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Method Development for Vitamin C Quantification in Two Complex Matrices

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METHOD DEVELOPMENT FOR VITAMIN C QUANTIFICATION

IN TWO COMPLEX MATRICES

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METHOD DEVELOPMENT FOR VITAMIN C QUANTIFICATION IN TWO COMPLEX MATRICES

By

Hannah D. Hutt

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A THESIS

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THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Hannah D. Hutt, I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

Dr. L. Brian Perkins, Assistant Research Professor Date

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METHOD DEVELOPMENT FOR VITAMIN C QUANTIFICATION IN TWO COMPLEX MATRICES

By Hannah D. Hutt

Thesis Advisor: Dr. L. Brian Perkins

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
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The following liquid chromatographic (LC) method developments and applied research studies were done using two complex food matrices, potatoes and elderberries, which are common to the state of Maine. Potatoes are Maine's largest agricultural crop, a staple food in most U.S. households, and are, from an analytical standpoint, considered a complex matrix due to the high starch content that can be difficult to remove without degrading or removing nutrients in the process. Elderberries are an emerging crop in the U.S. because of their antioxidant and anti-viral properties and are found growing wild, throughout Maine. Elderberries are also considered a complex matrix because of the large number of compounds naturally present in the berries, including a range of flavonoids. Many flavonoids have similar chemical structures to vitamin C, which makes removing them without degradation or removing the vitamin C difficult. Both methods described in this thesis were created for use with high performance liquid chromatography and use Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP) as the reducing agent.

The experiments that follow the method development for these matrices center around accurate nutrient reporting and interest in nutrient variation. The potato method was applied to a research question of inter-variation in vitamin C content in a single,

consume- available purchase package of potatoes. For this study eight different varieties of potatoes were purchased from a local supermarket and measured for ascorbic acid, dehydroascorbic acid, and total vitamin C concentrations. The results showed a significant variation between potatoes from the same purchase package. The variety with the largest variation in total vitamin C had concentrations ranging between 3.90 – 23.38 mg/100g. Additional research on other commercially available individual produce will allow the USDA to report nutrient ranges for foods as opposed to the single nutrient content, as are currently listed in the National Nutrient Database for Standard Reference.

The applied elderberry experiment focused on whether wild elderberries grown in different locations throughout Northern Maine have significant differences in vitamin C content. For this study fourteen frozen elderberry samples and seventeen freeze-dried elderberry samples were used, all collected throughout Northern and Central Maine. Samples were measured for ascorbic acid, dehydroascorbic acid, and total vitamin C. Results show significant variation in total vitamin C levels between samples grown in different locations. Reasons for this variation are unknown but research to investigate differences such as, soil conditions (including pH, moisture, nutrient levels) and other factors that affect nutrient content in produce grown in the same region may contribute to the source of the variation (Hepperly et al., 2009).

DEDICATION

This thesis is dedicated to my grandmother and grandfather, Selma and Harry Eigner, who worked so hard to make sure all of their grandchildren could get the education they wanted. You inspired me and gave me the opportunity to excel where others insisted I would fail, for that I cannot thank you enough.

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LIST OF ABBREVIATIONS

AA = Ascorbic Acid

DHAA = Dehydroascorbic Acid

MPA= Metaphosphoric Acid

TFA = Trifluoroacetic Acid

THF= Tetrahydrofuran

TCEP = Tris(2-carboxyethyl)phosphine Hydrochloride

DTT = Dithiothreitol

HPLC = High Performance Liquid Chromatography

LC- Liquid Chromatography

LIST OF EQUATIONS

Equations for Potato Analysis

Standard Concentration. 10µL injection

25mg dissolved into 25mL, of which 0.1mL was then diluted into 25mL

Step 1.A: 0.1mL * (1000µg / 1mg) * (1000ng / 1µg) = 100000ng

Step 1.B: 100000ng / 25mL * (1000mL / 1µL) = 4.0ng / µL

*Step 1.*C: 10µL injected = 4.0ng / µL * 10µL = 40ng STD injected

Ascorbic Acid Concentration. 10µL injection

Step 2.A: The diluted sample weight = 1.00g (± 0.01) = Y

Step 2.B: 10mL (dilution) + Y = V_T

Step 2.C: (Sample AUC/Standard AUC) * 40ng (Standard Concentration) = X

Step 2.D: X= ng AA in the sample

Step 2.E: V_T * (1000µL / 1mL) * (X / 10µL) = Z

Step 2.F: (homogenized potato weight + extraction solution volume) * Z * (1mg / 1000µg) * (1µg / 1000ng) = W

Step 2.G: W / (homogenized potato weight) = mg / g AA * 100 = mg / 100g AA

Dehydroascorbic Acid Concentration. 10µL injection

Step 3.A: Follow calculation steps for AA

Step 3.B: Multiply the final mg / 100g by 2 (to account for the 1:1 dilution with TCEP)

Step 3.C: Subtract this number from the number calculated in Step 2.G[1].

Step 3.D: This is the concentration of DHAA in mg/100g

Total Vitamin C Concentration. 10μL injection

Step 4.A: Add the result of 2.G and 3.D (AA + DHAA)

Step 4.B: This is the concentration of Total Vitamin C in mg/100g

Equations for Elderberry Analysis

Standard Concentration. 10μL injection

25mg dissolved into 25mL, of which 0.1mL was then diluted into 25mL

Step 1.A: 0.1mL * (1000μg / 1mg) * (1000ng / 1μg) = 100000ng

Step 1.B: 100000ng / 25mL * (1000mL / 1μL) = 4.0ng/μL

*Step 1.*C: 10μL injected = 4.0ng / μL * 10μL = 40ng injected

Ascorbic Acid Content. 10μL injection

Step 2.A: % Dry weight (DW) = % moisture - 1

Step 2.B: 1.00g (±0.01) sample * % DW = Y

Step 2.C: (Sample AUC/Standard AUC) * 40ng (Standard Concentration) = X

Step 2.D: X= ng AA in the sample

Step 2.E: Dilution + $Y=V_T$

The dilution in step 2.E for frozen berries was 10mL and 20mL for freeze-dried berries

[1] *Note: For both studies anything under 0.5mg/100g was considered not a significant amount of DHAA*

[2] Excellent source refers to any food that provides 20% or more of the RDA of a nutrient

Step 2.F: V_T x (1000µL / 1mL) * (X / 10µL) = Z

Step 2.G: ((Homogenized Elderberry Weight * % DW) + Extraction Solution Volume (10mL)) * Z * (1mg / 1000µg) * (1µg / 1000ng) = W

Step 2.H: W / (Homogenized Elderberry Weight * % DW) = mg / g AA * 100 = mg / 100g AA

<u>Dehydroascorbic Acid Content.</u> 10µL injection

Step 3.A: Follow calculation steps for AA

Step 3.B: Multiply the final mg / 100g by 2 (to account for the 1:1 dilution with TCEP)

Step 3.C: Subtract this number from the number calculated in Step 2.G.

Step 3.D: This is the concentration of DHAA in mg / 100g

<u>Total Vitamin C Content.</u> 10µL injection

Step 4.A: Add the result of 2.G and 3.D (AA + DHAA)

Step 4.B: This is the concentration of Total Vitamin C in mg / 100g

CHAPTER ONE

INTRODUCTION

Vitamin C is one of the nine essential water-soluble vitamins in the human diet, and has been studied dating back as early as 1550 B.C.E. Only in the past 25 years, however, have researchers really begun to fully understand this nutrient. Vitamin C plays a role in nearly every function of the human body, from gene regulation to cell growth to prevention of cardiovascular disease. It plays a role in regulating some of the most important functions of the body (Sauberlich, 1996).

Vitamin C can be a difficult nutrient to quantify due to degradation both inside and outside of the food matrix. Factors such as presence of oxygen, oxidizing and reducing agents, light sensitivity, time between extraction and measurement, temperature, and pH all affect vitamin C stability, both inside and outside the food matrix (Nováková et al., 2008). If these factors are not controlled prior to and during extraction the resulting vitamin C contents will be artificially low.

For the following research studies two Maine food crops were used, potatoes and wild elderberries. These items were chosen because they represent different types of agricultural revenue for the state of Maine. Potatoes are an established source of revenue, the most valuable agricultural crop grown in Maine (USDA, 2013), and elderberries are an emerging source of revenue. One cup of either item is considered an excellent source of vitamin C[2] (USDA, 2011). White potatoes are also listed in the 2003- 2006 NHANES list of food items that contribute most substantially to the vitamin C intake of the average American (O'Neil et al., 2012). Both food items are considered complex matrices because of their varied chemical composition, which includes a large variety of compounds with similar chemical structure that can prevent accurate recovery of vitamin C from the food matrix. In potatoes, one of the major obstacles is high starch content. “Cloudy” or starchy samples that are injected into the liquid chromatography (LC) system can damage the system by leaving residue in the system and in the column, which can lead to high repair costs. During cooking the starch in the potato sample gelatinizes, which can cause inaccuracies in measurement if the sample forms a mass during homogenization. The formation of such a mass can prevent the ascorbic acid or dehydroascorbic acid from being fully extracted from the matrix. It is also very difficult to remove the gelatinized starch from the sample extract. In elderberries starch is not as much of a concern but interactions of vitamin C with other similar nutrients found naturally in the sample leads to difficulty isolating the ascorbic acid peak (Kaack & Austed, 1998). This can also be a concern for in pigmented potato varieties, which contain flavonoids.

[2] Excellent source refers to any food that provides 20% or more of the RDA of a nutrient in a 2,000-calorie diet (FDA, 2013). According to the USDA’s National Nutrient Database for Standard Reference raw white potatoes contain 23% of the RDA and raw elderberries contain 87% of the RDA (USDA, 2011).

CHAPTER TWO

LITERATURE REVIEW

<u>Potatoes</u>

White potatoes are one of the five most common sources of vitamin C in the American diet (Sinha et al., 1993). Americans alone consume 116lbs of potatoes per capita (National Potato Council, 2014). In Maine, potatoes are one of the largest agricultural crops and potato sales represent a significant amount of the state's income, with the 2013 state crop valued at more than 167,000,000 U.S. dollars (USDA, 2013).

Potatoes (*Solanum tuberosum* L.), are categorized as a starchy vegetable, meaning that they have a high ratio of starch to other macronutrients. They are in the *Solanaceae* family, which includes other common vegetables such as tomatoes, eggplant, and peppers. The starch content of potatoes can make them a difficult matrix to work with. One of the major obstacles that arises when analyzing potatoes for nutrient content is a direct result of the high starch content. Samples injected into the liquid chromatography (LC) system without removing the starch can damage the system by leaving residue in both the system and in the column, which can lead to high repair costs. Additionally, when potatoes are cooked the starch begins to gelatinize. Gelatinization occurs when starch granules absorb moisture, swell, and break apart to form a gel-like substance (Ratnayake & Jackson, 2009). Gel formation can lead to difficulty fully extracting nutrients from the matrix in two ways, both related to sample dilution. If the sample is not diluted enough, or is not fully soluble in the extraction solution, a mass can form, which prevents the extraction solution from fully penetrating the sample. This can result in an artificially low reporting of nutrient content. Alternatively, if the sample is too dilute, in an attempt to prevent a mass from forming, the sample could be too dilute to accurately detect and measure nutrients such as AA.

Nutritionally, potatoes are important to the U.S.; they are a significant source a variety of nutrients in the average American diet (King, 2005; Slavin, 2013; Johnson & Gee, 1996). Potatoes contain significant levels of both resistant starch and fiber (Slavin, 2013). Resistant starch is similar to fiber, but is actually a type of amylose, where as fiber is typically a cellulose. Both resistant starch and fiber are resistant to digestive amylase, allowing them to bypass digestion in the small intestine and travel to the large intestine where they are fermented by the gut micro flora (Johnson & Gee, 1996). These indigestible starches have been linked to numerous health benefits such as protection against obesity and type two diabetes, (Yoon et al., 2008; Slavin, 2013; Howarth et al., 2001), protection against diventricular disease (Aldoori et al., 1998), protection again cardiovascular disease (Theuwissen & Mensink, 2008; Steemburgo et al., 2009; King, 2005), and protection against metabolic syndrome (Steemburgo et al., 2009 & McKnewn et al., 2002). Potatoes and potato products, with skin, contain an average of three grams of fiber per 100g sample (USDA, 2011).

Potatoes are much more than an excellent source of carbohydrates. They also contain significant protein, a small amount of fat, and variety of micronutrients. A common misconception among dieters today is that potatoes are high in fat. In fact, during the 1980s potatoes were popular among dieters due of their low fat content (Johnson and Gee, 1996). Potatoes are actually a naturally low fat item; they are often seen as a high fat item because fat is commonly added to potatoes during processing or preparation. 100g if a plain baked potato contains only 0.13g of lipids, where as and 100 grams of French fries contain 2.59g of lipids and 100 grams of potato chips contain a staggering 9.5g of lipids (USDA, 2011). In addition to beneficial carbohydrates and low fat content, potatoes contain an average protein content of 2.5 g per 100g sample (USDA, 2011). The benefit of this protein is that it has a high biological value, between 90-100 (Buckenhüskes, 2005), which means that the protein found in potatoes is actually more readily available than the protein found in soy protein isolate, which has a biological value of 83 (Gazeava, 1985).

The micronutrients of note found in potatoes include vitamin B6, vitamin C, potassium, and magnesium. Vitamin B6 (pyridoxine) is needed for a variety of cellular functions including metabolism of amino acids, nucleic acids, glycogen, and lipids (Kant & Block, 1990). Vitamin C (AA and DHAA) is important for many bodily maintenance functions. 100g if a plain baked potato contains 12.6mg of vitamin C as ascorbic acid, equating to 16% of the average RDA for adults (80 mg). Potatoes eaten with the skin intact are considered a good source of both potassium and magnesium. Potatoes are the most potassium dense vegetable (King & Slavin, 2013). Potassium, in combination with sodium, is required in the diet to regulate blood pressure. Recent studies, including that of Drewnowski et al. (2012), have found that 99.985% of Americans are over-consuming sodium and under-consuming potassium. This imbalance could be having a significant impact on the number of individuals with hypertension in the United States. By consuming foods high in potassium (potatoes), this imbalance could be reduced. Appendix A lists the macro and micronutrient values found in potatoes and common processed potato products, as listed in the USDA National Nutrient Database for Standard Reference, release 27 (USDA, 2011).

