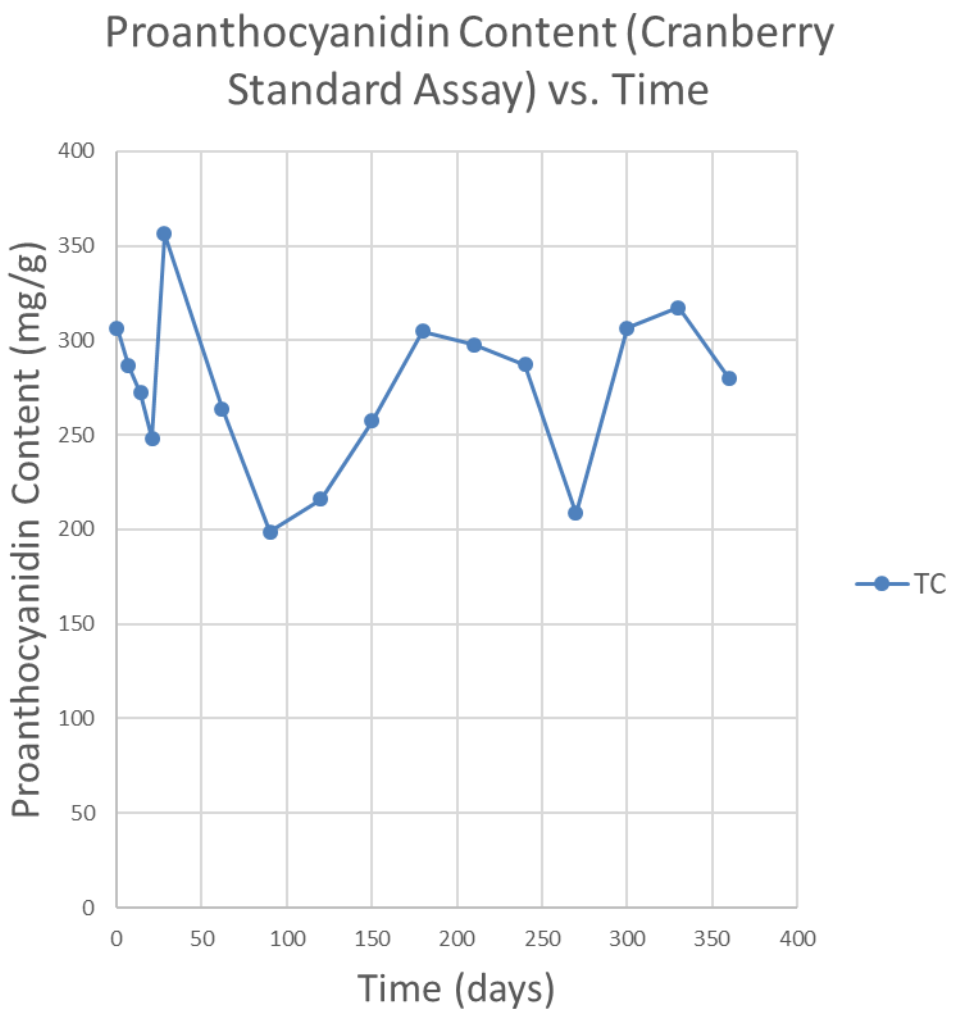

Figure 3.5 Proanthocyanidin content (Cranberry Standard Assay) versus time of TC

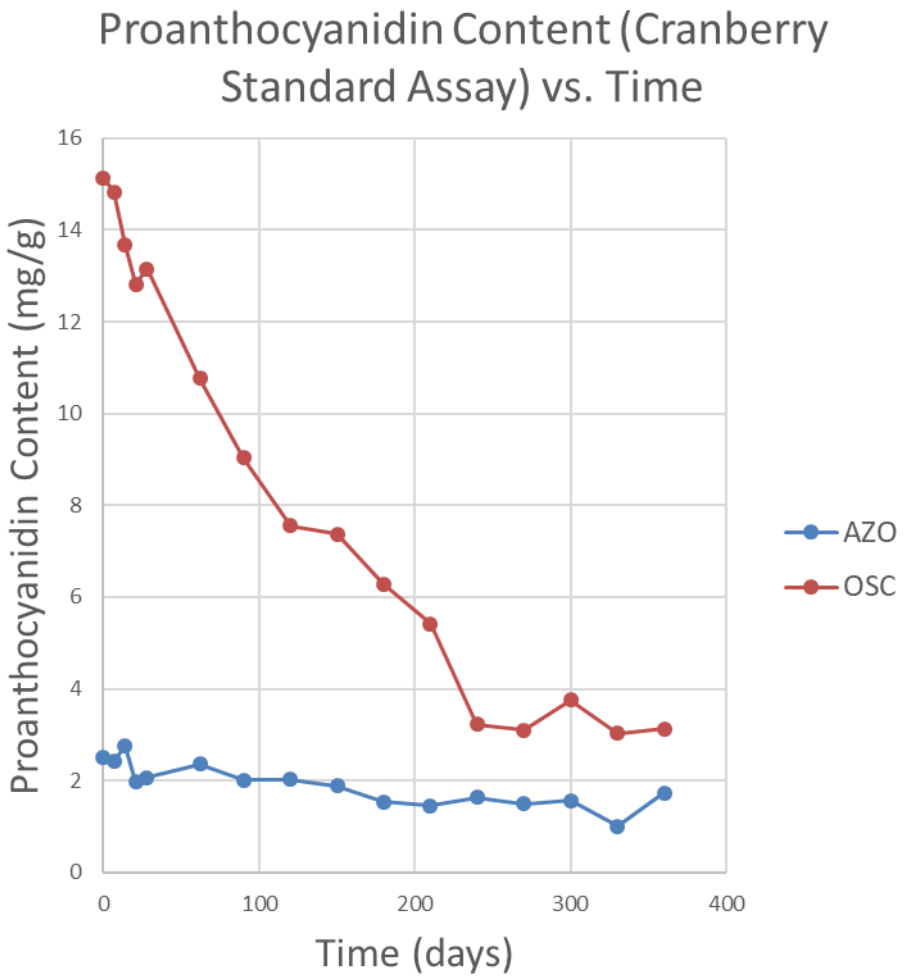

Figure 3.6 Proanthocyanidin content (Cranberry Standard Assay) versus time of OSC and AZO

Anthocyanin Content

TC was the only treatment with no significant ($p>0.05$) difference between initial (3862.67 ppm) and final (3938.69 ppm) anthocyanin content, indicating that spray dried encapsulated supplements provide higher anthocyanin stability than gummy supplements (Table 3.5) (Figure 3.7) (Figure 3.8). TC had a significantly higher initial anthocyanin content than OSC and AZO treatments (270.69 ppm and 24.51 ppm, respectively) as well as a significantly higher final anthocyanin content than OSC and AZO treatments (9.02 ppm and 0.00 ppm, respectively). TC also had a positive rate of change of anthocyanins compared to AZO and OSC which has negative rates of anthocyanin change (Table 3.2). As seen with proanthocyanidins, OSC and AZO both are processed with longer exposure to heat and slower water removal which destabilize the active components, as well as both contain other filler ingredients which reduces the concentration of active components, resulting in less anthocyanin content, where TC is a spray dried extract which concentrates and stabilizes the anthocyanins resulting in larger anthocyanin content. Another cause of anthocyanin degradation could be polyphenol oxidase (PPO) - a naturally occurring enzyme that causes browning in the presence of oxygen (Siddiq & Dolan, 2017) (Fang, et al., 2007) (Institute of Food Science and Technology, 2017). PPO can degrade anthocyanins to brown, black, or yellow colors from the natural red, purple, and blue pigments (Bermejo-Prada & Otero, 2016). Processing, such as drying, as well as storage conditions, can promote PPO reactions causing most color changes and anthocyanin polymerization reactions (Siddiq & Dolan, 2017) (Bermejo-Prada & Otero, 2016) (Jaiswal, et al., 2010). Anthocyanins in the gummy format of cranberry supplements are extremely unstable due to processing conditions and product matrix, decreasing 96.7% in OSC and 100% in AZO compared to TC which saw no significant change in anthocyanins and remained stable over time.

A study with bayberry polyphenols reported that spray-drying of bayberry juice preserved anthocyanins 94% indicating spray-drying as an adequate method for anthocyanin preservation (Fang & Bhandari, 2011). The stability of anthocyanins and lack of degradation throughout the study in TC compared to OSC and AZO confirms that spray-drying is a more stable method for anthocyanins than heat treatment using depositing technology seen in the gummy treatments. Within the gummy supplement treatment, both OSC and AZO had differences, indicating that even within product matrix for gummy supplements, there are significant differences in the anthocyanin content over time. As with proanthocyanidins, the cranberry extract type and amount in the two gummy treatments may impact initial amount of anthocyanins, and as OSC has fewer filler ingredients to reduce the concentration of anthocyanins, resulting in a larger quantity of anthocyanins initially compared to AZO as well as at the end of the study, where there was no anthocyanin content for AZO, but OSC had 9.02 ppm. While the anthocyanin content within the gummy supplement treatments were significantly different initially and finally due to product matrix and composition, there was no significant difference in the overall rate of degradation of the two treatments (decrease of 96.7% in OSC and 100% in AZO) signifying that with regards to anthocyanins, packaging does not impact rate of degradation. OSC was stored individually wrapped, ideal packaging as it limits oxygen exposure over the study, compared to AZO which were stored in a bulk container and were exposed to more oxygen when the container was opened during each time point. Since this difference in packaging did not significantly impact the decrease in anthocyanins within the gummy treatments, product composition is likely responsible for the negative impact on anthocyanin content in gummy supplements.