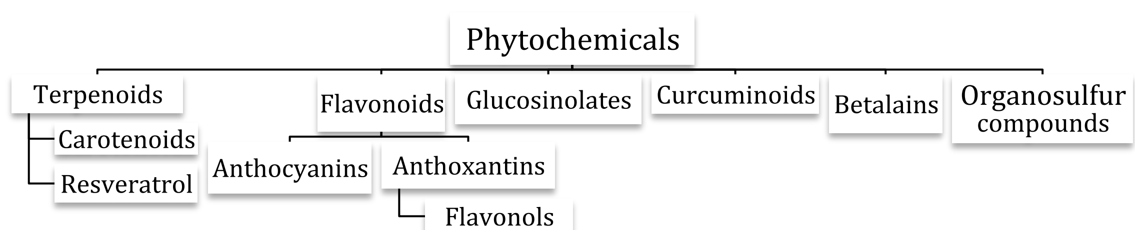
Elderberries

Elderberries are currently not a well-known crop across North America, but have gained interest recently because of potential anti-viral, anti-inflammatory, and anti-cancer properties (McKay, 2004). A search of Cornell's Department of Horticulture's database resulted in a myriad of recent publications detailing both commercial and non-commercial cultivation of elderberries in Oklahoma, Missouri, New York State, Ohio, Oregon, and Vermont (Stafne, unlisted; Byers, 2005; Way, 1981; Brownlee & Stedman, 2013). Although there is some commercial production of elderberries in Maine, it is not uncommon to see wild elderberry bushes growing on roadsides.

As commercial production of elderberries is so new in the U.S., there is little available information or research about elderberries, as compared to potatoes. However, fresh elderberries have made their way onto the USDA'S National Nutrient Database for Standard Reference and some databases for personal diet tracking. As mentioned previously, fresh elderberries are an excellent source of vitamin C, containing 87% of the RDA of vitamin C, as total AA, in a single cup. Elderberries are also considered a good source of fiber, containing 10g per cup (USDA, 2011). Additionally, elderberries contain more phosphorus and potassium than any other temperate crop (Cornell, 2013). A single cup of elderberries contains 8% of the RDA (for adults) of phosphorus and 9% of the RDA (for adults) of potassium. As previously discussed in the nutrient discussion for potatoes, potassium is a nutrient of concern in the U.S., with most adults under-consuming the nutrient (Drewnowski et al., 2012). Appendix B lists the macro and micronutrient values for elderberries.

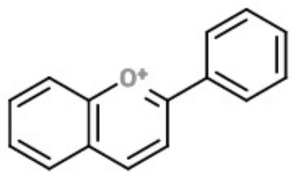
While elderberries contain a number of beneficial macro and micronutrients, their phytochemical profile sets them apart from many other fruits. Phytochemicals are a newly recognized category of bioactive compounds. They are secondary plant metabolites created by plants in response to environmental stresses (American Institute for Cancer Research, 2008). Figure 2.1 (a simplified list) shows some of the different groups of phytochemicals and their subgroups.

Figure 2.1. Phytochemical Classifications



Elderberries contain a wide variety of flavonoids, most notably, anthocyanins (Kaack & Austed, 1998). There are many types of anthocyanins and they vary in chemical structure; however, the general chemical structure consists of three benzene rings with a positively charged oxygen molecule on the B-ring (Figure 2.2). Anthocyanins are named and categorized based on the glycosylation location on the benzene rings. Like vitamin C, they are a heat-labile class of nutrients (Patras et al., 2010). However, again like vitamin C, heat is not the only factor influencing anthocyanin content pH, amount and type of other anthocyanins present, storage conditions, temperature, and presence of oxygen, enzymes, proteins, and/or metallic ions also influence anthocyanin stability (Rein et al., 2005).

Figure 2.2. Anthocyanin General Chemical Structure



This figure was created using emolecules.com

An indicator of anthocyanin stability is color, which is highly dependent on pH. At a pH below 3, anthocyanins are red and are most stable. At a pH between 3 and 6, anthocyanins tend to be colorless and not as stable as anthocyanins below 3. At a pH of 6 or above, anthocyanins tend to be blue and are the least stable. A list of the four most commonly measured flavonoids found in elderberries is listed in table 2.1.

Table 2.1. Commonly Measured Elderberry Flavonoids

Flavonoid	Reported Content (mg/100g)
cyanidin-3-glucoside (Cy-3-G)	361-1266 mg/100g
cyanidin-3-sambubioside (Cy-3-Sa)	269-656 mg/100g
cyanidin-3-sambubioside-5-glucoside (Cy-3-Sa-5-G)	5-47 mg/100g
cyanidin-3,5-diglucoside (Cy-3.5-dG)	5-47 mg/100g

This table was created from information from (Kaack & Austed, 1998).

As mentioned previously, chemical similarities between AA and flavonoids can create difficulties when trying to separate individual peaks for quantification. To preserve AA, a pH below 4 is needed (Golubitskii et al., 2007), however, at a pH below 4 anthocyanins are most stable, making them difficult to remove from the matrix without degrading the AA. In order to create a method to accurately measure AA in these samples, a method of adequately separating the flavonoid peaks from the AA peaks needed to be created. Currently published methods for vitamin C quantification in elderberries are not sufficient as most do not use high performance liquid chromatography (HPLC) (González et al., 2012), measure only AA, or total vitamin C (Sadilova et al., 2009).

There are numerous health benefits associated with phytochemicals; one of the most notable is the potential role they play in cancer prevention (Thole et al., 2006; Choi et al., 2014). The long-term role of phytochemicals as cancer prevention agents has not yet been researched (Wang & Stoner, 2008). Wang and Stoner's (2008) review of the effectiveness of anthocyanins in cancer prevention concluded that the absorption of anthocyanins is very limited in humans. Due to the limited ability to absorb many phytochemicals, the ability to provide cancer prevention may also be limited. However, it has been noted that anthocyanins are effective in preventing cancer in areas of the body that some of these compounds come in direct contact with, such as skin, stomach, small intestine, and colon (Paturi et al. 2012; Madiwale et al., 2012; Liao et al., 2003).

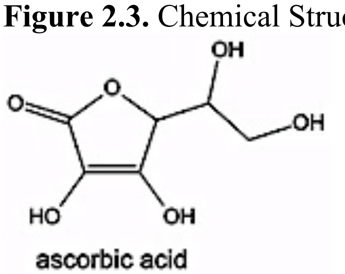
Elderberries have also been investigated for potential anti-viral properties (Kinoshita et al., 2012; Roschek et al., 2009; Krawitz et al., 2011). Kinoshita et al. (2012) found that twice-daily consumption of concentrated elderberry juice had a protective effect against the influenza virus in female BALB/c mice. Similarly, Roschek et al. (2009) found that specific flavonoids present in elderberries, 5,7,3,4-tetra-*O*-methylquercetin and 5,7-dihydroxy-4-oxo-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-3,4,5-trihydroxycyclohexanecarboxylate, bind to the H1N1 influenza virus to prevent infection *in vitro*. Additionally, Krawitz et al. (2011) found that *in vitro* elderberry extract showed an inhibitory effect against both A and B strains of the human influenza virus and in both *Streptococcus pyogenes* and *Branhamella catarrhalis* bacteria.

Vitamin C

Vitamin C is one of the nine water-soluble vitamins that are essential in the human diet. It is perhaps best known for its role in the prevention of scurvy, which is the deficiency disease of vitamin C in humans. However, it also participates in a wide variety of biological functions such as formation of collagen, wound healing, and maintenance of bones, teeth, skin, gums, muscles, and cartilage. Vitamin C activity can be seen all the way down at the genomic level with regulation of gene expression and at the cellular level with influences in cellular growth (Sauberlich, 1996). Vitamin C is found naturally in a wide variety of fruits and vegetables and is also widely used in food production as a preservative because of its role as a strong antioxidant (FDA, 1979). There has been some recent concern about overconsumption of antioxidants. However, Sauberlich (1996) cites vitamin C as being “the most effective and least toxic antioxidant present in the human body.”

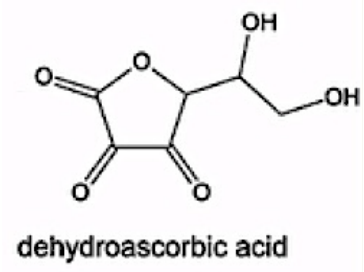
Vitamin C can be found in one of three forms within fresh fruits and vegetables. These forms are ascorbic acid (AA), dehydroascorbic acid (DHAA), and diketogulonic acid (Prest et al., 2003). AA is the non-oxidized, or reduced, form of vitamin C that is commonly used interchangeably with the term vitamin C and is often measured in items such as food supplements and food items to represent the available vitamin C in the item. DHAA is the first oxidized form of vitamin C and at this stage can be reduced back to AA with the addition of a reducing agent. The bioactivity of DHAA within the human body is unclear, with reports ranging from 100% activity as compared to AA (Elmadfa & Koenig, 1996) to only 10% of the activity of AA (Ogiri et al., 2002). The sum of the AA and DHAA content in a food sample is called the total vitamin C. Total vitamin C is often reported for foods in databases such as the USDA's National Nutrient Database for Standard Reference. Diketoglyonic acid is the second oxidized form of vitamin C. At this stage it cannot be reduced back to AA and is typically excreted from the body through the kidneys (Nishikimi & Yagi, 1996). AA, DHAA, and total vitamin C were measured in the following research studies. Chemical structures of each of these are depicted in Figures 2.3, 2.4, and 2.5.

Figure 2.3. Chemical Structure of Ascorbic Acid



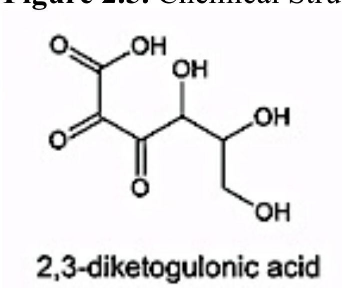
This figure was sourced from Prest et al., 2003

Figure 2.4. Chemical Structure of Dehydroascorbic Acid



This figure was sourced from Prest et al., 2003

Figure 2.5. Chemical Structure of Diketogulonic Acid



This figure was sourced from Prest et al., 2003

There is fourth form of vitamin C, isoascorbic acid or erythorbic acid. It is derived from fruit and vegetable sources but is typically added to processed foods as a preservative (FDA, 1979). Isoascorbic acid is only 5% bioactive as compared to ascorbic acid (Elmadfa & Koenig, 1996). This form was not measured in the following thesis studies because the samples used in the studies were raw fresh produce samples.

Vitamin C is an essential nutrient because, like monkeys and guinea pigs, humans have lost the ability to produce L-gulono-γ-lactone oxidase (GLO). GLO is an integral protein found in the liver of most mammals, with the exception of the aforementioned mammals. It is necessary to the final stage of vitamin C synthesis from glucose (Nishikimi & Yagi, 1996). Despite the inability to synthesize vitamin C in the human body, the 2011-2012 NHANES survey noted that vitamin C is among the nutrients that are consumed in adequate amounts by most Americans (USDA, 2014). The U.S. RDA for vitamin C is depicted in the table 2.2. Individuals who smoke are recommended to consume the listed RDA plus an additional 35mg/day to compensate for the reduced absorbance of vitamin C (NIH, 2013). The most commonly consumed food items that contribute to vitamin C in the American diet are fruit juice (28.6%), fruit (15.3%), fruit drinks and ades (14.0%), tomatoes and tomato juices (7.2%), broccoli, spinach and greens (5.1%), white potatoes (4.5%), ready-to-eat cereals (2.4%), and other vegetables (11.3%) (O'Neil et al., 2012).

Table 2.2. RDA for Vitamin C by Age

Age (years)	**Male**	**Female**	**Pregnant**	**Lactating**
1-3	15mg	15mg		
4-8	25mg	25mg		
9-13	45mg	45mg		
14-18	75mg	65mg	80mg	115mg
19+	90mg	75mg	85mg	120mg

This table was adapted from the NIH Vitamin C Fact Sheet for Health Professionals (NIH, 2013). RDAs are not available for infants under 12 months of age.

While many aspects of vitamin C have been well researched, laboratory work to accurately quantify vitamin C from fresh produce samples still has a number of hurdles that researchers must account for. One common factor that leads to degradation inside the food matrix is post harvest treatment (Phillips, et al., 2010), which often is not or cannot be controlled by processing, researchers, or consumers. Other common factors that lead to degradation outside of the food matrix revolve around the very unstable nature of vitamin C in aqueous solution. Environmental factors such as presence of oxygen, presence of oxidizing and reducing agents, light permeability of the storage container and location, time between extraction and measurement, temperature, and pH all effect vitamin C stability both inside and outside the food matrix (Nováková et al., 2008; Assiry et al., 2006). Assiry et al., (2006) reported that AA follows a first order degradation model for conventional heating, ohmic heating, and pH change. AA is most stable at a pH range of 1-4. A pH of 5 or above causes AA to be oxidized to DHAA and further irreversibly oxidized to diketogulonic acid (Golubitskii et al., 2007). For vitamin C analysis, control of these reactions during sample preparation is of the utmost importance.

Current methods for quantifying vitamin C in potatoes are insufficient as they use expensive equipment, which may be unattainable for the average researcher; most measure AA or total vitamin C only, and do not quantify DHAA (Yang et al., 1998; Bushway et al., 1988); they have not been proven to work with starchy (Gökmen et al., 2000; Odriozola et al., 2007; Nisperos et al., 1992; Zapata & Dufour, 1992), cooked (Gökmen et al., 2000; Odriozola et al., 2007; Bushway et al., 1988; Zapata & Dufour, 1992), or blue pigmented samples (Gökmen et al., 2000; Vanderslice et al., 1990; Odriozola et al., 2007; Bushway et al., 1988; Nisperos et al., 1992; Zapata & Dufour, 1992), or were run at a pH range that were not suitable for many LC systems (Yang et al., 1997; Wu et al., 2003; Yang et al., 1998). Some examples of expensive and time consuming methods required by current publications include, use of simultaneous dual detectors (Zapata & Dufour, 1992; Nisperos et al., 1992), post column dervitization (Vanderslice et al., 1990), or mass spectroscopy (Zhang et al., 2002).

An additional area for improvement in the quantification of vitamin C in potatoes is developing a method that works for both raw and cooked samples. Published methods were developed for raw potatoes, despite typical preparation including cooking. During the cooking process the vitamin C content of the potatoes changes. When the potato is cooked without the skin, AA, DHAA, and total vitamin C contents all decrease. However, when the potatoes are cooked with the skin intact, AA, DHAA, and total vitamin C content depend on the method they were cooked (Vanderslice et al., 1990).

CHAPTER THREE

METHOD DEVELOPMENT FOR VITAMIN C QUANTIFICATION IN POTAOTES

Introduction

There are many methods for quantifying vitamin C in potatoes. Most recent publications use prohibitively expensive equipment, which may be unattainable for the average researcher; many others measure ascorbic acid (AA) only, failing to differentiate dehydroascorbic acid (DHAA) or total vitamin C, ignoring the difference between DHAA and AA. Most other published methods cannot be applied to cooked or pigmented samples. The purpose of this research was to design a method to measure AA, DHAA, and total vitamin C in raw, cooked, and pigmented samples that could be used with simple, less expensive high performance liquid chromatography (HPLC) systems. A common difficulty for researchers updating equipment, a lapse in equipment modernization at many research facilities can lead to an inability to preform the functions necessary to use recently published methods. The goal of this is to enhance the capacity of research laboratories by creating a method that can be used with less advanced HPLC technology.