The significantly larger amount of anthocyanins in TC combined with the stability that TC anthocyanins have throughout shelf life indicate that anthocyanin copigmentation and stacking occurs at this larger anthocyanin level (Gordillo, et al., 2012). Copigmentation of various anthocyanins cause a π-π stacking effect, which insulates the anthocyanin compounds from oxidation and provides enhances stability (Gordillo, et al., 2012). OSC and AZO do not have anthocyanin compounds at the larger levels seen in TC, so no insulation effect from stacking of anthocyanin was observed, resulting in significant decreases in anthocyanin over time. HPLC anthocyanin spectra for treatments initially and finally can be seen in Figures 3.9-3.14.

Compared to raw cranberry anthocyanin content (604.2 ppm), both OSC and AZO have significantly less anthocyanin content while TC has a significantly larger anthocyanin content (Haytowitz, et al., 2018). By concentrating and stabilizing the cranberry anthocyanins into a spray dried encapsulated form, TC can increase the amount of anthocyanin by 6x that of raw cranberries. Consuming cranberry supplements in a spray dried encapsulated pill form compared to eating 6x w/w of raw cranberries is a more convenient alternative though it may be cost prohibitive for some consumers as TC is $0.92 per serving compared to AZO ($0.32/serving) and OSC ($0.90/serving). However, as Millennials move away from medicinal-like supplements, consuming multiple gummies may be more acceptable and affordable to consumers.

Table 3.5 Anthocyanin content of cranberry supplements over time

TIME (DAYS)	OSC	AZO	TC
0	$270.69^{bA} \pm 21.79$	$24.51^{cA} \pm 4.72$	$3862.67^{aABC} \pm 36.00$
7	$243.12^{bA} \pm 22.56$	$12.34^{cBC} \pm 2.62$	$3962.17^{aAB} \pm 63.53$
14	$154.07^{bB} \pm 32.74$	$14.74^{cAB} \pm 0.15$	$3998.40^{aAB} \pm 65.18$
21	$141.09^{bB} \pm 3.89$	$5.29^{cCD} \pm 1.27$	$3922.69^{aAB} \pm 16.71$
28	$130.25^{bB} \pm 17.93$	$8.94^{cBCD} \pm 3.70$	$4098.46^{aA} \pm 109.92$
60	$83.26^{bC} \pm 4.00$	$12.38^{cABC} \pm 3.49$	$3841.22^{aABC} \pm 33.44$
90	$59.59^{bCD} \pm 3.01$	$9.93^{cBCD} \pm 1.47$	$3917.61^{aAB} \pm 100.84$
120	$31.25^{bDE} \pm 1.84$	$3.93^{cCD} \pm 1.81$	$4110.86^{aA} \pm 44.86$
150	$26.59^{bDE} \pm 1.22$	$3.83^{cCD} \pm 0.08$	$3490.76^{aCD} \pm 194.37$
180	$22.48^{bDE} \pm 3.73$	$0.00^{cD} \pm 0.00$	$3630.52^{aBCD} \pm 60.08$
210	$16.23^{bE} \pm 1.00$	$3.33^{cD} \pm 3.45$	$3756.22^{aABC} \pm 69.94$
240	$12.49^{bE} \pm 0.76$	$0.00^{cD} \pm 0.00$	$3797.22^{aABC} \pm 69.94$
270	$10.70^{bE} \pm 1.10$	$0.00^{cD} \pm 0.00$	$3278.12^{aD} \pm 446.24$
300	$9.68^{bE} \pm 1.11$	$0.00^{cD} \pm 0.00$	$3873.21^{aABC} \pm 28.54$
330	$10.91^{bE} \pm 0.42$	$0.00^{cD} \pm 0.00$	$3908.50^{aAB} \pm 6.81$
360	$9.02^{bE} \pm 0.61$	$0.00^{cD} \pm 0.00$	$3938.69^{aAB} \pm 38.44$

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing an uppercase letter are significantly ($p<0.05$) different. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