Materials and Methods

Chemicals

There were a variety of chemicals used in this study. Meta-phosphoric acid (MPA) was purchased from Sigma Aldrich, St. Louis, MO, certified ACS grade. The acetonitrile (EM Science, Gibbstown, NJ), tetrahydrofuran (THF) (ACROS Organics, Geel, Belgium), and trifluoric acid (TFA) (Fisher Scientific, Fair Lawn, NJ) were all 99% pure. The potassium dihydrogen phosphate (99.9% pure) and phosphoric acid (85% pure) were purchased from Fisher Scientific, Fair Lawn, NJ. The tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Thermo Scientific, Rockford, IL. The mobile phase, 3% meta-phosphoric acid and 3% acetonitrile dissolved in HPLC grade water, was stable at ambient temperature for three days. The extraction solution was a 0.2M phosphate buffer solution prepared weekly. The extraction solution was prepared by dissolving 54g of potassium phosphate and 55mL of phosphoric acid in 2L of HPLC grade water. The solution later referred to as the, dilution solution, was 3% MPA dissolved in HPLC-grade water and was prepared every three days. The TCEP solution was also prepared every three days by dissolving 67mg, 134mg, or 268mg into 10mL of 3% MPA.

Standards for AA and DHAA were purchased from Baker Analyzed and Sigma Aldrich, respectively. Standards and standard curves were prepared daily with 3% MPA as a diluent. Stock standards were made by dissolving 25mg of the standard in 25mL of 3% MPA. The AA standard curve was made by diluting 0.025mL, 0.05mL, 0.1mL, 0.2mL, and 0.4mL of AA stock solution in 25mL of 3% MPA.

Equipment

An Agilent Technologies (Santa Claram CA) HPLC system modified with units from Hewlett-Packard (Palo Alto, CA) was used for this study. The degasser (110 series), thermostatted column compartment (1200 series), and diode array (1200 series) were from Agilent Technologies. The pump (1100 series ISO pump, upgraded to a quat pump) and the autosampler (1100 series) were from Hewlett-Packard. The computer used with this system was a Dell Optiflexx 755 formatted with Windows XP Professional version 5.1.2600. Measurements were done using ChemStation for LC by 3D Systems, version 8.0401(481) (Agilent Technologies).

Three different homogenization tools were used for the trial portion of this study. A Kinematica, Luzern, Switzerland, polytron equipped with a Superior Electronic, Bristol, CT, POWERSTAT autotransformer was used in the matrix trials. A Waring, Odessa, FL, Commercial Blender with a 40oz glass insert was used for both the trial and the applied studies. A Magic Bullet, by Capital Brands, Los Angeles, CA, was used for the applied studies, for samples that weighed under 100g. Samples were centrifuged with an Eppendorf centrifuge model 5430 (Hamburg, Germany). An Emerson (Hackensack, NJ), 1050W home model microwave was used to steam samples.

HPLC System Trials

Creating a new HPLC method requires balancing of all of the components of HPLC. This includes choosing the proper combination of detector setting, column and mobile phase, as well as flow rate and run time. One method that works with a specific column will likely need adjustments in order to work on a different column. Similarly if flow rate is increased or decreased run time will need to be adjusted as well.

The detector was set to wavelengths 244 and 254nm because they are the wavelength where AA is most highly absorbed (Bushway et al., 1988). Three columns and two mobile phases were tested for this method. The columns tested were Thermoscientific Hypersil Gold, Phenomenex Kinetex EVO, and Agilent Technologies PLRP-S. All columns were 5μ, 100A, 250 x 4.6mm. These columns were chosen because they are rated for use with a wider pH range than other columns, this was necessary because of the low pH needed to preserve the AA in the sample. The two mobile phases tested were 3% MPA and 0.1% TFA. Flow rate and run time was chosen once all other factors were determined, based on peak separation and elution.

Final Analysis Method

The detector was set to UV-Vis, measuring 244 and 254nm. A flow rate of 0.7mL/min with a run time of 10 minutes was used and 10μL of both samples and standards were injected into the system. The mobile phase was 3% MPA and 3% Acetonitrile, dissolved in HPLC grade water. The column used was a ThermoScientific Hypersil Gold C18 column 5μ 100A (250x4.6mm) operated at ambient temperature.

Samples

The samples for this study were full sized russet, gold fleshed, red skinned, and blue-fleshed potatoes donated from growers working in conjunction with the University of Maine. Hannaford-brand russet and red skinned potatoes were also used and purchased from the local Hannaford supermarket. Multiple varieties of potatoes were used for this study because different potato varieties can have different chemical interferences. Using different varieties resulted in the creation of a stronger and more widely applicable method.

Method Testing

Six different studies were done, between January 2015 and May 2015, to test the validity of the sample preparation and LC run method. They are described below.

Ascorbic Acid Standard Curve. A standard curve was developed by preparing independent dilutions of AA at the following concentrations, 5ng, 10ng, 20ng, 40ng, and 80ng.

Potato Matrix. One concern with using a sample that contains a complex matrix is ensuring that the nutrients are fully extracted from the matrix. To ensure that all of the vitamin C was removed from the potato matrix, the differences between diluting and hand-shaking to combine, and diluting and polytronning to combine were studied. Both hand-shaking and polytronning were done for 30 seconds immediately after diluting the sample. Polytronning was accomplished using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50. All other sample preparation followed the method listed. Composite samples were assayed in duplicate.

Ascorbic Acid Fortification. Samples were spiked with AA to determine AA recovery rates from the potato matrix. This was done by determining the average amount of AA in the sample and then calculating and adding enough AA to increase the AA peak area by two fold and five fold. This was accomplished by adding the concentrated AA stock solution to the potato homogenate prior to sample dilution. The homogenate was weighed to 1.00 (± 0.01g) and spiked with 0.05mL (x2) or 0.25mL (x5). Samples were assayed with 20ng and 100ng AA standards as well as an unspiked "control" potato sample. Composite samples were assayed in duplicate for this study.

Dehydroascorbic Acid Reduction. To ensure that all of the DHAA in the sample was being converted to AA by the TCEP reduction procedure, different concentrations of TCEP and reaction times were tested, both with potato samples and DHAA standards. Samples were prepared using the method described in the Sample Preparation section. 0.5 mL of prepared sample was placed in a 2mL HPLC vial; then 0.5mL of the prepared TCEP solution (67mg, 134mg, or 368mg dissolved in 10mL of 3% MPA) was added and allowed to react for either 60 minutes or 120 minutes in the autosampler tray. Duplicate composite samples were assayed. This reduction process was repeated with 0.5mL of DHAA stock solution.

Dehydroascorbic Acid Reduction in the Potato Matrix. This study followed the procedure outlined in the AA spiking study. DHAA was added to the potato matrix after homogenization and prior to dilution in order to ensure that the DHAA permeated the potato matrix. Samples were extracted, diluted, and assayed by using the AA procedure, as previously described.

Confirmation of Method Applicability to Cooked and Pigmented Samples. Four potato varies were used for this study. Two white fleshed varieties (russet and superior), one variety with red skin and yellow flesh (dark red), and one variety with blue skin and flesh (Adirondack blue). Cooked samples followed the same procedure developed for raw samples (and with a minor change to the homogenization method) were microwave-steamed after washing. Whole potatoes were steamed for 10 minutes with 10mL of deionized (DI) in a 600mL glass beaker using a standard 1050W home model Emerson microwave set to full power.

Final Sample Preparation Method

All potatoes were homogenized with 0.2M phosphate buffer at a ratio of 1:1 (W:V) for raw potatoes and 1:2 (W:V) for cooked potatoes. Subsamples of the homogenate were then weighed (1.00 (± 0.01g)) into a 50 mL centrifuge tube and diluted with 10mL of 3% MPA. This was centrifuged for 10 minutes at 7,745 x g. 1.5mL of supernatant was then transferred to a 2mL centrifuge vial and again centrifuged for 10 minutes at 30,156 x g. 0.5mL of supernatant was transferred to a 2mL HPLC vial and immediately injected into the LC system for AA analysis. Concurrently, another 0.5mL of supernatant was transferred to a 2mL HPLC vial and 0.5 mL of 67mg/10mL TCEP was added and allowed to react for 60 minutes before HPLC analysis for DHAA content.

Calculations

All results were reported on a fresh weight basis.

__**Standard Concentration.**__ 10µL injection

25mg dissolved into 25mL, of which 0.1mL was then diluted into 25mL

Step 1.A: 0.1mL * (1000µg / 1mg) * (1000ng / 1µg) = 100000ng

Step 1.B: 100000ng / 25mL * (1000mL / 1µL) = 4.0ng / µL

*Step 1.*C: 10µL injected =4.0ng / µL * 10µL= 40ng STD injected

__**Ascorbic Acid Concentration.**__ 10µL injection

Step 2.A: The diluted sample weight=1.00g (±0.01) = Y

Step 2.B: 10mL (dilution) + Y = V_T

Step 2.C: (Sample AUC / Standard AUC) * 40ng (Standard Concentration) = X

Step 2.D: X= ng AA in the sample

Step 2.E: V_T x (1000µL / 1mL) * (X / 10µL) = Z

Step 2.F: (homogenized potato weight + extraction solution volume) * Z *

(1mg / 1000µg) * (1µg / 1000ng) = W

Step 2.G: W / (homogenized potato weight) = mg / g AA *100 = mg / 100g AA

Dehydroascorbic Acid Concentration. 10µL injection

Step 3.A: Follow calculation steps for AA

Step 3.B: Multiply the final mg/100g by 2 (to account for the 1:1 dilution with TCEP)

Step 3.C: Subtract this number from the number calculated in Step 2.G[3].

Step 3.D: This is the concentration of DHAA in mg / 100g

Total Vitamin C Concentration. 10µL injection

Step 4.A: Add the result of 2.G and 3.D (AA + DHAA)

Step 4.B: This is the concentration of Total Vitamin C in mg / 100g

Results and Discussion

Detector

Wavelength 244nm was chosen for calculations because of additional interference seen at 254nm. Results from 254nm were used in conjunction with 244nm results for mobile phase trials.

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[3] *Note: For this study anything under 0.5mg/100g was considered not a significant amount of DHAA*

Other published methods include using dual detectors, UV and Fluorescence, to measure AA and DHAA simultaneously (Zapata & Dufour, 1992; Nisperos et al., 1992). Similarly other methods use post-column derivitization (Vanderslice et al., 1990), neither dual detector nor post-column derivitization methods are compatible with all HPLC systems. For these reasons a pre-column reduction of DHAA to AA was chosen.

Column and Mobile Phase

The chemically bonded C18 columns, ThermoScientific Hypersil Gold and Phenomenex EVO, provided superior peak resolution and better specificity than the physically bonded C18 column, Agilent Technologies PLRP-S. The two chemically bonded columns gave similar results, however, samples assayed with the ThermoScientific Hypersil Gold column had slightly better peak resolution than samples assayed with the Phenomenex EVO.

An equal percentage of acetonitrile was added as a modifier to the mobile phase, to improve peak resolution. The mobile phase was chosen after reviewing the peak high ratio at 244nm and 254nm of samples vs. standards. Samples and standards should have equal peak ratios. Ratios that are not similar is an indication of interference from co-eluting compounds. The ratios for 3% MPA were very similar for both the samples and the standards, however, the ratios for the 0.1% TFA samples and standards were not similar, as shown in table 3.1.

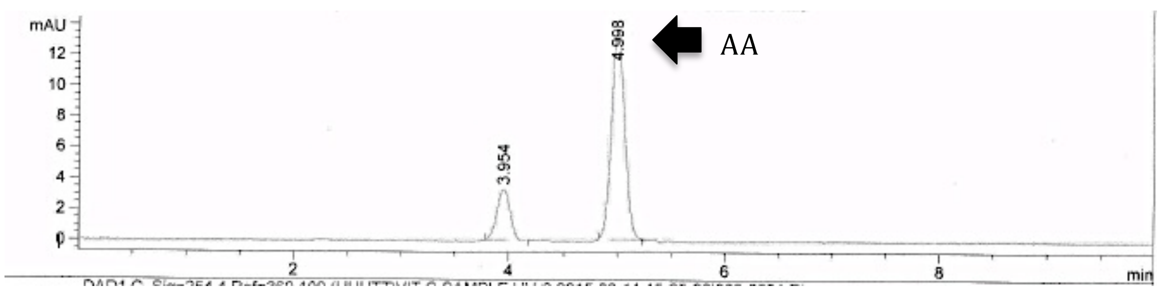
See Figures 3.1, 3.2, and 3.3, ascorbic acid standard (40ng), white potato sample, and white potato sample with TCEP added, respectively, for examples of chromatograms generated using this method.

Table 3.1. Mobile Phase Trial Results for the Potato Method

Mobile Phase	Sample	Area		Ratio
		244nm	254nm	
0.1% TFA	AA STD 40ng	152.87	111.04	1.38
	Potato Sample	272.67	352.20	0.77
3% MPA	AA STD 40ng	64.28	47.20	1.36
	Potato Sample	367.39	271.20	1.35

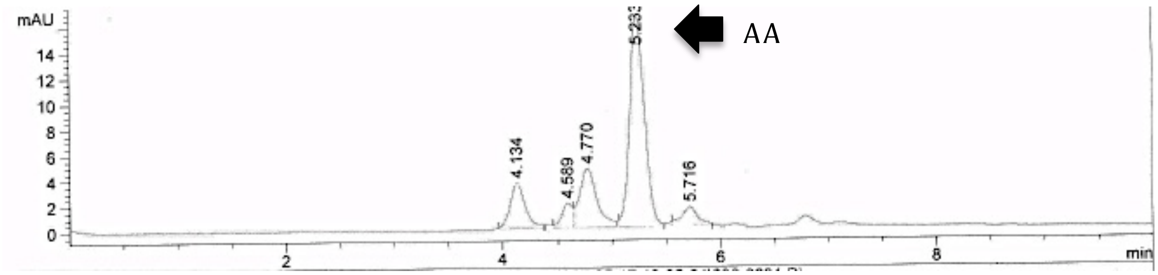
Average nutrient values listed for duplicate samples are listed

Figure 3.1. Chromatogram of the Ascorbic Acid Standard using the Potato Method



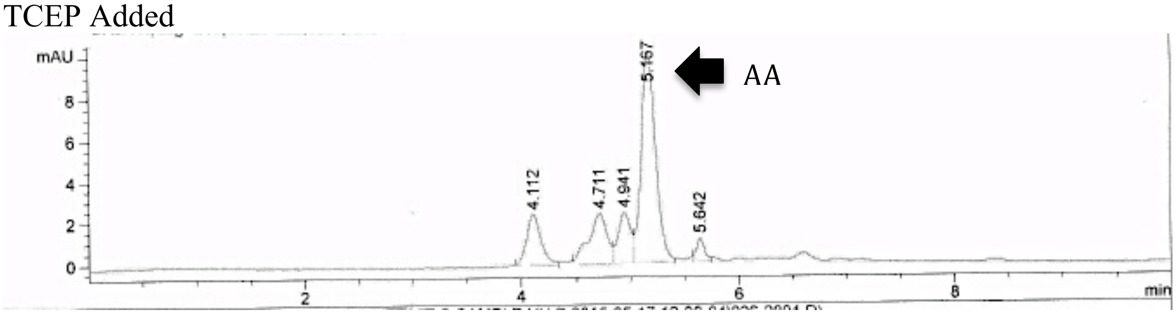
Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Figure 3.2. Chromatogram of a White Potato Sample using the Potato Method



Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Figure 3.3. Chromatogram of a White Potato Sample using the Potato Method with TCEP Added



Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Method interferences with AA, TCEP, and mobile phase were tested. Neither TCEP nor the mobile phase interfered with AA separation.