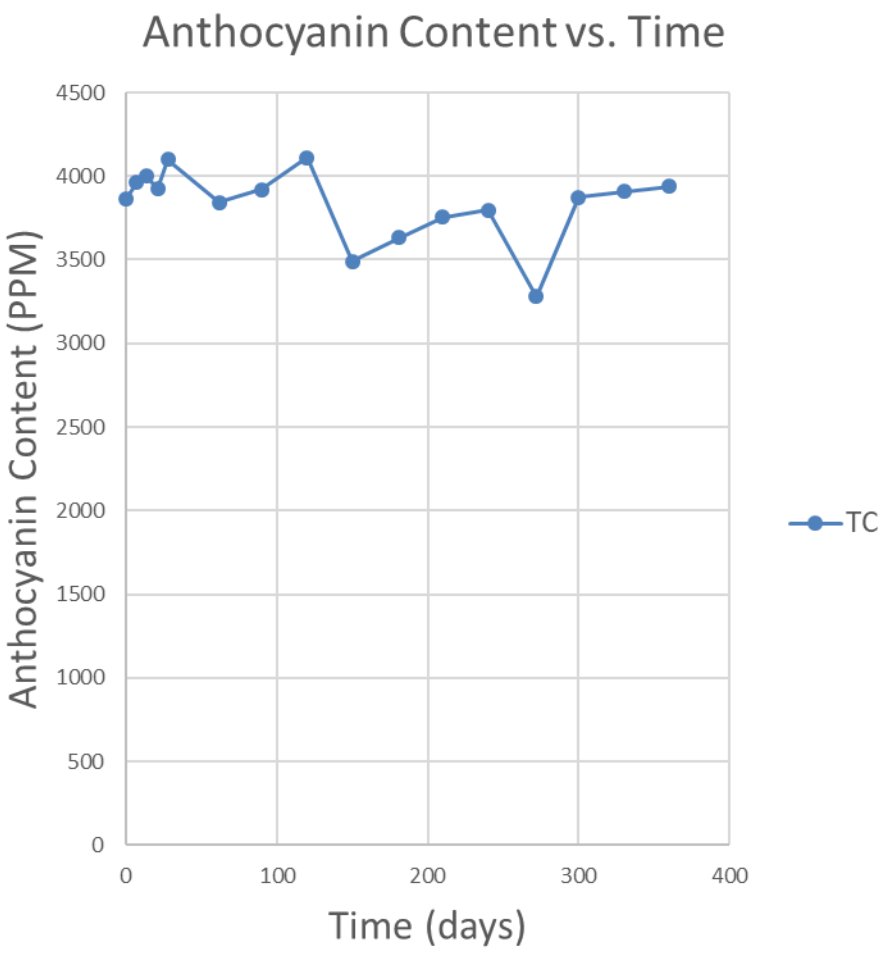

Figure 3.7 Anthocyanin content versus time of TC

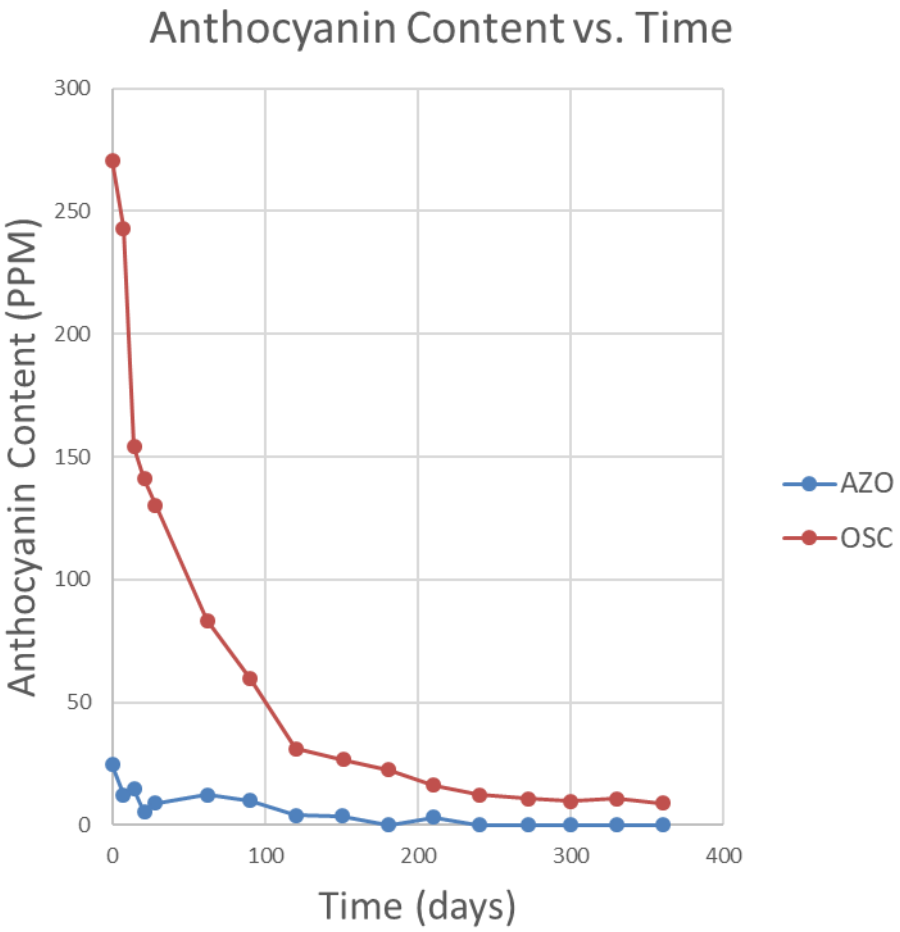

Figure 3.8 Anthocyanin content versus time of OSC and AZO

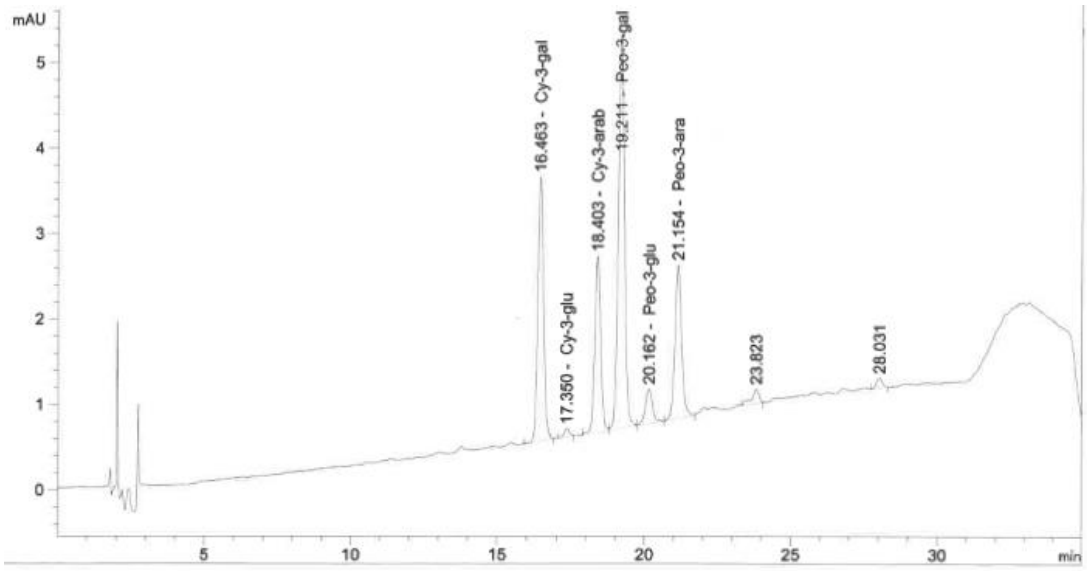

Figure 3.9 Initial anthocyanin profile of OSC

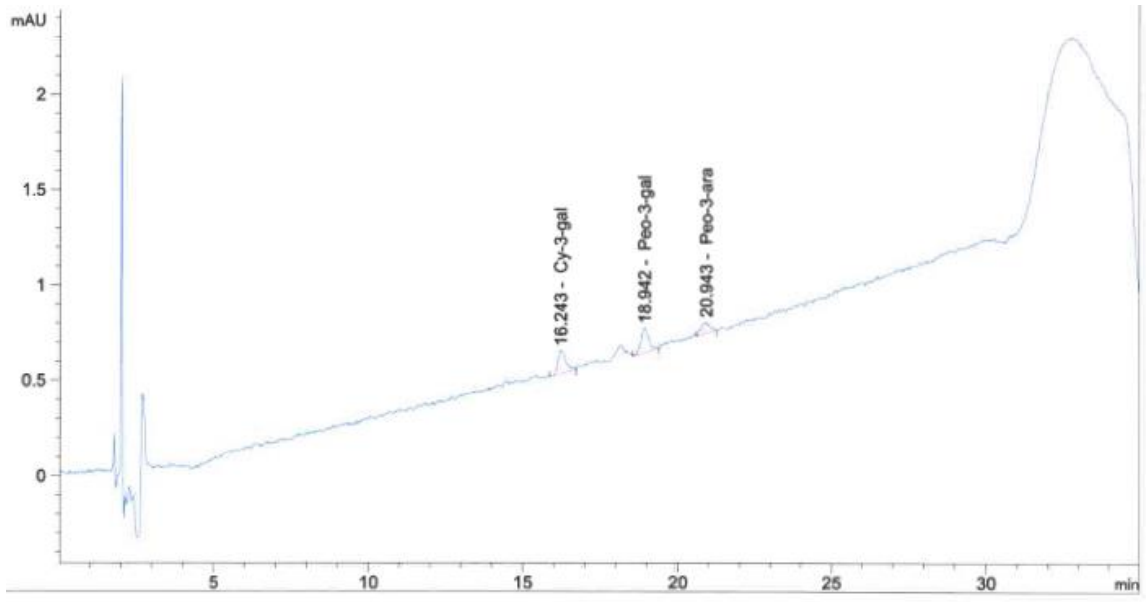

Figure 3.10 Final anthocyanin profile of OSC

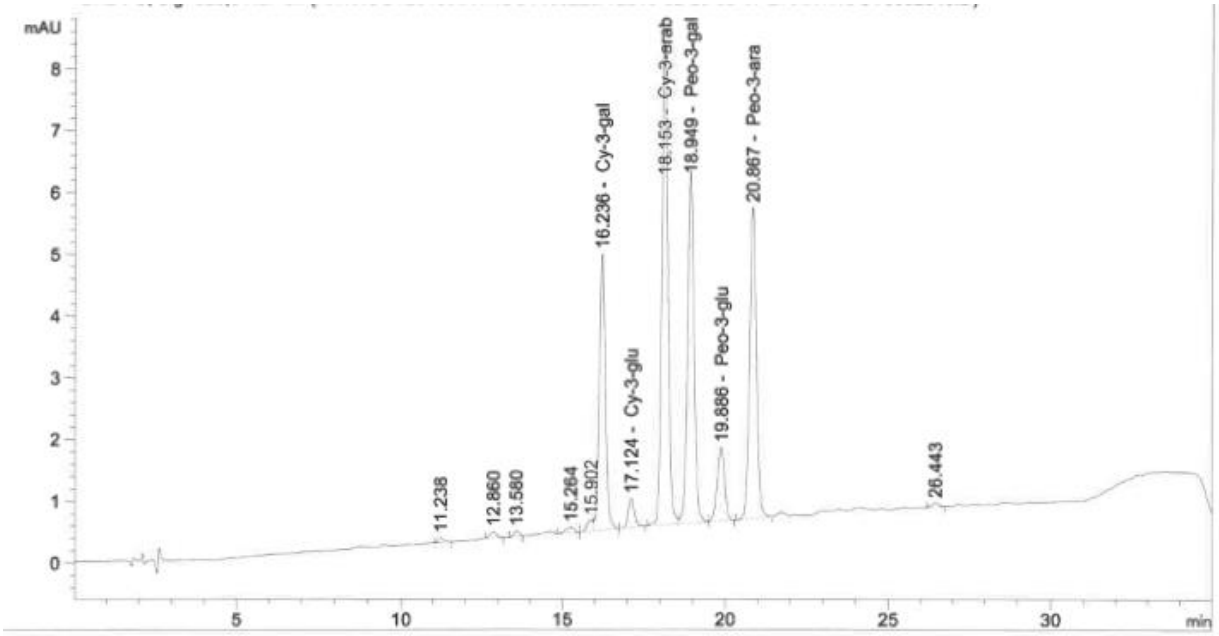

Figure 3.11 Initial anthocyanin profile of TC

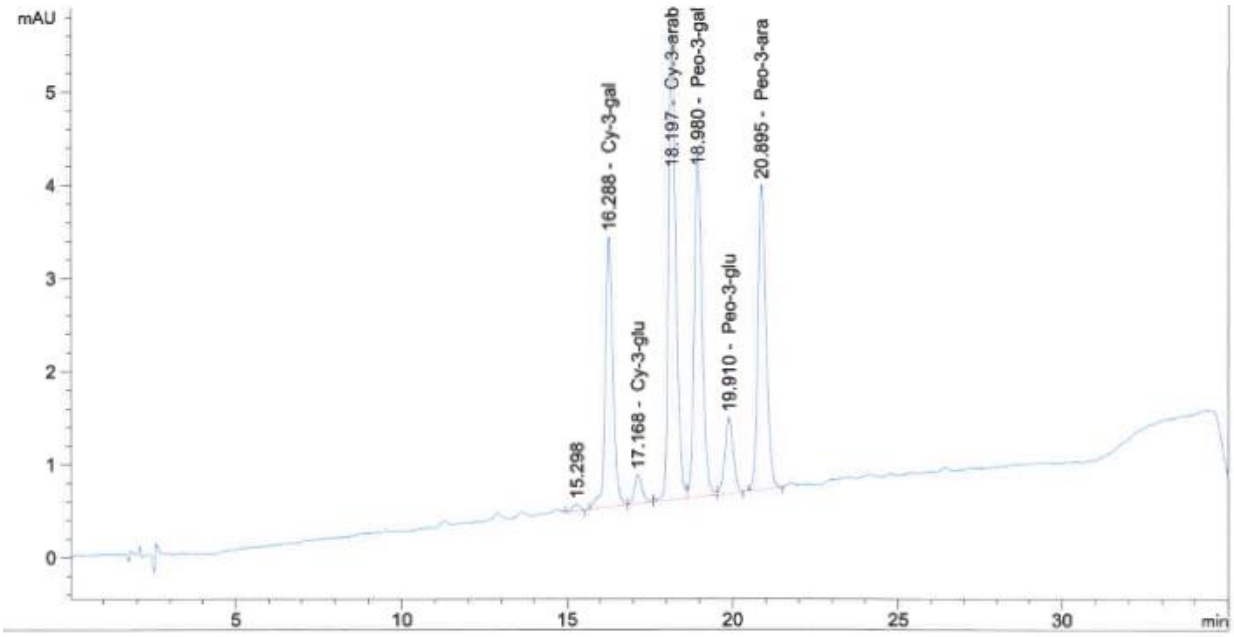

Figure 3.12 Final anthocyanin profile of TC

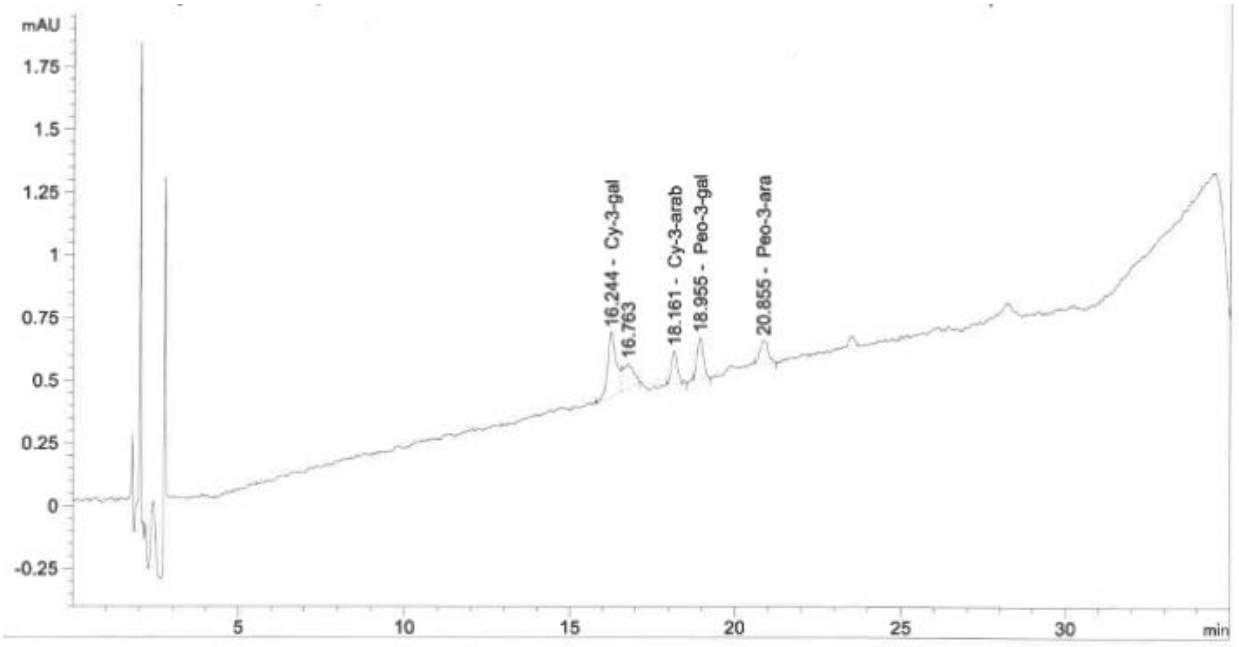

Figure 3.13 Initial anthocyanin profile of AZO

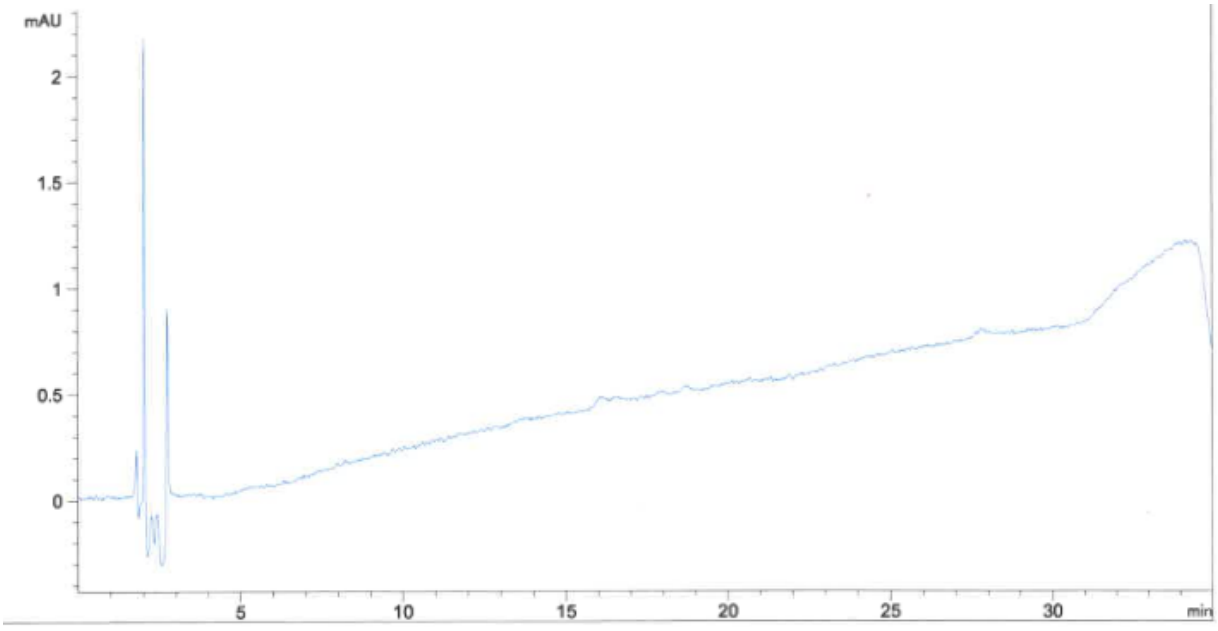

Figure 3.14 Final anthocyanin profile of AZO

Total Phenolic Content

OSC was the only treatment without a significant ($p>0.05$) decrease in total phenolic content, indicating that the addition of apple juice concentrate and apple paste to OSC may stabilize total phenolic content and have more stability than other phenolic compounds found in TC or AZO (Table 3.6) (Figure 3.15) (Figure 3.16). Overall, as seen in both proanthocyanidin and anthocyanin content, TC had a significantly higher initial total phenolic content (220.18 mg/g) compared to OSC and AZO (18.31 mg/g and 4.45 mg/g, respectively), as well as a significantly higher final total phenolic content (184.28 mg/g) compared to OSC and AZO (16.73 and 3.82 mg/g, respectively). As previously discussed with proanthocyanidins and anthocyanins, spray-drying is a superior method to gummy manufacturing for preserving phenolic compounds during processing, resulting in larger total phenolic content in TC compared to OSC and AZO. The addition of apple paste and apple juice concentrate, which contain high amounts of phenolic compounds, in OSC could make the total phenolic content in OSC significantly more stable than AZO and TC. It has been reported that apple phenolic compounds are stable in varying ranges of heat, validating this hypothesis (Chen, et al., 2012). Stability of OSC stemming from additional apple juice concentrate and apple paste is not mirrored in anthocyanin content or proanthocyanidin content as the major polyphenols seen in apples are chlorogenic acid and quercetin which would only increase total phenolic content stability in the increased amounts compared to AZO and TC (Gliszczynska-Swiglo & Tyrakowska, 2003). Quercetin has been shown to increase total phenolic content stability and result in larger total phenolic content (Michalska, et al., 2018) (Aherne & O'Brien, 2002) (White, et al., 2011). The rates of degradation of total phenolic content was not significantly different at the lower levels of phenolics seen in AZO and OSC, however TC had the largest rate of degradation, stemming

from the significantly larger total phenolic content (Table 3.2). While there are significant differences between the TC spray-dried treatment and the gummy treatments, differences within the gummy treatments also occurred, further indicating that product matrix is a significant factor in polyphenol content and stability. As previously seen with proanthocyanidins and anthocyanins, OSC product matrix resulted in higher total phenolic content compared to AZO due to product composition. OSC also showed no significant degradation between initial and final total phenolic content while there was a significant difference within AZO, signifying that apple stabilized phenolics. This difference within the treatments could also indicate that for total phenolic content the individually wrapped OSC did provide more protection than the bulk packaging of AZO. Although packaging was not a significant factor for proanthocyanidins or anthocyanins, the compounds that make up the majority of total phenolic content could be more sensitive to oxidation thus the individually wrapped OSC was more stable with regards to total phenolic content than AZO.

Compared to proanthocyanidins and anthocyanins content, total phenolic is the most stable phenolic measure for gummy cranberry supplements, only decreasing 8.6% for OSC and 14.2% for AZO, while TC saw a 16.3% decrease in total phenolic content. Compared to raw cranberries (0.307 mg/g total phenolic content), all supplements are better options for delivering phenolic content, with TC having the largest amount of total phenolic content (Haytowitz, et al., 2018).

Table 3.6 Total phenolic content of cranberry supplements over time

TIME (DAYS)	OSC	AZO	TC
0	$18.31^{bA} \pm 1.97$	$4.46^{cA} \pm 0.24$	$220.18^{aA} \pm 5.15$
7	$16.84^{bAB} \pm 0.98$	$3.98^{cAB} \pm 0.06$	$231.31^{aA} \pm 4.09$
14	$15.86^{bAB} \pm 0.17$	$3.99^{cAB} \pm 0.25$	$229.42^{aA} \pm 2.37$
21	$17.01^{bAB} \pm 0.46$	$3.56^{cBCD} \pm 0.18$	$214.78^{aA} \pm 2.99$
28	$16.64^{bAB} \pm 0.16$	$3.81^{cBC} \pm 0.20$	$184.84^{aB} \pm 5.44$
60	$16.93^{bAB} \pm 0.42$	$3.64^{cBCD} \pm 0.20$	$159.71^{aC} \pm 5.78$
90	$14.76^{bB} \pm 1.49$	$3.72^{cBCD} \pm 0.38$	$159.71^{aC} \pm 5.78$
120	$16.45^{bAB} \pm 0.19$	$3.32^{cCD} \pm 0.16$	$148.54^{aC} \pm 2.38$
150	$16.71^{bAB} \pm 0.21$	$3.44^{cBCD} \pm 0.16$	$182.95^{aB} \pm 6.09$
180	$18.40^{bA} \pm 1.13$	$3.56^{cBCD} \pm 0.20$	$181.75^{aB} \pm 7.08$
210	$17.52^{bAB} \pm 0.29$	$3.36^{cCD} \pm 0.22$	$185.18^{aB} \pm 5.22$
240	$16.27^{bAB} \pm 0.09$	$3.75^{cBC} \pm 0.19$	$187.71^{aB} \pm 3.58$
270	$16.54^{bAB} \pm 0.21$	$3.75^{cBC} \pm 0.09$	$196.74^{aB} \pm 11.89$
300	$15.94^{bAB} \pm 0.04$	$3.14^{cD} \pm 0.10$	$192.49^{aB} \pm 4.76$
330	$16.52^{bAB} \pm 0.27$	$3.46^{cBCD} \pm 0.16$	$186.41^{aB} \pm 5.72$
360	$16.73^{bAB} \pm 2.28$	$3.82^{cBC} \pm 0.16$	$184.28^{aB} \pm 3.97$