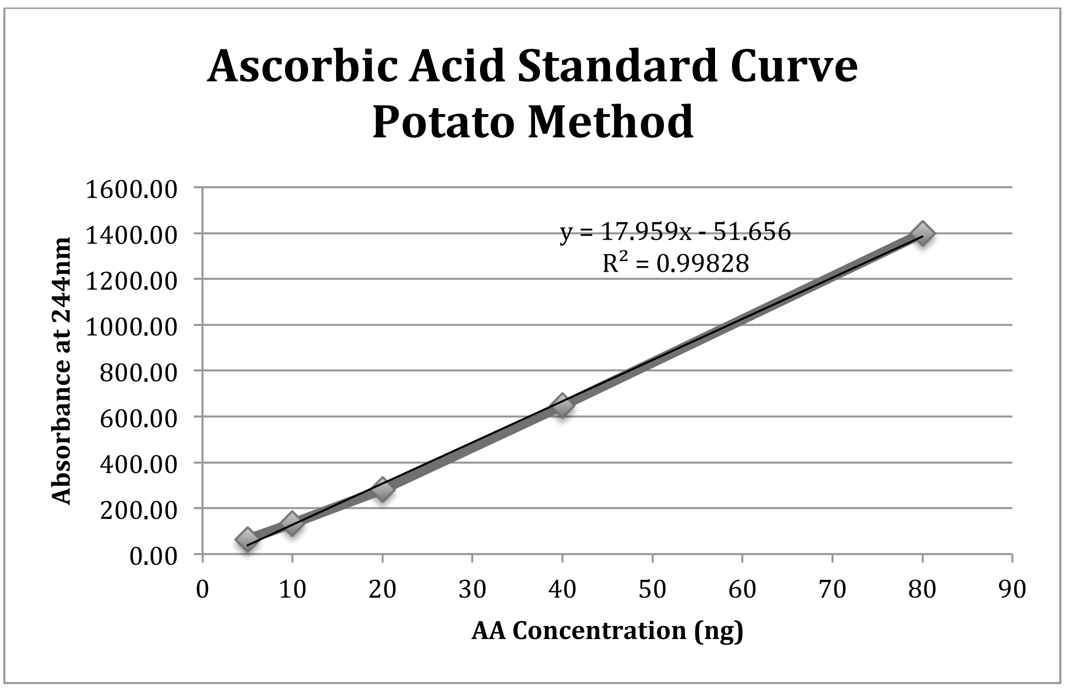
Flow Rate and Run Time

The flow rate and run time were determined based on the samples and peak separation. The flow rate that provided optimal peak separation was 0.7mL/min. At this flow rate there were no late eluting peaks after six minutes. To allow the column to fully clear before the next sample was injected a run time of 10 minutes was chosen.

Ascorbic Acid Standard Curve

The standard curve showed a linear response with an R^2 value of 0.998. This is shown in Figure 3.4.

Figure 3.4. Ascorbic Acid Standard Curve using the Potato Method



Standard curve using 5ng, 10ng, 20ng, 40ng, and 80ng AA concentrations

Sample Preparation

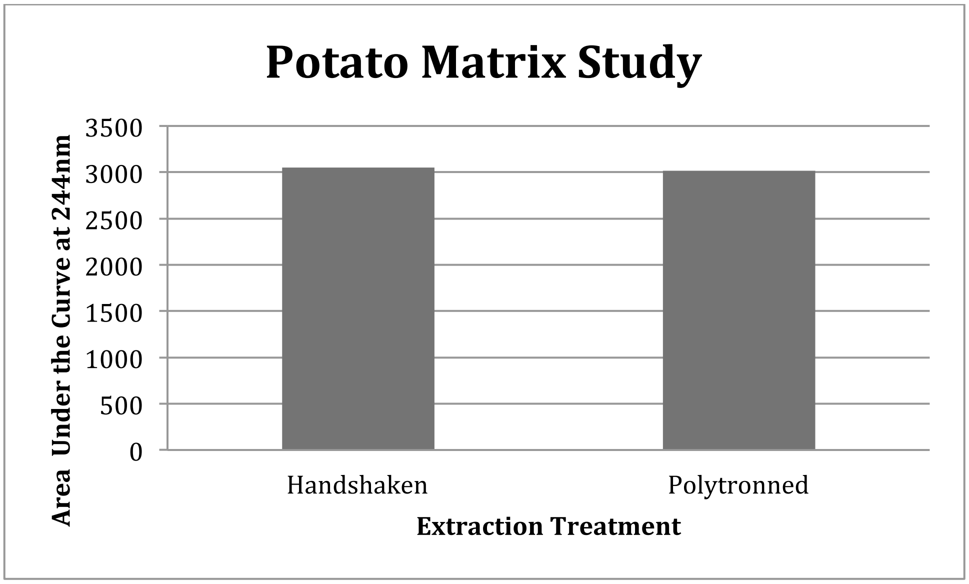
Samples were comprised of a composite of a representative sample from three different potatoes and were washed to remove residual sediment left from harvest. The sample was then homogenized with extraction solution at a ratio of 1:1 (W:V) in a Waring Commercial Blender using a 40 oz. glass insert.

Once homogenized, 1.00g (± 0.01g) of the composite sample was weighed into a 50mL centrifuge tube and diluted with 10mL of dilution solution. Samples were then hand-shaken for 30 seconds to combine and centrifuged for 10 minutes at 7,745 x g. The sample was still slightly cloudy, so 1.5mL of supernatant was then removed and placed into a 2mL centrifuge tube and centrifuged again for 10 minutes at 30,156 x g. This additional step yielded a clear sample that was both easier to work with and less damaging to run through the LC column.

Potato Matrix

Results, depicted in figure 3.5, show that both hand-shaking and polytronning were sufficient methods to extract the vitamin C before removing the starch matrix by centrifuging. This method uses hand-shaking because it was found to be equally efficient for extraction and more widely available to researchers.

Figure 3.5. Reduction of DHAA to AA in the Potato Matrix



Average absorbance values listed for duplicate samples are listed

Ascorbic Acid Fortification

Recovery rates for the ascorbic acid fortification trials were calculated to be 100%. No AA is lost in the potato matrix.

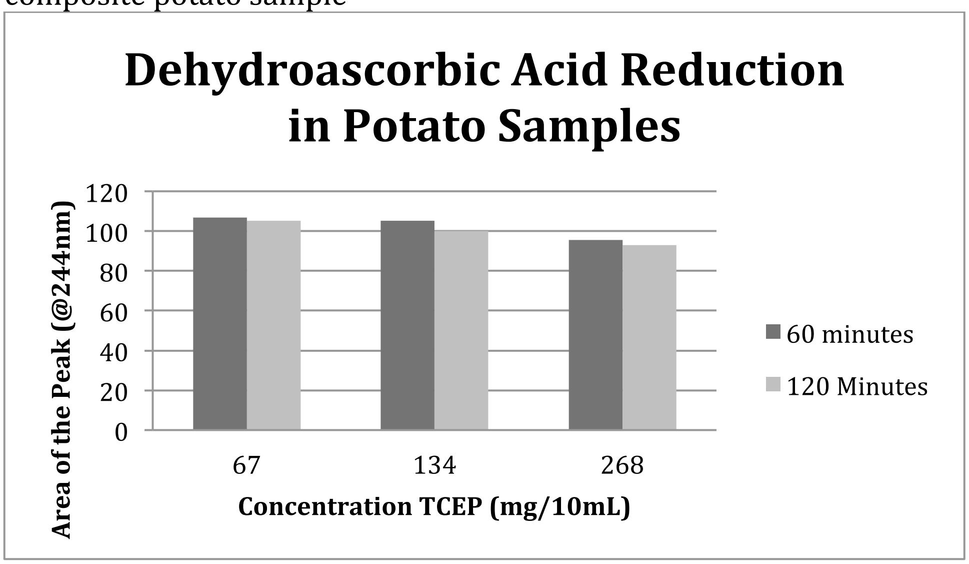
Dehydroascorbic Acid Reduction

A 60 minute reaction time and 67mg dissolved in 10mL of 3% MPA was found to be sufficient for a full reaction of DHAA in both potato samples and concentrated DHAA samples. Concentrated DHAA was used because it was nearly 100x more DHAA than was found in potato samples to ensure that complete conversion of DHAA to AA would occur even in potato varieties with significantly higher DHAA than the composite used for method development. Figures 3.6 and 3.7 show the results for potato samples and

DHAA concentrate. The reduction method showed a slight decrease in total vitamin C after reduction when a more concentrated TCEP solution was used. This could be a due to a slight interference from a peak that eluted 0.2 minutes prior to the AA peak in both the potato samples and the DHAA samples. This peak is an unknown interference; it does not occur in the blank samples, in the TCEP only samples, or in the AA STDs.

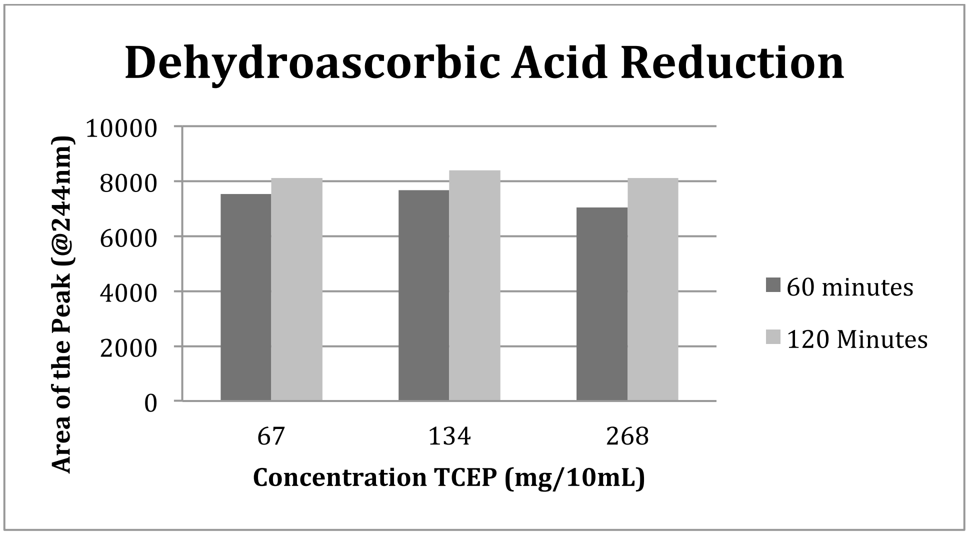
Results for DHAA reduction in the potato samples are presented in Figure 3.6, results for DHAA reduction in the concentrated DHAA sample are presented in Figure 3.7.

Figure 3.6. Amount of TCEP needed and time needed to reduce DHAA to AA in a composite potato sample



Average absorbance values listed for duplicate samples are listed

Figure 3.7. Amount of TCEP needed and time needed to reduce DHAA to AA in a concentrated DHAA standard



For this study the DHAA concentration used was (25mg/25mL)

TCEP was used instead of dithiothreitol (DTT) for a number of reasons. TCEP has a longer self-life in solution, it can be used in a wider range of pH environments, and it is odorless; DTT has a strong unpleasant odor. TCEP also has a shorter reaction time than DTT and does not require refrigeration for the reaction (Odriozola et al., 2007).

Dehydroascorbic Acid Reduction in the Potato Matrix

Recovery rates for the dehydroascorbic acid reduction trials were calculated to be 100%. No DHAA was lost in the potato matrix.

Confirmation of Method Applicability for Cooked and Pigmented Samples

The samples were homogenized with extraction solution at a ratio of 1:2 (W:V). A ratio of 1:1 (W:V) was attempted but it was found that the sample was too thick to completely remove the starch via centrifuging and resulted in a cloudy sample.

This method was effective for both cooked and raw samples. The results are summarized in Table 3.2.

Table 3.2. Amount of Total Vitamin C found in Cooked vs. Raw Potatoes.

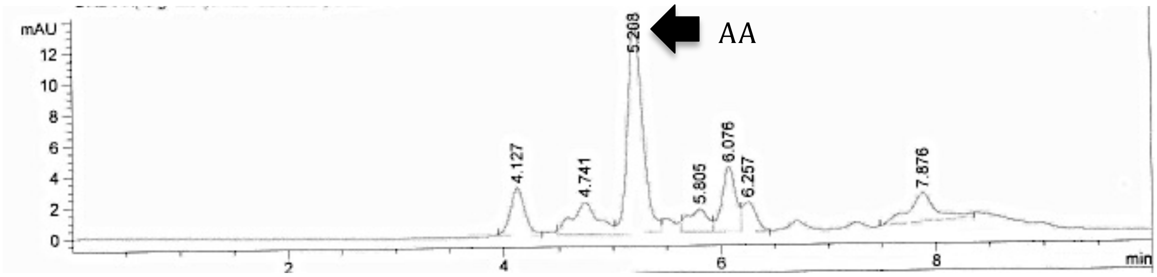
Total Vitamin C Content **Cooked vs. Raw (mg/100g)**				
	Russet Burbank	**Superior**	**Dark Red**	**Adirondack Blue**
Raw	6.19	9.32	6.92	7.97
Cooked	4.47	6.08	9.65	9.57

Average nutrient values listed for duplicate samples are listed

Only total vitamin C was listed in table 3.2 as no significant levels of DHAA were found in any of the samples. The results of this study show that cooking does not inherently increase or decrease vitamin C in potatoes, however there is a trend of increasing vitamin C content in darker pigmented potatoes and decreasing vitamin C content in lighter pigmented potatoes with cooking. More research would need to be conducted to confirm this trend.

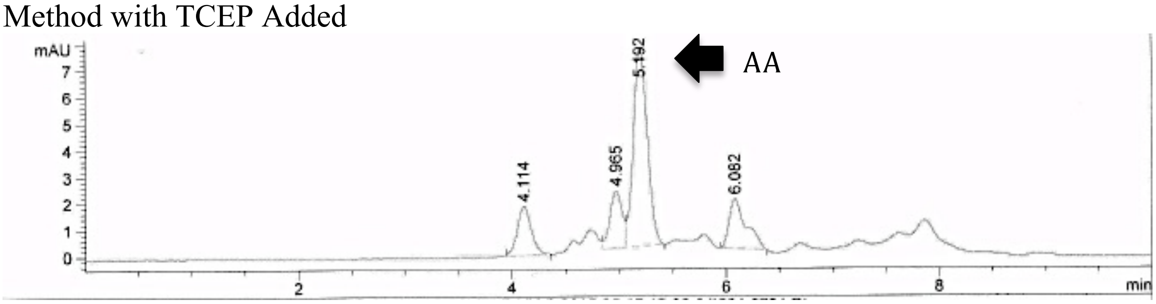
Figures 3.8, 3.9, 3.10 and 3.11 show chromatograms of a cooked white potato sample, a cooked white potato sample with TCEP added, a pigmented potato sample, and a pigmented potato sample with TCEP added.

Figure 3.8. Chromatogram of a Cooked White Potato Sample using the Potato Method



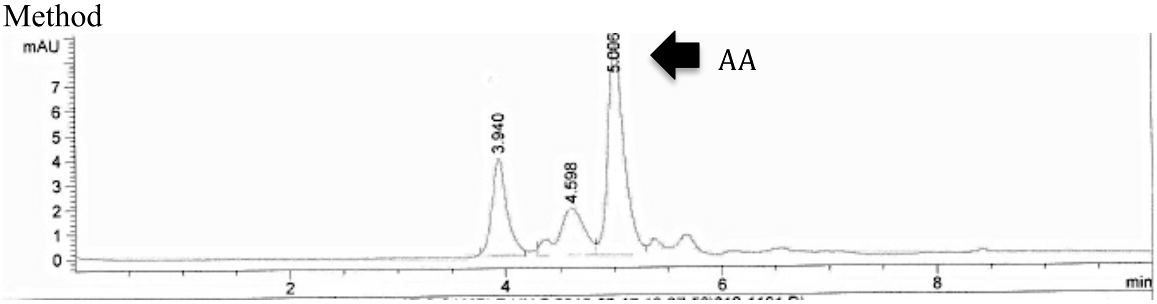
Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Figure 3.9. Chromatogram of a Cooked White Potato Sample using the Potato Method with TCEP Added



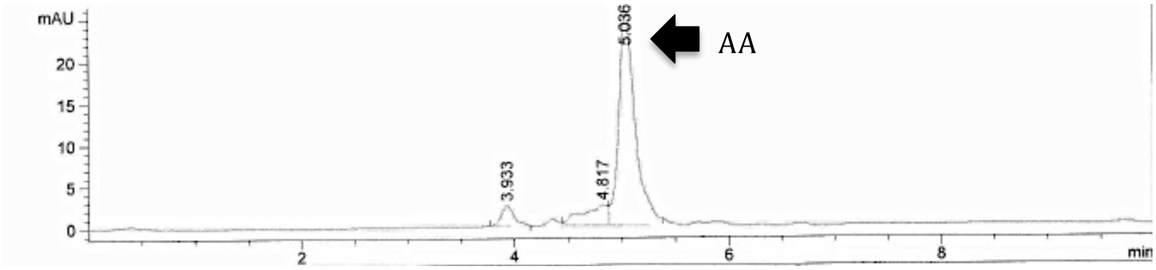
Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Figure 3.10. Chromatogram of a Pigmented Potato Sample using the Potato Method



Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

Figure 3.11. Chromatogram of a Pigmented Potato Sample using the Potato Method with TCEP Added



Sample was run at a flow-rate of 0.7mL/min, with 3% MPA and 3% acetonitrile as the mobile phase, ThermoScientific Hypersil Gold column, 10µL injection, at 244nm wavelength

CHAPTER FOUR

INTER-VARIATION OF VITAMIN C CONTENT IN POTATOES FROM THE SAME PURCHASE PACKAGE

Introduction

Vitamin C is adequately consumed in the average American diet; however, there are some concerns about problems estimating vitamin C intakes. One of the concerns that Sinha et al. (1993) noted was that the method that NHANES uses to measure nutrient consumption is through consumption surveys. Consumption surveys are known to be prone to issues associated with reporting errors (Sinha et al., 1993). Another concern, which is discussed in the applied potato method research study of this thesis, is that nutrient intakes are estimated from amounts listed in the USDA's National Nutrient Database for Standard Reference (NNDSR) (USDA, 2014). While this database does contain results that were most congruent with results from published data, it is concerning that individual variances of nutrient content in foods are not taken into consideration. Due to large variances in nutrient values of produce samples, the USDA should consider utilizing a nutrient range instead of a single listing for foods. The USDA's NNDSR does currently have a section available for listing nutrient ranges, however, with further inspection of the database it appears that this column is not frequently utilized (USDA, 2011).

In researching reported nutrient composition it was noted that common nutrient tracking software, such as USDA's SuperTracker, MyFitnessPal, and SparkPeople, listed the vitamin C content in raw white potatoes as more than double that of what was listed in the USDA's NNSDR (USDA, 2011; SparkPeople, Inc., Unlisted; MyFitnessPal, Inc., Unlisted; USDA, Unlisted). Table 4.1 summarizes the current published vitamin C content in raw, white potatoes. In consideration of these differences, health professionals should use caution when recommending a patient use diet tracking software. This could potentially pose a significant health risk to those who rely on such software.

Table 4.1. Current Published Vitamin C Content in Raw, White Potatoes

Nutrient Database	Reported Vitamin C content (mg/1 cup diced)
USDA's NNSDR	13.6
USDA's SuperTracker	30
MyFitnessPal	29.4
SparkPeople	29.5

All reported values were based off of raw, white potato, skin and flesh data. Reports that were listed %RDA of a 2,000 calorie diet were converted to mg. All nutrient values were obtained in June of 2015.

Materials and Methods

Chemicals

The same chemicals and preparations were used in this study as were used in the method development study with the exception of the meta-phosphoric acid (MPA), which was purchased from ACROS Organics, Geel, Belgium.

Equipment

The same HPLC system, scales, and centrifuge were used for this study as were used for the method development study. However, all samples weighing more than 100g were homogenized using the Waring, Odessa, FL, Commercial Blender with a 40oz glass insert. The Magic Bullet, Capital Brands, Los Angeles, CA, was used to homogenize all samples that weighed less than 100g.

Analysis Parameters

The detector was set to UV-Vis, measuring 244 and 254nm. The injection was set to 10µL with a flow rate of 0.7mL/min and a run time of 10 minutes. The mobile phase was 3% MPA and 3% Acetonitrile dissolved in HPLC grade water. The column used was a ThermoScientific Hypersil Gold C18 column 5µ 100A (250x4.6mm) operated at ambient temperature.

Samples

Potato samples were purchased from a local grocery store, Hannaford Brothers Company, to mimic what would typically be available to consumers. Eight different varieties were chosen. With the exception of the blue-skinned cultivar, all tubers were conventionally grown. Appendix C lists the details of each potato variety used in this study.

To prevent bias during analysis, replicate samples were blinded with three-digit codes. Variety, run-order, and individual sample runs were randomized.

Sample Preparation

Varieties were tested by analyzing each individual tuber from the purchase package separately, each tuber was numbered, weighed, sampled, and analyzed separately. All potatoes were homogenized with 0.2M phosphate buffer at a ratio of 1:1 (W:V). Subsamples of the homogenate were weighed (1.00 (± 0.01g)) into 50 mL centrifuge tubes and diluted with 10mL of 3% MPA. These were hand-shaken for 30 seconds and then centrifuged for 10 minutes at 7,745 x g. 1.5mL of supernatant was then transferred to a 2mL centrifuge vial and again centrifuged for 10 minutes at 30,156 x g. 0.5mL of supernatant was transferred to a 2mL HPLC vial and immediately injected into the LC system for AA analysis. Concurrently, another 0.5mL of supernatant was transferred to a 2mL HPLC vial and 0.5 mL of 67mg/10mL TCEP was added and allowed to react for 60 minutes before analysis for DHAA content.

Calculations and Statistical Analysis

All sample runs included a standard curve with an R^2 value of 0.994 or higher. Vitamin C contents were calculated based on a single point AA standard of 40ng. Calculations were performed as described in the method development section. Nutrient contents were calculated on a fresh weight basis, as the average consumer would not consider moisture content when choosing a potato to consume.

Samples were unblinded and replicate results were reviewed to ensure similar values for the same sample. Some variability was noted between duplicates; this could be attributed to sampling error. Part of the variability could be due to the employment of three different student research technicians to prepare the samples. Due to the speed with which vitamin C metabolizes, student help was necessary to complete the work.

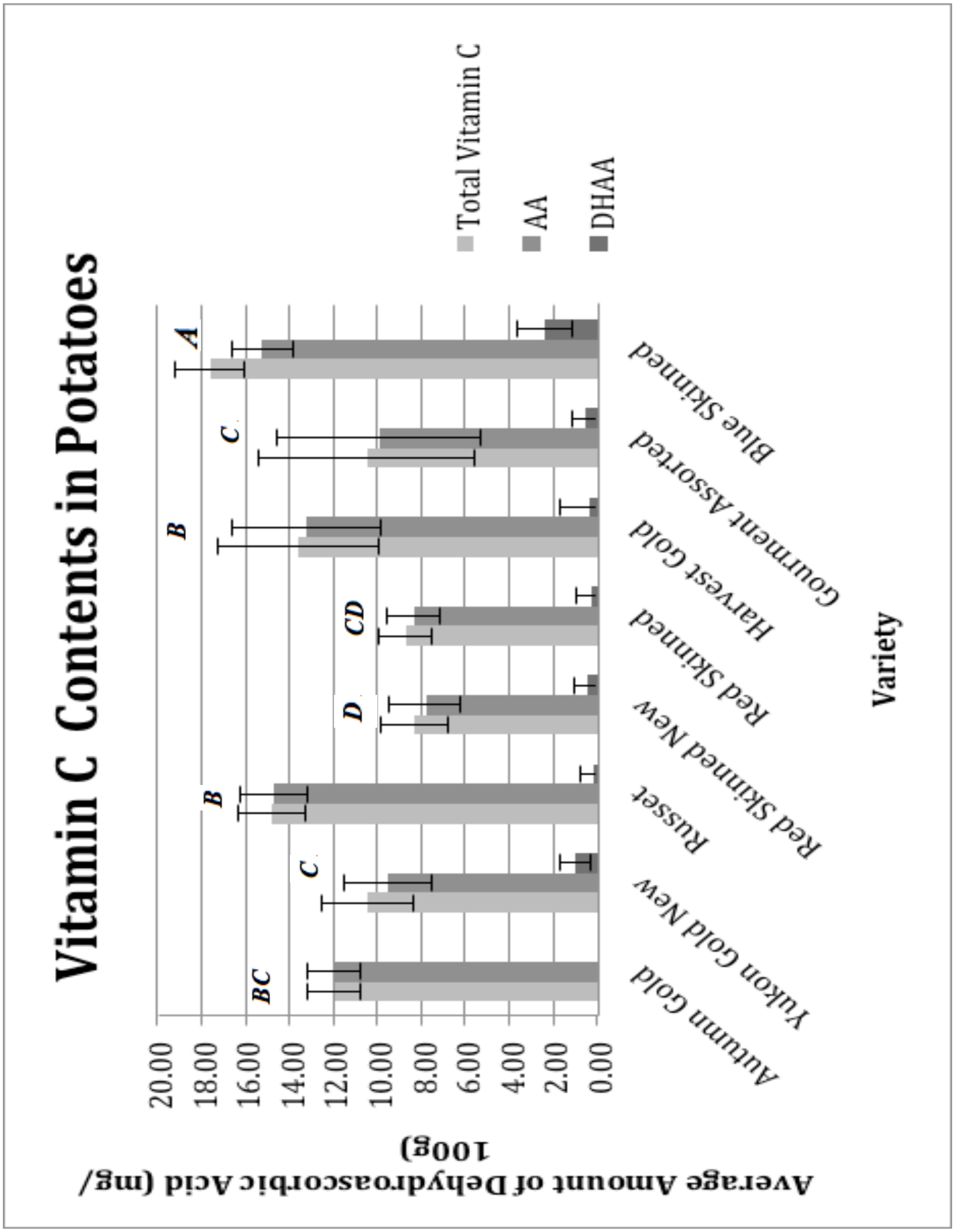
Nutrient contents were analyzed based on both differences between potato varieties and individual bag differences. SYSTAT 12 (Cranes Software International Ltd.) was used for statistical analysis. Comparisons were made using ANOVA with a confidence interval of 95% (0.05 significance level) within varieties and between varieties. For between variety calculations, ANOVA was followed by Tukey's HSD for pairwise comparisons. Charts are based on average nutrient contents found in samples and variation between samples as calculated by SYSTAT.

Results and Discussion

Contents of AA, DHAA, and total vitamin C were analyzed by variety and between varieties. There were significant differences in AA, DHAA, and total vitamin C contents between varieties. Figure 4.1 shows the average AA, DHAA, and total vitamin C content of each potato variety. The Blue Skinned cultivar had significantly more AA than all other varieties except Russet, and significantly more DHAA and total vitamin C than all other varieties.

All potatoes had significant differences between AA and total vitamin C contents, and between potatoes in the same purchase package. There were significant differences between DHAA content in all varieties except Autumn Gold, Harvest Gold, Red Skinned and Russet. These varieties did not differ significantly in DHAA content because they had a low or non-detectable DHAA content. Inter-variation in AA, DHAA, and total vitamin C is listed by potato variety in Table 4.2 and Figure 4.2. Gourmet assorted fingerling potatoes had the largest variance in AA and total vitamin C, with a range of 17.21 and 23.79, respectively, (contents between 3.79 – 21.00g/100g for AA and 3.90 – 23.38 for total vitamin C); Harvest Gold had the second largest variance in AA and total vitamin C with a range of 17.71 and 13.258, respectively, (contents between 7.89 – 25.60mg/100g for AA and 7.89 – 27.04 mg/100g for total vitamin C). Harvest Gold had the largest variance in DHAA with a range of 8.04 (contents between 0.00 – 8.04 mg/100g); Blue Skinned had the second largest variance in DHAA with a range of 1.467 (contents between 0.29 – 5.30 mg/100g).