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing an uppercase letter are significantly ($p<0.05$) different. Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

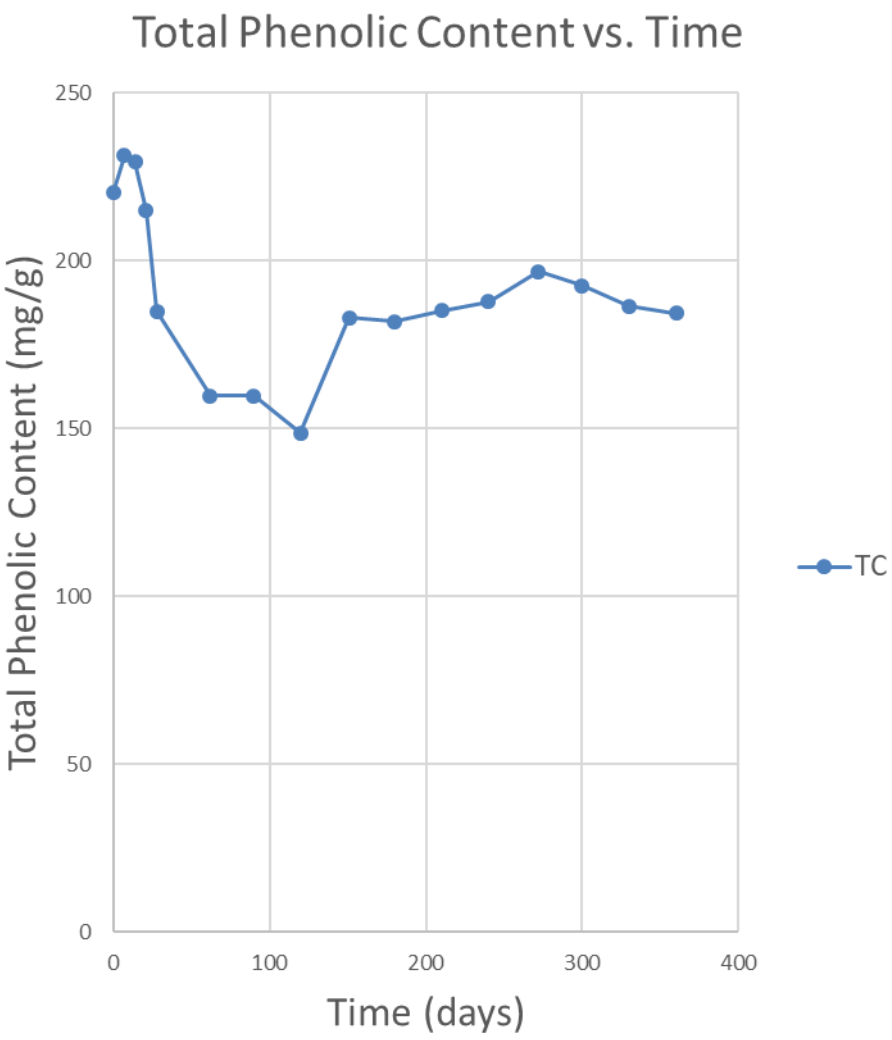

Figure 3.15 Total phenolic content versus time of TC

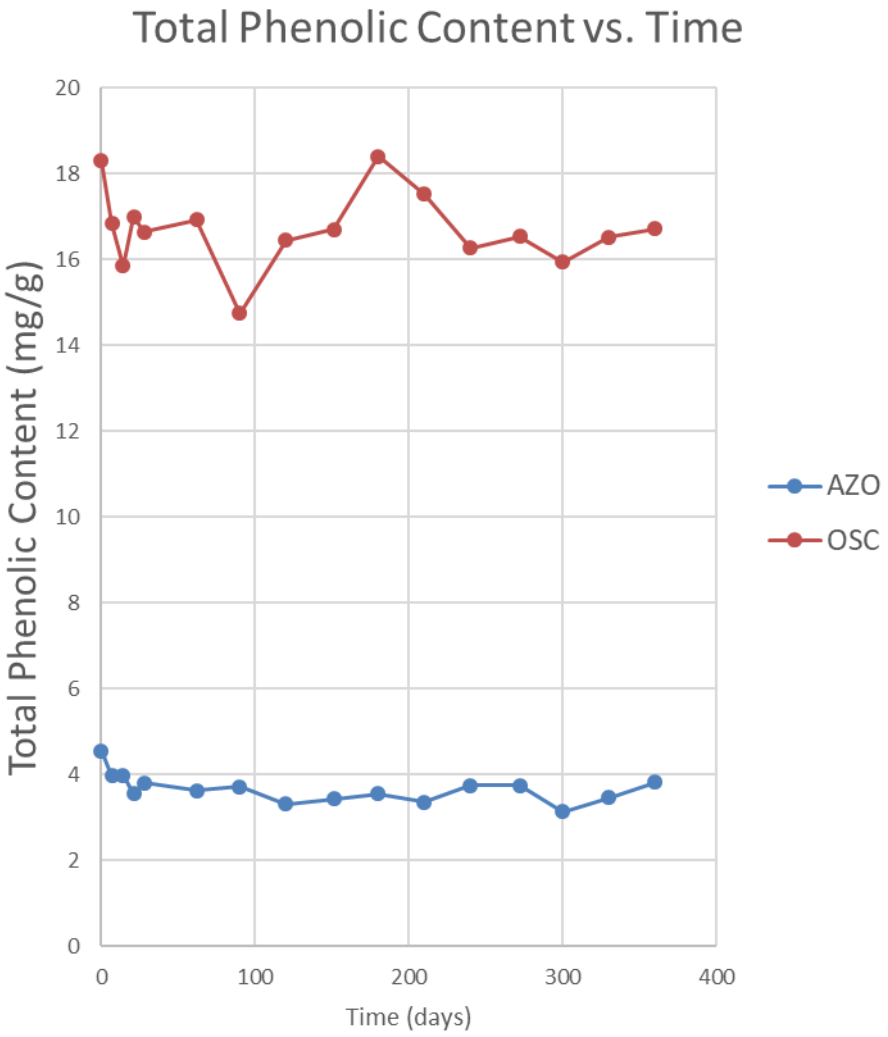

Figure 3.16 Total phenolic content versus time of OSC and AZO

Antioxidant Activity

All treatments had significantly ($p<0.05$) different maximum DPPH scavenging (%) and IC_{50} values (mg/mL) (Table 3.7). TC demonstrated the ability to scavenge 15.0% more DPPH free radicals than OSC and 62.8% more DPPH free radicals than AZO. TC also has the lowest IC_{50} value, indicating that it has the most antioxidant capacity. Comparatively, ascorbic acid standard had a maximum DPPH scavenging of 95.3% with an IC_{50} of 79.84 mg/mL, indicating that TC and OSC are both not as potent an antioxidant as ascorbic acid. Studies have reported

that cranberries are among the fruits with the highest levels of antioxidants (177.0 mol/g vitamin C equivalents compared to apples at 97.6 mol/g vitamin C equivalents) (Sun, et al., 2002). The larger amount of DPPH scavenging antioxidant compounds seen in both TC and OSC compared to AZO confirm that while not as potent an antioxidant as ascorbic acid, cranberry supplements do display antioxidant capacity. More studies on how DPPH and antioxidant capacity *in vitro* compares to *in vivo* models should be done, as previous studies have indicated that different polyphenol pathways and bioavailability of those polyphenols react differently *in vitro* than *in vivo*, so the antioxidant effect cannot be directly translated to how antioxidant pathway would work in the human body (Prior, et al., 2007) (da Silva Pinto, et al., 2010). Further analyzing a complete phenolic profile may assist in this determination of antioxidant capacity between *in vivo* and *in vitro* models by isolating the most abundant phenolics in each treatment (da Silva Pinto, et al., 2010).

Table 3.7 Antioxidant activity of cranberry supplements

TREATMENT	DPPH SCAVENGING (%)	IC_{50} (MG/ML)
OSC	69.17 ± 2.32^{b}	$8.55 \times 10^{4} \pm 1.41 \times 10^{4b}$
AZO	21.47 ± 5.55^{c}	$1.27 \times 10^{9} \pm 1.64 \times 10^{9a}$
TC	84.28 ± 1.70^{a}	$1.07 \times 10^{3} \pm 229.61^{c}$

Each value is the average ± standard deviation (n=48) except for AZO (n=15). Treatments were stored at for 360 days at 21°C. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Water Activity and Moisture Content

Water Activity

TC was the only treatment with a significant ($p<0.05$) decrease in initial (0.366) and final (0.282) A_w (Table 3.8) (Figure 3.17). The significantly lower initial A_w of TC (0.366 compared to 0.588 for OSC and 0.500 for AZO) and significantly lower final A_w of TC (0.282 compared to 0.581 for OSC and 0.491 for AZO) is due to processing and composition differences, with TC being a spray-dried extract compared to OSC and AZO which are manufactured with other high moisture ingredients and subjected to different target moisture contents. Despite the differences in A_w between the treatments, there was no significant difference in their rates of change, indicating that A_w is stable analytical measurement for cranberry supplements (Table 3.2). From a microbial standpoint, stability in A_w is desired as there is certain microbial threshold that the A_w cannot exceed from a food safety and spoilage perspective. However, studies have reported that lower A_w may result in more stable phenolic compounds (Fang & Bhandari, 2011). Spray-dried bayberry polyphenols showed that at A_w of 0.11 to 0.33, TPC were significantly more stable than above 0.33 A_w (Fang & Bhandari, 2011). The lower A_w of TC compared to OSC and AZO, despite being stored at the same temperature, validates this hypothesis that lowering A_w can aid in preservation of total phenolic content during processing, once again making spray-drying a superior method to long heat exposure and slow water removal gummy manufacturing for phenolic stability. Although initial A_w within the gummy treatments showed significant differences, there was no significant difference in the final A_w within the gummy treatments, indicating that A_w throughout these gummy treatments will stabilize over time. Initial variation in A_w between OSC and AZO can be attributed to differing processing parameters that would dictate the A_w of the product. Even though packaged differently the A_w of both treatments were

not significantly different, so packaging did not affect A_w which reach a similar point for both treatments.