Figure 4.1. Average Vitamin C Contents of Different Potato Varieties



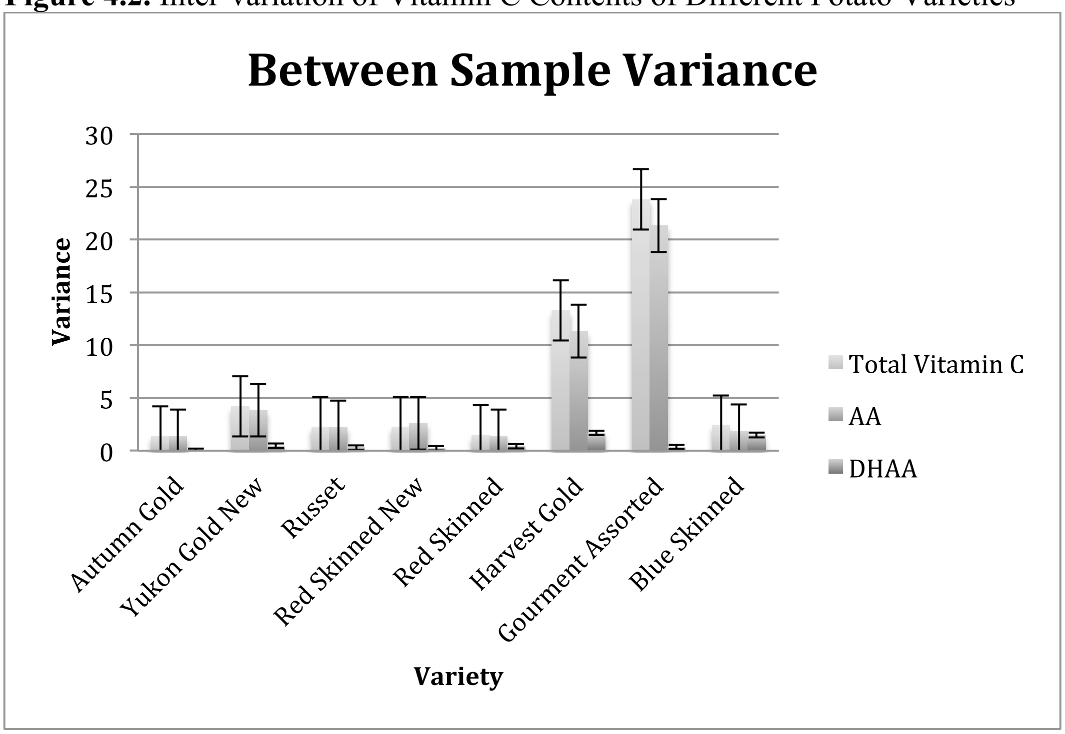
Average nutrient contents listed for each variety based on statistics run in SYSTAT. Error bars and significance indicators were created from standard deviations and Tukey's HSD calculations created using statistics run in SYSTAT. Average nutrient values listed for duplicate samples are listed. Significance indicators are listed for total vitamin C only.

Table 4.2. Vitamin C Contents of Different Potato Varieties

Potato Variety	AA Content			DHAA Content			Total Vitamin C Content		
	Min	Max	Variance	Min	Max	Variance	Min	Max	Variance
Autumn Gold	10.6	14.2	1.4	0.0	0.0	0.0	10.6	14.2	1.4
Yukon Gold New	5.4	13.9	3.8	0.0	3.0	0.4	6.7	14.9	4.2
Russet	12.2	18.4	2.2	0.0	2.7	0.3	12.2	18.1	2.3
Red Skinned New	5.6	12.4	2.6	0.0	1.8	0.2	5.8	10.9	1.4
Red Skinned	6.7	10.8	1.4	0.0	3.0	0.4	6.8	10.9	1.4
Harvest Gold	7.9	25.6	11.3	0.0	8.0	1.7	7.9	27.0	13.3
Gourmet Assorted	3.8	21.0	21.3	0.0	2.4	0.3	3.9	23.4	23.8
Blue Skinned	11.5	18.7	1.8	0.3	5.3	1.5	15.4	21.5	2.4

Nutrient contents are listed in mg/100g units

Figure 4.2. Inter-variation of Vitamin C Contents of Different Potato Varieties



Nutrient variations listed for each variety based on statistics run in SYSTAT. Average nutrient values listed for duplicate samples are listed. Error bars are based on standard error.

Conclusions

The findings for difference in vitamin C contents were congruent with previously published results (Shakya et al., 2006; Nassar et al., 2014; Leo et al., 2008). However, the Blue Skinned variety had significantly higher vitamin C contents as compared to all other varieties, which could be due to a number of different factors. One factor that separates this variety from all others used in this study was that this variety is organic. In a review of current findings Washington (2001) found that on average, organically-grown produce had significantly higher vitamin C levels than conventionally grown produce. However, this difference in vitamin C content could also be due to a number of other factors, such as harvest date, storage method, transportation time, etc.

The findings for the differences in vitamin C contents between potatoes from the same purchase package were larger than expected. The data show the importance of consumer understanding that nutrient values vary significantly for a number of reasons. One common reason that nutrient values vary in potatoes is storage conditions. The variables discussed by Külen et al. (2013) were storage duration and temperature. In their study, Külen et al., found that even in potatoes stored between 3-5°C there was a significant decline in vitamin C content from harvest through seven months of storage. However, the average consumer stores potatoes at room temperature, which is significantly higher than 5°C, leading to additional degradation. However, this would likely not have influenced the data, as potatoes from the same purchase package should have been stored identically.

In order to bridge the gap between consumer expectations and scientific understanding, it would be beneficial for the USDA to utilize the minimum and maximum values for nutrients in the National Nutrient Database for Standard Reference instead of listing a single average nutrient content. A concern with nutrient tracking is that unless measured in the lab, nutrient values for foods are an estimation. This can lead to problems when consumers "diet track" and become overly concerned with getting exact intakes of certain nutrients. By listing the range of nutrients provided by a food, consumers may gain a better understanding of the variation in nutrient value found in produce.

In order to truly achieve improved consumer and professional awareness of variation in nutrient values found in produce it is necessary for additional research to be done on other types of produce.

CHAPTER FIVE

METHOD DEVELOPMENT FOR VITAMIN C QUANTIFICATION IN ELDERBERRIES

Introduction

Like many fruits and vegetables, elderberries are considered a complex matrix due to components in the berries that have chemical similarities. Ascorbic acid (AA) and some flavonoids can be difficult to separate by liquid chromatography (LC). In order to preserve AA, a pH below 4 is needed (Golubitskii et al., 2007), however, at a pH below 4 anthocyanins are chemically stable as well, making them difficult to remove them from the sample matrix without degrading the AA as well. Current published methods for vitamin C quantification in elderberries do not include high performance liquid chromatography (HPLC) (González et al., 2012) and quantify only AA or total vitamin C (Sadilova et al., 2009), not DHAA alone. It is likely that common preservation methods of elderberries, such as freezing and freeze-drying, will result in substantial amounts of total vitamin C dehydroascorbic acid (DHAA) form. The distinction between AA and DHAA is important because there may be a significant difference in how effectively the human body can utilize the compound as compared to AA (Ogiri et al., 2002). In order to create a method to accurately measure AA in these samples a method that can adequately separate the flavonoid peaks from the AA peaks needs to be developed.

Materials and Methods

Chemicals

A variety of chemicals were used in this study. The meta-phosphoric acid (MPA) was purchased from ACROS Organics, St. Louis, MO, certified ACS grade. The acetonitrile (EM Science, Gibbstown, NJ), tetrahydrofuran (THF) (ACROS Organics, Gell, Belgium), methanol and trifluoric acid (TFA) (Fisher Scientific, Fair Lawn, NJ) were all 99% pure. The potassium dihydrogen phosphate (99.9% pure) and phosphoric acid (85% pure) were purchased from Fisher Scientific, Fair Lawn, NJ. The tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Thermo Scientific, Rockford, IL. The mobile phase, 0.3% TFA dissolved in HPLC grade water, was prepared weekly. The extraction solution, 0.2M phosphate buffer, was prepared once a week as well. The extraction solution was prepared by dissolving 54g of potassium phosphate and 55mL of phosphoric acid into 2 L of HPLC grade water. The dilution solution, 2% MPA dissolved in HPLC grade water, was prepared every three days. The TCEP solution was prepared every three days by dissolving 67mg into 10mL of 2% MPA.

Standards for AA and DHAA were purchased from Baker, Phillipsburg, NJ, and Sigma Aldrich, St. Louis, MO, respectively. Standards and standard curves were prepared daily in 2% MPA. Stock standards were made by dissolving 25mg of the standard in 25mL of 2% MPA. The AA standard curve was then made by diluting 0.05mL, 0.1mL, 0.2mL, 0.4mL, and 0.8mL of AA stock solution in 25mL of 2% MPA.

The SepPaks used in the filtration study were purchased from Waters Corporation (Milford, MA), they were silica packed c18 Sep-Paks were prepped with methanol, HPLC-grade water, and 2% MPA.

Equipment

An Agilent Technologies (Santa Clara, CA) HPLC system modified with Hewlett-Packard (Palo Alto, CA) components was used for this study. The degasser (110 series), thermostatted column compartment (1200 series), and diode array (1200 series) were from Agilent Technologies. The pump (1100 series ISO pump, upgraded to a quat pump) and the autosampler (1100 series) were from Hewlett-Packard. The computer used with this system was a Dell Optiflexx 755 formatted with Windows XP Professional version 5.1.2600. Measurements were done using ChemStation for LC by 3D Systems, version 8.0401(481) (Agilent Technologies).

Two different homogenization tools were used for the trial portion of this study. A Magic Bullet, by Capital Brands, Los Angeles, CA, was used to blend whole freeze-dried elderberry samples into a powder. A Kinematica, Luzern, Switzerland, polytron equipped with a Superior Electronic, Bristol, CT, POWERSTAT autotransformer was used to homogenize all samples. Samples were centrifuged with an Eppendorf (Hamburg, Germany) centrifuge, model 5430.

Samples were freeze-dried using a VirTris Ultra 35EL by SP Scientific, Stone Ridge, NY.

HPLC System Trials

The detector was set to wavelengths 244 and 254nm as they are the wavelengths where AA is most highly absorbed (Bushway et al., 1988). Three columns and seven mobile phases were tested for this method. The columns tested were Thermoscientific Hypersil Gold, Phenomenex Kinetex EVO, and Sielc: Primesep 100. The first two columns were 250 x 4.6mm, with 5µ, 100A packing material. The Sielc column had specifications of 150 x 4.6mm, with 5µ, 100A packing material. These columns were chosen because they are rated for use with a wider pH range than other columns. The Sielc: Primesep 100 column was chosen because it has a unique chemistry which included ion-exchange, ion-exclusion, and pi-pi interactions. The seven mobile phases tested were: 3% MPA, 2% MPA, 0.2% TFA, 0.3% TFA, 0.3% TFA and 0.3% Methanol, 0.3% TFA and 0.3% THF, and 0.3% TFA and 0.3% acetonitrile.

Final Analysis Method

The detector was set to UV-Vis, measuring 244 and 254nm. The injection was set to 10µL with a flow rate of 0.5mL/min, with a run time of 30 minutes. The mobile phase was 0.3% TFA dissolved in HPLC grade water. The column used was a Phenomenex Kinetex EVO C18 column 5µ 100A (250x4.6mm) operated at ambient temperature.

Samples

Wild elderberry samples grown in four counties in Maine; Penobscot, Piscataquis, Somerset, and Aroostook. Samples were collected by Dr. Rodney Bushway. Two types of preservations methods were tested in this study, freezing and freeze-drying. For Freezing, composite samples were used from 14 different growing sites, for freeze-drying, composite samples were used from 17 different growing sites. The specific locations were Bradford (Penobscot county) and Barnard (Piscataquis county). Both freeze-dried and frozen samples were analyzed as whole berries.

Freeze-Dried. Samples for freeze-drying were collected in the weeks between August 18th and September 14th 2013. Samples were frozen and stored in a -20°C freezer until they were freeze-dried with a SP Scientific, VirTris Ultra 35EL using the method as described in table 5.1. Freeze-drying was accomplished in nine batches between December and May of 2013. After freeze-drying stems and leaves were removed and the berries were stored whole at -80°C. A composite sample of all 17 growing locations was created and stored at -80°C until used for these studies. Bradford and Barnard growing site samples were stored separately at -80°C until used for these studies.

Table 5.1. Freeze Drying Method for Elderberries

Temperature	Duration (hours)
-30°C	24
-20°C	24
-10°C	24
0°C	24
10°C	24
20°C	24
25°C	24

Samples were processed in 7 day cycles and kept under a vacuum of 100mT

Frozen. Samples for fresh-frozen analysis were collected during a span of two weeks between August 31st 2014 and September 14th 2014. A composite sample of all 14 growing locations was created and stored at -20°C until used for these studies. Bradford and Barnard growing site samples were stored separately at -20°C analysis.

Method Testing

Five different studies were done in order to test the validity of the sample preparation method.

Ascorbic Acid Standard Curve. A standard curve was developed by preparing dilutions of AA at the following concentrations, 5ng, 10ng, 20ng, 40ng, and 80ng.

Berry Matrix. Freeze-dried samples were analyzed as whole, dried berries. The two extraction methods tested were hand blending with a mortar and pestle, and mechanically grinding, with a Magic Bullet. For this study ~5g samples were either blended for 30 seconds using a Magic Bullet, a Scientific Industrial Division RSI 64 at 1500 rpm, or they were hand blended, with a mortar and pestle, until they reached the desired consistency. Composite berry samples were used from the same location as those for the frozen berry samples for this study.

Ascorbic Acid Fortification. Samples were fortified with AA to determine AA recovery rates within the berry matrix. This was done by determining the average amount of AA in the sample and then calculating and adding enough AA to increase the AA peak by two fold and five fold. These concentrations were different for frozen and freeze-dried elderberries. The concentrated AA stock solution was added to the elderberry homogenate prior to diluting and polytronning. The 1.00 (± 0.01g) subsamples of the homogenate were spiked with 0.05mL (x2) or 0.25mL (x5) for frozen berries and 0.15mL (x2) and 0.80mL (x5) for freeze-dried berries. These samples were run with 20ng and 100ng (frozen) and 60ng and 320ng (freeze-dried) AA standards, as well as an unspiked "control" frozen and freeze-dried sample.

Dehydroascorbic Acid Reduction. This study was performed in the same way as the AA spiking study; DHAA was added to the elderberry matrix after homogenization and prior to dilution in order to ensure that the DHAA permeated the berry matrix. Samples were extracted, diluted, and run normally.

Filtration. Filtration of the samples was tested as a means to reduce AA interference. SepPaks were prepared with HPLC grade water, Methanol, and 2% MPA. Prepared samples were run through the prepared SepPaks and then assayed normally using LC.

Final Sample Preparation Method

Freeze-Dried. Whole freeze-dried berry samples were blended for 30 seconds using a Magic Bullet blender or hand-blended using a mortar and pestle until powdered. Samples were then extracted, a 1.00g (± 0.01g) sample was weighed into a 50mL centrifuge tube with 20mL of 0.2M phosphate buffer, it was hand-shaken for 30 seconds, and let sit for 5 minutes. The sample was then polytronned for 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50.

Once homogenized, two 1.00g (± 0.01g) subsamples were weighed into 50mL centrifuge tubes and diluted with 10mL of 2% MPA. Samples are then centrifuged for 10 minutes at 7,745 x g. 0.5mL of prepared sample was placed into a 2mL HPLC vial and run immediately for AA content using LC. Concurrently, another 0.5mL of sample was placed in another 2mL HPLC vial. 0.5mL of TCEP was added. The vial was then placed in the HPLC autosampler tray for an hour, to reduce, prior to running for total vitamin C content using LC.

Frozen. Frozen whole berry samples were homogenized with 0.2M phosphate buffer extraction solution at a ratio of 1:1 (W:V). Samples were weighed and stems and leaves were quickly removed (less than 30 seconds). Samples were then homogenized for 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50.

Once homogenized, a 1.00g (± 0.01g) subsample was weighed into a 50mL centrifuge tube and diluted with 10mL of 2% MPA. Samples were then hand-shaken for 30 seconds and polytronned for 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50. Samples were then centrifuged for 10 minutes at 7,745 x g. 0.5mL of the prepared sample was placed into a 2mL HPLC vial and assayed immediately for AA content using LC. Concurrently, another 0.5mL of sample is removed and placed into another 2mL HPLC vial. 0.5mL of

TCEP is added and the vial was placed in the HPLC autosampler tray and allowed to react for an hour prior to running for total vitamin C content by LC.

Calculations

All calculations were done on both a fresh and dry weight basis.

<u>Standard Concentration.</u> 10µL injection

25mg dissolved into 25mL, of which 0.1mL was then diluted into 25mL

Step 1.A: 0.1mL * (1000µg / 1mg) * (1000ng / 1µg) = 100000ng

Step 1.B: 100000ng / 25mL * (1000mL / 1µL) = 4.0ng / µL

*Step 1.*C: 10µL injected = 4.0ng / µL * 10µL= 40ng injected

<u>Ascorbic Acid Content.</u> 10µL injection

Step 2.A: % Dry weight (DW) = % moisture -1

Step 2.B: 1.00G (±0.01) sample * % DW = Y

Step 2.C: (Sample AUC / Standard AUC) * 40ng (Standard Concentration) = X

Step 2.D: X = ng AA in the sample

Step 2.E: Dilution + Y = V_T

The dilution in step 2.E for frozen berries was 10mL and 20mL for freeze-dried berries

Step 2.F: V_T * (1000µL / 1mL) * (X / 10µL) = Z

Step 2.G: ((Homogenized Elderberry Weight * % DW) + Extraction Solution Volume (10mL)) * Z * (1mg / 1000µg) * (1µg / 1000ng) = W

Step 2.H: W / (Homogenized Elderberry Weight * % DW) = mg / g AA * 100 = mg / 100g AA

Dehydroascorbic Acid Content. 10µL injection

Step 3.A: Follow calculation steps for AA

Step 3.B: Multiply the final mg / 100g by 2 (to account for the 1:1 dilution with TCEP)

Step 3.C: Subtract this number from the number calculated in Step 2.G[4].

Step 3.D: This is the concentration of DHAA in mg/100g

Total Vitamin C Content. 10µL injection

Step 4.A: Add the result of 2.G and 3.D (AA + DHAA)

Step 4.B: This is the concentration of Total Vitamin C in mg/100g

Results and Discussion

Detector

As was done for the potato study, a UV detector was used and measured wavelengths 244 and 254nm. These wavelengths were used because they are the wavelength where AA is best absorbed, while absorbance of interfering peaks is

[4] *Note: For this study concentrations 0.5mg/100g and below were considered non-significant concentration of DHAA*

minimized. For the work described in this thesis 254nm was chosen for calculations, as there was additional interference at 244nm that made peak integration more difficult.

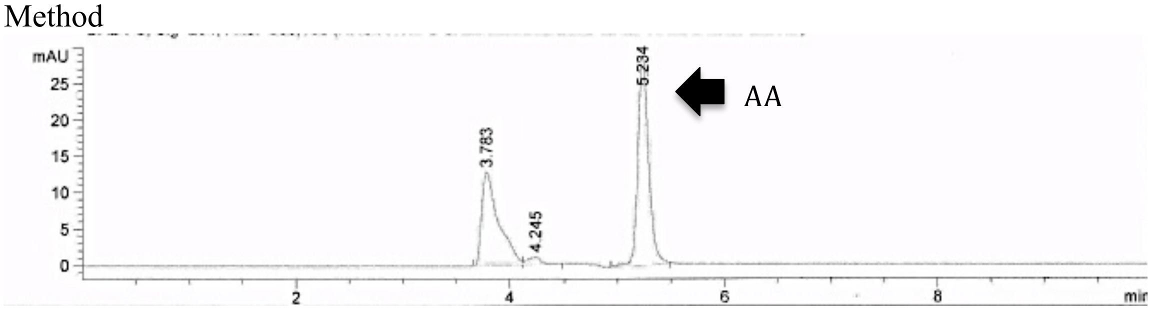
Column and Mobile Phase

Three columns and seven different mobile phases were tested for this method. The three columns tested were Thermoscientific Hypersil Gold, Phenomenex Kinetex EVO, and Sielc: Primesep. Both the Thermoscientific Hypersil Gold and the Phenomenex Kinetex EVO are mixed mode columns, C18 and ion-exchange, with the following specifications 5µ, 100A, 250x4.6mm. The Sielc: Primesep column is an ion-exchange column with the specifications 5µ, 100A, 150x4.6mm. These columns were chosen because they are rated for use with a wider pH range than other columns. This was necessary because of the low pH needed to preserve the AA in the sample. The Phenomenex Kinetex EVO column produced better peak resolution and separation then the Thermoscientific Hypersil Gold column, this could be because the Thermoscientific Hypersil Gold column had just been used for a large quantity of potato samples, which may have degraded the efficiency of the column. The resolution and peak separation on the Sielc: Primesep 100 column was inferior to the Phenomenex Kinetex EVO.

The seven mobile phases tested for this method can be placed into three categories; TFA, MPA, and organic modifiers. With all mobile phases, the sample dilution solution was changed to match the mobile phase being used, the extraction solution stayed the same. The 3% MPA results did not produce the separation needed for quantification. The MPA concentration was reduced to 2% but still did not produce workable results. 0.3% TFA resulted in peaks that showed the best peak separation of the four mobile phases tested to this point. Samples diluted with 2% MPA were run with 0.3% TFA as the mobile phase. This combination provided significantly better peak separation and specificity than previous methods. Three organic modifiers; methanol, acetonitrile, and THF, were then added to the 0.3% TFA, in equal concentration to the TFA, in an attempt to further improve peak resolution. All three modifiers tested resulted in reduced peak separation than that of 0.3% TFA alone.

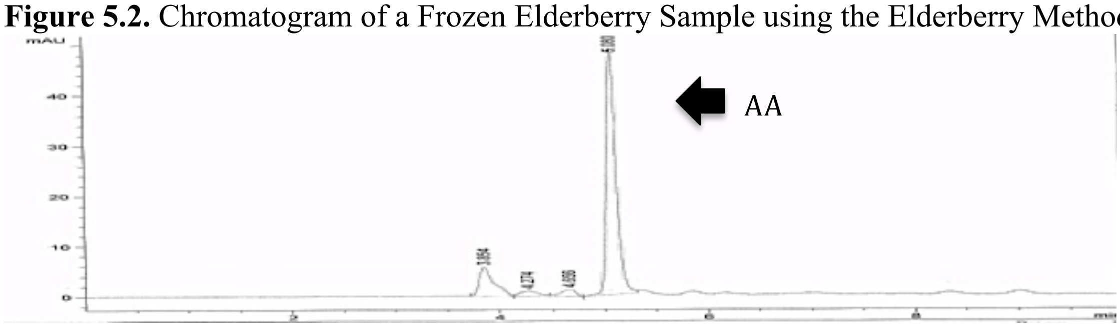
Figures 5.1, 5.2, 5.3, 5.4, and 5.5 show chromatograms generated using this method.

Figure 5.1. Chromatogram of the Ascorbic Acid Standard Using the Elderberry Method



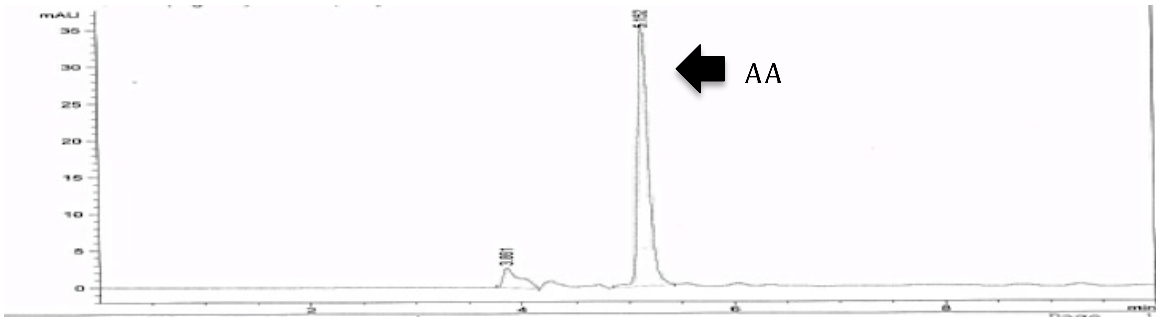
Sample was run at a flow-rate of 0.5mL/min, with 0.3% TFA as the mobile phase, Phenomenex EVO column, 10μL injection, at 254nm wavelength

Figure 5.2. Chromatogram of a Frozen Elderberry Sample using the Elderberry Method



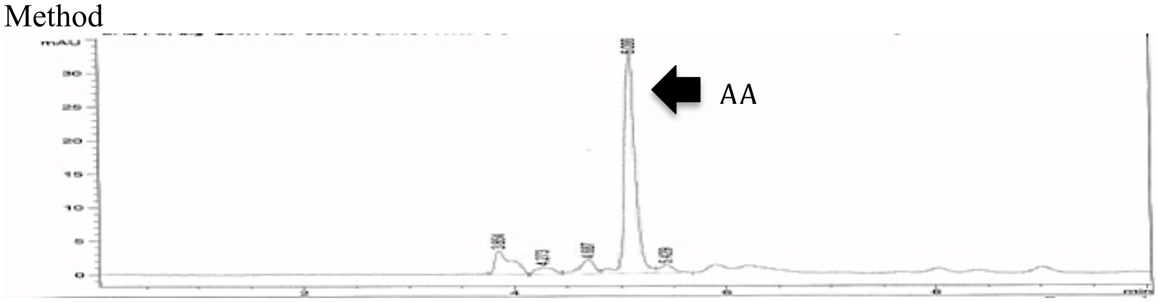
Sample was run at a flow-rate of 0.5mL/min, with 0.3% TFA as the mobile phase, Phenomenex EVO column, 10μL injection, at 254nm wavelength

Figure 5.3. Chromatogram of a Frozen Elderberry Sample using the Elderberry Method with TCEP Added



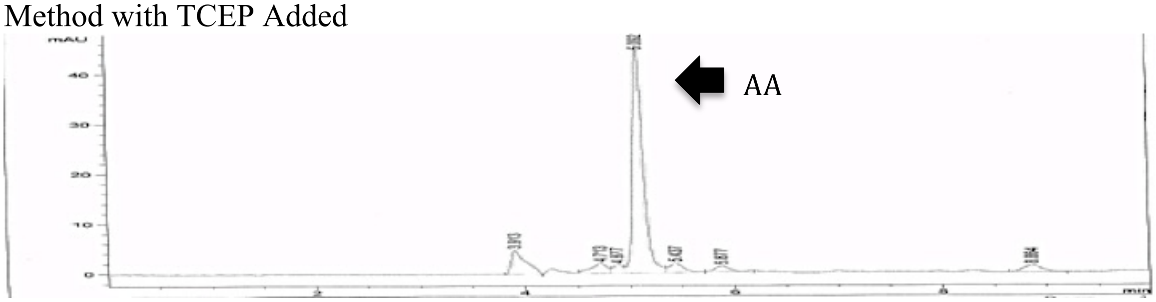
Sample was run at a flow-rate of 0.5mL/min, with 0.3% TFA as the mobile phase, Phenomenex EVO column, 10µL injection, at 254nm wavelength

Figure 5.4. Chromatogram of a Freeze-Dried Elderberry Sample using the Elderberry Method



Sample was run at a flow-rate of 0.5mL/min, with 0.3% TFA as the mobile phase, Phenomenex EVO column, 10µL injection, at 254nm wavelength

Figure 5.5. Chromatogram of a Freeze-Dried Elderberry Sample using the Elderberry Method with TCEP Added



Sample was run at a flow-rate of 0.5mL/min, with 0.3% TFA as the mobile phase, Phenomenex EVO column, 10µL injection, at 254nm wavelength

Method interferences with the AA standard, TCEP, and mobile phase were tested. Neither TCEP nor the mobile phase interfered with AA separation.

Flow Rate and Run Time

A flow rate of 0.7mL/min was used, however this flow rate resulted in poor resolution of the ascorbic acid peak. So the flow was decreased the rate to 0.5mL/min. As a result, some interference between samples run in succession was noted. To better understand where the interferences were coming from, freeze-dried and frozen samples were injected separately and each was allowed it to elute for 60 minutes. In both samples, late eluting peaks were noted at 25 minutes. To allow samples to fully clear the column before the next sample injection began; a run time of 30 minutes was chosen.[5]

Sample Preparation

Freeze-Dried. All freeze-dried samples from the same growing site were combined to make a composite sample of that growing location. Samples were powdered as needed by either blending with a mortar and pestle or processing in a Magic Bullet for 30 seconds. Samples were then extracted by weighing two 1.00g (± 0.01g) samples into a 50mL centrifuge tube with 20mL of extraction solution. They were hand-shaken for 30 seconds, and let sit for 5 minutes to allow for complete extraction. The samples were then polytronned for 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50.

[5] Chromatograms shown in Figures 5.2, 5.3, 5.4, and 5.5 show the first 10 minutes of the sample run only as AA elutes shortly before 5 minutes.

Once extracted and homogenized, two 1.00g (± 0.01g) subsamples were weighed into 50mL centrifuge tubes and diluted with 10mL of dilution solution. Samples were then centrifuged for 10 minutes at 7,745 x g. 0.5mL was removed and placed into a 2mL HPLC vial and assayed immediately for AA content. Concurrently, another 0.5mL of sample was placed into a 2mL HPLC vial. 0.5mL of TCEP was added and the vial was placed in the HPLC autosampler tray for an hour to allow the reaction to occur. Once the reaction was complete samples were assayed for total vitamin C content by LC.

Frozen. 5g (± 0.01g) samples were weighed into 50mL centrifuge tube sand homogenized 1:1 (W:V) using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer for 30 seconds.

Stems and leaves were removed to ensure accurate vitamin C quantification. During the course of the study it was noted that samples picked more carefully, and left on the countertop thawing for longer periods of time, had significantly reduced levels of vitamin C in the sample. Handpicking the berries for leaves and stems at room temperature is not recommended.

A 1.00g (± 0.01g) subsample was weighed into a 50mL centrifuge tube and 10mL of dilution solution was added. Samples were then hand-shaken for 30 seconds and polytronned for an additional 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50. Samples were then centrifuged for 10 minutes at 7,745 x g. 0.5mL was removed and placed into a 2mL HPLC vial and assayed immediately by LC for AA content. Concurrently, another 0.5mL of sample was removed and placed in another 2mL HPLC vial. 0.5mL of TCEP was added to the vial.