Further work to potentially stabilize compounds in OSC and AZO could be to evaluate glass transition temperatures of the products, and ensure that storage temperatures of the treatments are below the glass transition temperatures, as this has been shown to significantly aid in total phenolic content retention, as it slows down the rate of reactions in the product (Fang & Bhandari, 2011). Glass transition temperature is the temperature at which a material goes from the glassy state to rubbery state, which can have effects on the reactions that occur within a system (Bell & Hageman, 1994).

Table 3.8 Initial and final comparison of A_w of cranberry supplements

SAMPLE	INITIAL	FINAL
OSC	0.588 ± 0.006^{aA}	0.581 ± 0.006^{aA}
AZO	0.500 ± 0.018^{bA}	0.491 ± 0.036^{aA}
TC	0.366 ± 0.001^{cA}	0.282 ± 0.006^{bB}

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

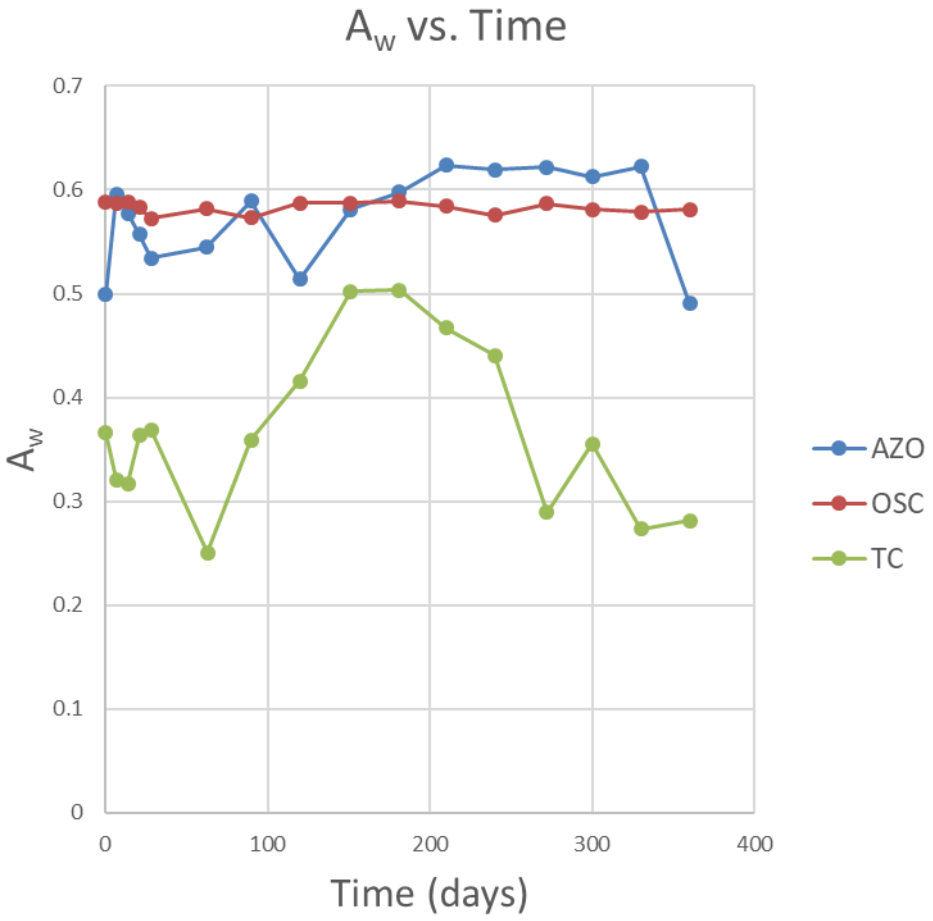

Figure 3.17 A_w versus time of cranberry supplements

Moisture Content

All treatments had a significant ($p<0.05$) difference between initial and final moisture content indicating that moisture changes in cranberry supplements occurs regardless of product matrix or packaging (Table 3.9) (Figure 3.18). TC saw an increase in initial moisture content (0.01% to 0.02%) while OSC and AZO both saw reductions in moisture content (19.98% to 14.93% for OSC and 15.07% to 12.96% for AZO, respectively). The spray-dried nature of TC left it more susceptible to moisture integration as powders can undergo plasticization and increased glass transition due to an increase in moisture content caused by water vapor from the

atmosphere or other ingredients such as gelatin calcium silicate, or silicon dioxide that may have available water to increase moisture content (Montes, et al., 2011) (Sandler, et al., 2010). The product matrices of OSC and AZO which are more complex, composed of high moisture ingredients, and not dried to the same initial moisture level as TC, leads to higher initial moisture content. However, unlike TC which gained moisture over time and had a positive rate of change, OSC and AZO decreased in moisture content with OSC moisture content degrading significantly faster than AZO (Table 3.2). This loss in moisture content could be a result of diffusivity during storage which is affected by the glass transition temperature and could result in a decrease in moisture content over time (Miranda, et al., 2014).

Packaging also could be a factor in moisture content changes. OSC were individually wrapped while AZO were in a bulk container which was opened and closed for each time pull similarly to TC. The opening and closing of the container could have resulted in an increase in moisture content in TC, as spray dried capsules are more likely to pick up moisture in the air resulting in clumping, plasticization, and increased moisture content due to the smaller particle size of the powder compared to a larger, solid, gummy (Montes, et al., 2011). While this is true for the spray-dried powder, there did not appear to be a significant difference in the package between gummy supplements, as both lost moisture over time.

Table 3.9 Initial and final comparison of moisture content of cranberry supplements

SAMPLE	INITIAL (%)	FINAL (%)
OSC	19.95 ± 0.07^{aA}	14.93 ± 0.70^{aB}
AZO	15.07 ± 0.71^{bA}	12.96 ± 0.13^{bB}
TC	0.01 ± 0.00^{cB}	0.02 ± 0.00^{cA}

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

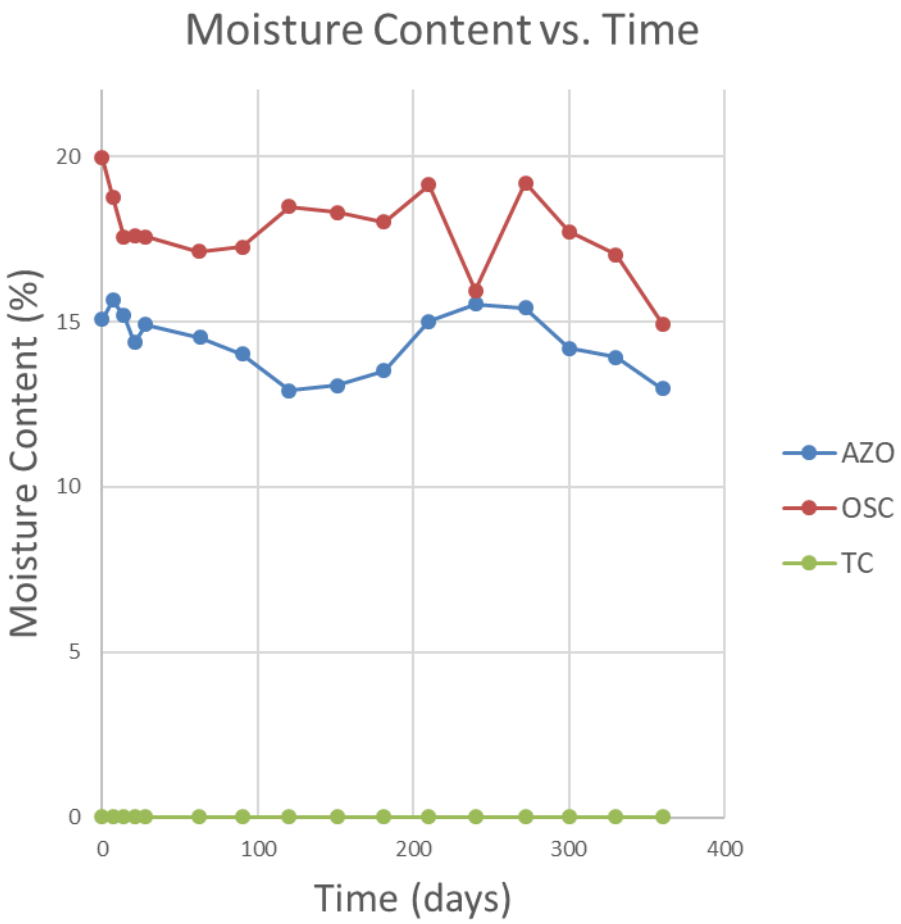

Figure 3.18 Moisture content versus time of cranberry supplements

Colorimetric and Texture Analyses

Color

AZO had a significant ($p<0.05$) increase in a* while OSC saw no significant impact to a*, indicating that AZO redness was increased compared to OSC (Table 3.10) (Table 3.11) (Figure 3.19). OSC had a significantly lower initial a* (1.80) compared to AZO (5.02) and a significantly lower final a* (1.19) compared to AZO (6.62). a* represents the red color in a sample, with larger positive a* values indicating that the sample is more red. Despite the OSC treatment being darker than AZO and the AZO treatment looking purple in color, AZO was measured as being more red. This discrepancy is because OSC is extremely dark, almost black in color, so a* value is not as relevant as it is in AZO, likely attributed to different types of cranberry extract, filler ingredients, and processing conditions. There was both a strong negative correlation between initial a* and initial anthocyanins and final a* and final anthocyanins despite the large decrease in anthocyanins content compared to a* which did not change for OSC and increased 31.8% for AZO. There was an overall color change (ΔE) in all treatments, which takes into consideration L*, a* and b* values (L* and b* data not shown) (Table 3.11). OSC had a significantly higher change in overall color (3.31) compared to AZO (1.94) and had a significantly higher rate of change compared to AZO (Table 3.2). ΔE encompasses any deviation in color from the initial sample to the final sample with regards to all aspects of the Hunter L*a*b* scale, so while OSC had a significantly larger change in overall color compared to AZO, the cause of the color change could be from different causes such as different ingredient composition, different processing conditions, browning and color deterioration due to oxidation and heat (Burdurlu & Karadeniz, 2003) (Damasceno, et al., 2008).

Despite differences in color throughout the study, consumer acceptability of color differences may be different in supplements than in other food matrices. Supplements are consumed whole and often come in a form that may not be indicative of freshness compared to color changes that occur in raw fruits. More work should be done to determine the consumer detectable threshold for ΔE to understand at what point color changes become important to the consumer in supplements.

Table 3.10 Initial and final comparison of a* of cranberry supplements

SAMPLE	A* INITIAL	A* FINAL
OSC	1.80 ± 0.28^{bA}	1.19 ± 0.40^{bA}
AZO	5.02 ± 0.04^{aB}	6.62 ± 0.33^{aA}
TC	-	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

Table 3.11 Overall color change (ΔE) of cranberry supplements

SAMPLE	ΔE
OSC	3.31 ± 0.17^{a}
AZO	1.94 ± 0.33^{b}
TC	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

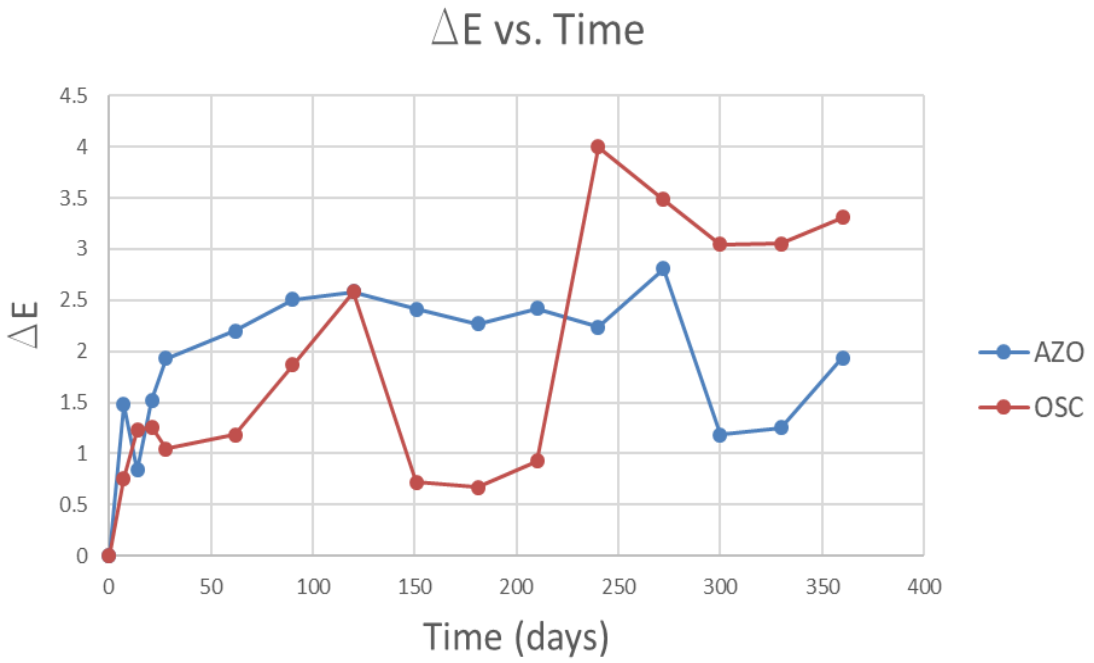

Figure 3.19 ΔE versus time for cranberry supplements

Texture

Across adhesion and toughness there were no significant ($p>0.05$) difference in initial and final values indicating that OSC and AZO both maintain textural adhesion and toughness properties throughout shelf life (Table 3.12) (Table 3.13) (Table 3.14) (Figure 3.20) (Figure 3.21) (Figure 3.22). Except for initial hardness where OSC and AZO had statistically the same hardness (308.80 for OSC and 474.07 for AZO, respectively), AZO had significantly more adhesion force and was significantly tougher than OSC. AZO had a significantly higher initial adhesion force (511.97 g) compared to OSC (124.88 g), and a significantly higher final adhesion force (654.00 g) compared to OSC (462.30) indicating that AZO overall was chewier than OSC. AZO also had a significantly higher initial toughness (5065.7 g/sec) compared to OSC, and a significantly higher final toughness (5441.6 g/sec) compared to OSC (3171.4 g/sec) indicating

that AZO is also significantly tougher than OSC. Hardness of both treatments were initially the same (308.80 g for OSC and 474.07 g for AZO, respectively) while AZO was significantly harder at the end of shelf life (654.00 g/sec compared to 462.30 g/sec for OSC). Despite OSC and AZO having A_w that were the same, OSC had a significantly higher initial and final moisture content than AZO, which would explain the treatment being less hard and tough compared to AZO. As the moisture content in the treatment is lower, it is drier because there is less water to soften the sample. AZO has a lower moisture content than OSC, which means it has less water and is drier, resulting in a harder and tougher treatment compared to OSC. As with moisture, packaging could be a factor in the increase in hardness and toughness in AZO compared to OSC. As AZO was packaged in a bulk container and dried out over time, since the package was open and shut multiple times throughout the study, it could have caused the treatment to dry out even more. OSC does not have this issue to the same degree as AZO, as the treatments were in individually wrapped packaging. Despite differences in initial and final textures across the treatments, the rate of change was not significantly different with the exception of OSC adhesion force which had a rate of change significantly slower than AZO (Table 3.2). Packaging conditions of OSC (individually wrapped versus bulk container) could have accounted for the slow rate of increase in hardness seen in OSC compared to AZO.

Table 3.12 Comparison of initial and final hardness of cranberry supplements

SAMPLE	HARDNESS INITIAL (G)	HARDNESS FINAL (G)
OSC	308.82 ± 46.83^{aB}	462.36 ± 47.97^{bA}
AZO	474.16 ± 131.40^{aA}	654.01 ± 69.24^{aA}
TC	-	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different excluding rate of change. Treatments were analyzed by ANOVA (Tukey's HSD).

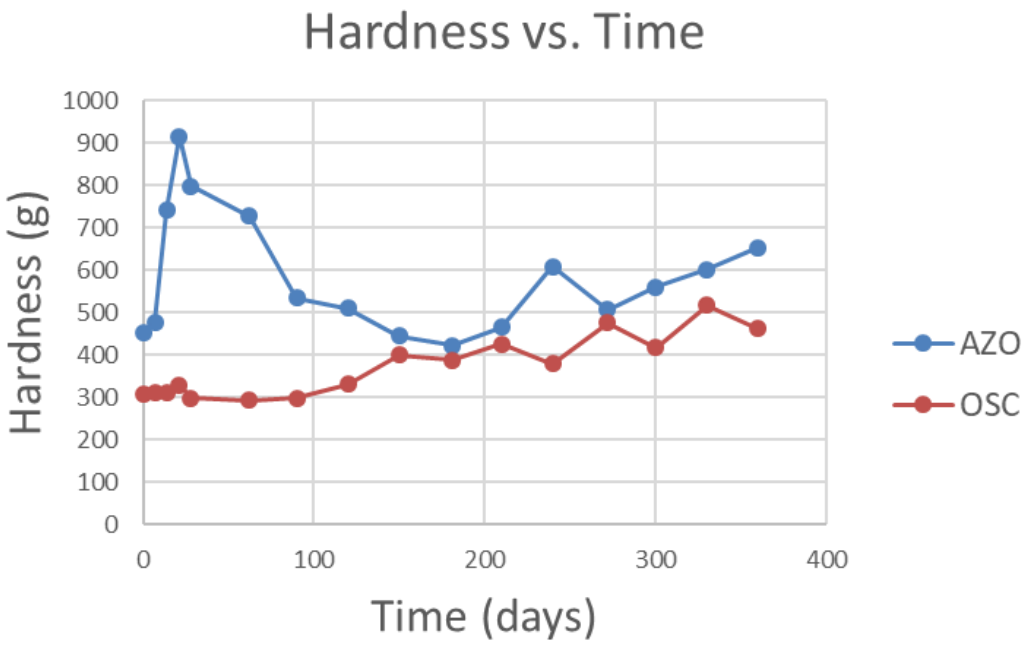

Figure 3.20 Hardness versus time of cranberry supplements

Table 3.13 Comparison of initial and final adhesion of cranberry supplements

SAMPLE	ADHESION INITIAL (G)	ADHESION FINAL (G)
OSC	124.90 ± 39.66^{bA}	216.10 ± 26.78^{bA}
AZO	512.01 ± 85.23^{aA}	718.13 ± 111.12^{aA}
TC	-	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21ºC. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different excluding rate of change. Treatments were analyzed by ANOVA (Tukey's HSD).

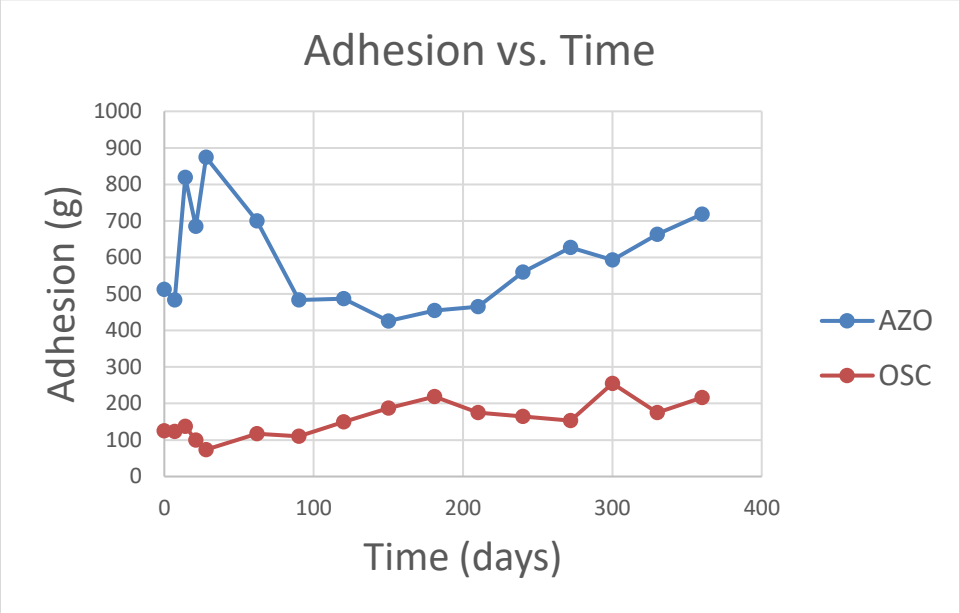

Figure 3.21 Adhesion versus time for cranberry supplements

Table 3.14 Comparison of initial and final toughness of cranberry supplements

SAMPLE	TOUGHNESS INITIAL (G/SEC)	TOUGHNESS FINAL (G/SEC)
OSC	2574.33 ± 371.76^{bA}	3171.36 ± 119.77^{bA}
AZO	5066.02 ± 395.54^{aA}	5442.28 ± 584.76^{aA}
TC	-	-

Each value is the average ± standard deviation (n=3). Treatments were stored at for 360 days at 21°C. OSC were stored in individually wrapped gummy supplements in a larger box. AZO and TC were stored in loose bulk containers and treatments were pulled and containers resealed after each time point. Bulk containers of AZO and TC were stored in larger boxes. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different excluding rate of change. Treatments were analyzed by ANOVA (Tukey's HSD).

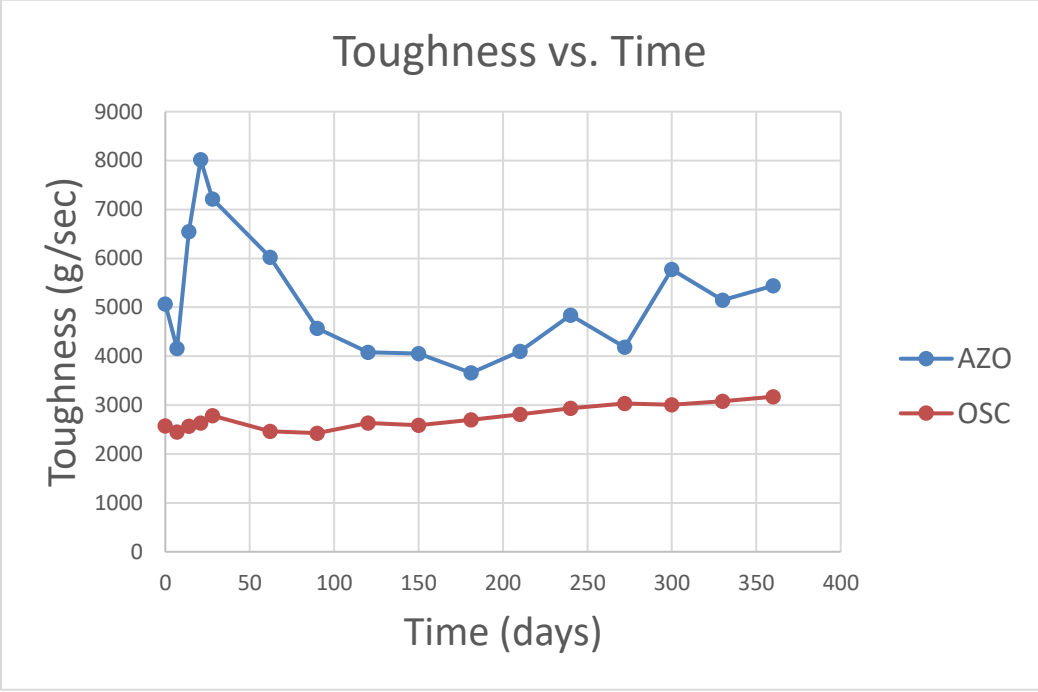

Figure 3.22 Toughness versus time of cranberry supplements

Conclusions

There were significant differences in the proanthocyanidin, anthocyanin, and total phenolic content between spray-dried supplement and gummy supplements, and even between the two gummy supplements studied, indicating that product matrix and processing conditions are significantly important for anthocyanin, proanthocyanidin, and total phenolic content stability in cranberry supplements. The spray-dried treatment showed significantly higher anthocyanins, proanthocyanidins, total phenolic content, and antioxidant activity compared to gummy supplements, indicating that spray-dried encapsulated cranberry supplements not only are better contributors of various polyphenols compared to gummy supplements, but they are also in a more stable configuration during processing than gummy supplements. Spray-drying and encapsulating cranberry extracts proved to be a more adequate way of polyphenol stabilization during processing than gummy supplements. Within the gummy treatments, product matrix proved to be important in initial and final polyphenol content, with the treatment with fewer filler ingredients having larger content and more antioxidant capacity. Rates of change across treatments followed a first order kinetic model. Cranberry supplements are more easily consumed than raw cranberries or other processed cranberry products like juices or dried fruits, so comparatively they may be a more viable option for consumers who want to add health benefits to their diet in a more convenient form than raw fruit. To further enhance the stability of cranberry supplements in gummy form, further research is needed to evaluate processing parameters and storage conditions.

Examples include 1) optimization of type and amount of filler ingredients to increase content and stability of active cranberry polyphenols 2) evaluation of glass transition

temperatures to observe optimal storage temperatures and conditions and 3) lowering A_w to further promote polyphenol stability.

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Chapter 4 - Overall Conclusions

Various sweetened dried cranberry products and cranberry supplements were evaluated over time for their polyphenol content (proanthocyanidin, anthocyanin, and total phenolic content), resulting in the conclusion that complex product matrices with increased A_w and moisture content lead to decreased stability of polyphenols and fewer initial polyphenol content.

Within sweetened dried cranberries it was proven that slicing cranberries increased infusion rate, leading to a large amount of initial polyphenol content. Whole sweetened dried cranberries had significantly fewer polyphenols as they are dried to a similar A_w and moisture content due to food safety and food quality guidance, leading to more degradation of polyphenols. Sweetened dried cranberries with ingredients proven to insulate and provide stability to polyphenols such as soluble corn fiber and glycerin resulted in the most polyphenol stability and highest polyphenol content. Due to processing conditions, sweetened dried cranberries lack antioxidant capacity when analyzed by DPPH assay. This assay, while used heavily in the food industry, yielded results that conflicted with literature on antioxidant capacity in raw cranberries, indicating that the mechanism in which cranberries inhibit oxidation may not be adequately represented by DPPH assay.

Within cranberry supplements spray-drying of cranberry extract powder into a capsule proved to be a superior method for yielding higher polyphenol content as well as increased stability compared to gummy supplements. Spray-drying by concentrating the polyphenols enhances the initial polyphenol content while also providing insulation from degradation of polyphenols. Gummy supplements did not have the stability or content that was seen with the spray-dried treatment. While spray-drying was the superior preservation method, within gummy cranberry supplements product formulation proved to significantly impact initial and final

polyphenol content, with the treatment with additional apple and fewer ingredients having larger polyphenol content. Despite the initially larger content of polyphenols, degradation of polyphenols was inferior to the other gummy treatment, indicating that larger amounts of polyphenols that may be too small for copigmentation to occur and provide insulation and may decrease at a faster speed than those with significantly smaller polyphenol content. Compared to raw cranberries supplements provide more total phenolic content, while only spray-drying provides increased proanthocyanidin and anthocyanin content. Consumer acceptance towards supplements compared to fresh fruit needs to be further explored.

Overall cranberries are a source of proanthocyanidins, anthocyanins, and other polyphenols, but the way in which they degrade and appear in product matrices is processing, packaging, and product dependent. Even within similar product matrices, differences occurred due to formulation and processing parameters, indicating that process has a significant impact on polyphenols. In order to leverage the polyphenols available in cranberries in these more convenient forms (sweetened dried cranberry snacks and cranberry supplements) processing parameters, product formulation, and packaging format needs to be further optimized and evaluated to understand the optimal combination to preserve polyphenols while providing a product that is appealing to the consumer.