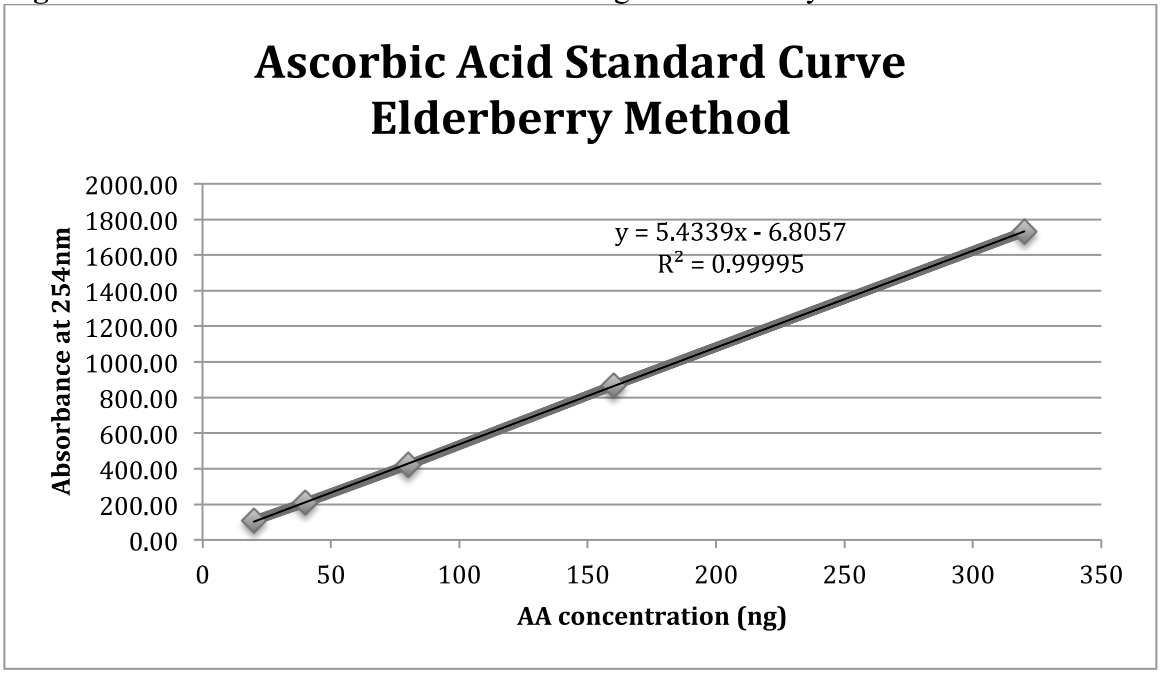
The vial was placed in the autosampler tray and allowed to react for an hour prior to being assayed by LC for total vitamin C content. The DHAA matrix study was not repeated for elderberries because DHAA content of elderberries was found to be negligible.

Preliminary studies showed that frozen elderberries contained a similar content of vitamin C to raw potatoes, so the method created for elderberries was adapted from the potato sample preparation method. One modification made to the method was that samples were polytronned to combine. This was done because homogenizing in a Magic Bullet did not fully break down the elderberry skins and seeds.

Ascorbic Acid Standard Curve

The standard curve showed a linear response with an R^2 value of 0.999. This is shown in Figure 5.6.

Figure 5.6. Ascorbic Acid Standard Curve using the Elderberry Method



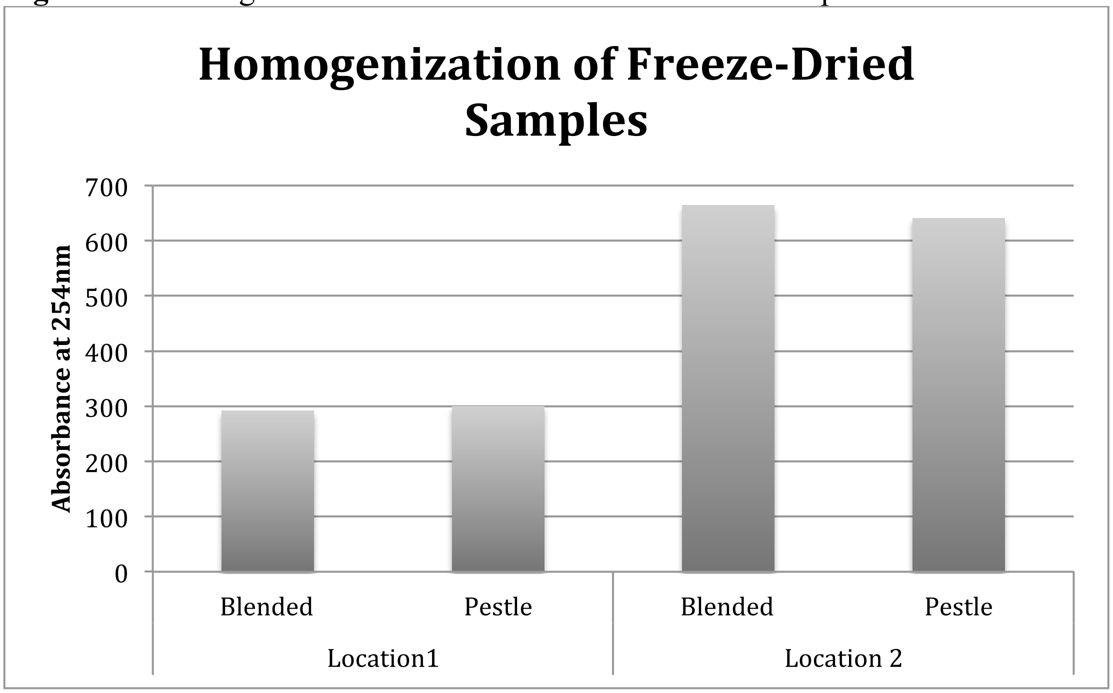
Standard curve using 20ng, 40ng, 80ng, 160ng, and 320ng AA concentrations

Berry Matrix

Freeze-dried samples were available as whole, dried berries. Two methods tried were compared; hand blending, with a mortar and pestle, and mechanically grinding, with a Magic Bullet. For this study ~5g samples were either blended for 30 seconds using a Magic Bullet, Scientific Industrial Division RSI 64 at 1500 rpm or they were hand blended into a powder. Composite berry samples were used from the same location as those used for the frozen berry samples for this study.

Results are presented in Figure 5.7.

Figure 5.7. Homogenization Trial Results of Freeze-Dried Samples



Average nutrient values listed for duplicate samples are listed.

Both methods resulted in similar vitamin C contents, but hand blending with the mortar and pestle was more labor-intensive and resulted in a product that was harder to work with. Grinding in the Magic Bullet was chosen over hand blending because of the shorter prep time.

Ascorbic Acid Fortification

Recovery rates were 100% for both frozen and freeze-dried samples. No AA was lost in the berry matrix.

Dehydroascorbic Acid Reduction

Mean recovery rates for frozen and freeze-dried berries were 100% and 85 %, respectively.

Filtration

After being filtered through the prepared SepPak samples showed interface in the sample area under the curve ratios (244nm/254nm) for AA but not DHAA measurements. UV absorbance ratios for standards were 1.36 as compared to ratios of AA measurements in samples, which ranged between 1.55-1.68. DHAA ratios in samples ranged between 1.33-1.36. This preparation method may have resulted in an oxidation reaction, which was reversed with the addition of TCEP to reduce the DHAA back to AA. Using the treated SepPak did not result in fewer interfering peaks.

While this method could have been tested further with other preparation methods, the cost of the SepPak could be a limiting factor and the additional sample preparation time could result in artificially decreased vitamin C content.

CHAPTER SIX

DIFFERENCES IN VITAMIN C CONTENT OF WILD ELDERBERRIES GROWN THROUGHOUT NORTHERN AND CENTRAL MAINE

Introduction

Previous research has been done to determine how nutrient values and chemical properties differ in different varieties of elderberries (Kaack et al., 2008; Thole et al., 2006; Mikulic et al., 2014; Lee & Finn, 2007). Both Lee & Finn (2007) and Thole et al. (2006) found that both *Sambucus nigra* and *Sambucus canadensis* had similar bioactive compounds. However, Lee & Finn determined that the *Sambucus canadensis* variety would be better suited for processing because of its red pigmentation, low pH, which resulted in more stable anthocyanins. Alternatively, Mikulic et al. (2014) and Kaack et al. (2008) found that different elderberry species and their hybrids had different levels of phytochemicals. This introduces the question if wild elderberries growing in different locations throughout Maine have different nutrient contents. The following research study focuses on the AA, DHAA, and total vitamin C content of elderberries grown throughout Northern and Central Maine. Additionally, samples that were freeze-dried and stored at -80°C were compared to those fresh-frozen and stored at -20°C.

Materials and Methods

Chemicals

The same chemicals and preparations were used in this study as were used in the method development study, with the exception of the MPA, which was purchased from Aldrich, St. Louis, MO, and was certified ACS grade.

Equipment

The same HPLC system, balances, and centrifuge were used for this study as was used for the method development study. However, instead of grinding with a Magic Bullet, whole freeze-dried berry samples were blended into a powder using a Scientific Industrial Division RS 64, Robot Coupe, Robot Coupe U.S.A., Inc., Ridgeland, MS. Whole frozen berries were also homogenized using the Scientific Industrial Division RS 64, Robot Coupe.

Samples

Wild elderberry samples grown throughout four counties of Maine and were collected by Dr. Rodney Bushway. Two types of preservations methods were tested in this study, freezing and freeze-drying. For Freezing, samples were picked from 14 different growing sites. For freeze-drying, samples were picked from 17 different growing sites, with 10 growing sites in common for both frozen and freeze-dried samples. Both freeze-dried and frozen samples were available as whole berries.

In this study samples from individual growing sites were compared. This differs from the method development study, which used composite frozen and freeze-dried samples from all locations.

Freeze-Dried. Samples for freeze-drying were collected in the weeks between August 18th and September 14th 2013. Samples were frozen in a -20°C freezer until they were lyophilized with a SP Scientific, VirTris Ultra 35EL freeze-drier, using the method as described in table 5.1. Freeze-drying was accomplished in nine batches between December and May of 2013. After freeze-drying stems and leaves were removed and the berries were stored whole at -80°C. The day of the study samples were transferred to the research laboratory freezer and stored until they were homogenized for use in the study.

Frozen. Samples for freezing were collected during a span of two weeks between August 31st 2014 and September 14th 2014. Samples were frozen in a -20°C freezer until they were used for these studies. The day of the study samples were transferred to the research laboratory freezer and stored until they were homogenized for use in the study.

Sample Preparation

Freeze-Dried. All freeze-dried samples from the same growing site were combined to make a composite sample representative of specific growing locations. The entire sample was powdered by processing in a Robot Coupe, Scientific Industrial Division RSI 64, for 30 seconds at 1500 rpm. Samples were then reconstituted and extracted by weighing two 1.00g (± 0.01g) samples into a 50mL centrifuge tube with 20mL of extraction solution. They were hand-shaken for 30 seconds, and left for 5 minutes to ensure compete combination with the sample matrix. The samples were then polytronned for 30 seconds using a 60Hz Kinematica Ploytron paired with a 50v POWERSTAT variable autotransformer set to 50.

Once extracted and homogenized, two 1.00g (± 0.01g) subsamples were weighed into 50mL centrifuge tubes and diluted with 10mL of dilution solution. Samples were then centrifuged for 10 minutes at 7,745 x g. 0.5mL was removed and placed into a 2mL HPLC vial and assayed immediately for AA content. Concurrently, another 0.5mL of sample was placed into a 2mL HPLC vial. 0.5mL of TCEP was added and the vial was placed in the HPLC autosampler tray for an hour to allow the reaction to occur. Once the reaction was complete, samples were assayed for total vitamin C content by LC.

Frozen. All frozen samples from the same growing site were combined to make a composite sample of that growing location. Approximately half of the total available sample was picked for stems and leaves and homogenized 1:1 (W:V) with extraction solution, for 30 seconds at 1500 rpm in a Robot Coupe, Scientific Industrial Division RSI 64. The picking process took no more than 30 seconds.

Once extracted and homogenized, two 1.00g (± 0.01g) subsamples were weighed into 50mL centrifuge tubes and diluted with 10mL of dilution solution. Samples were then centrifuged for 10 minutes at 7,745 x g. 0.5mL was removed and placed into a 2mL

HPLC vial and assayed immediately for AA content. Concurrently, another 0.5mL of sample was placed into a 2mL HPLC vial. 0.5mL of TCEP was added and the vial was placed in the HPLC autosampler tray for an hour to allow the reaction to occur. Once the reaction was complete samples were assayed for total vitamin C content by LC.

Moisture Content

Moisture content of samples was measured in duplicate using AOAC method 984.25. Due to limited sample availability after vitamin C analysis, this method was modified to use 5g samples instead of 10g samples. A composite sample was used for freeze-dried elderberries. Measuring individual moisture contents of freeze-dried samples would represent only information about the freeze-drying method, not information about the berries themselves.

Analysis Parameters

The LC detector was set to UV-Vis, measuring 244 and 254nm. The injection was set to 10µL with a flow rate of 0.5mL/min, with a run time of 30 minutes. The mobile phase was 0.3% TFA dissolved in HPLC grade water. The column used was a Phenomenex Kinetex EVO C18 column 5µ 100A (250x4.6mm) operated at ambient temperature.

Calculations and Statistical Analysis

All sample runs included a standard curve with an R^2 value of 0.994 or higher. Vitamin C contents were calculated based on a single point AA standard of 40ng. Calculations were done as described in the method development section. Nutrient contents were calculated based on both a dry weight basis.

Samples replicate results were reviewed to ensure similar measurements for the same sample. One growing location was removed from the sample set because there were not enough berries to complete all facets of the experimental procedure.

AA, DHAA, and total vitamin C amounts were compared based on both differences between growing locations and method of preservation. Statistical analysis was done using SYSTAT 12. Comparisons between the freeze-dried and frozen samples were made using a T-Test. Comparisons between growing locations were made using ANOVA with a confidence interval of 95% (0.05 significance) followed by a Tukey's HSD for pairwise comparisons. Charts are based on average nutrient contents found in samples.

Results and Discussion

The average moisture content of the frozen elderberries was 28%. As anticipated, the moisture content of the composite freeze-dried sample was very low, at 4%. Appendix F lists the moisture content and dry matter content for each growing location. This is not congruent with the moisture content of elderberries listed in the USDA's

National Nutrient database, which lists elderberries as being nearly 80% moisture. This could be due to the berries being frozen for a long period of time, however it is likely due to an improper method of moisture analysis. The AOAC method used for this study was originally created for frozen French fries, it does not take into account the many reducing sugars and organic compounds that interfere with moisture analysis. Instead of using the AOAC method, Červenka (2011) discusses a method of drying where berries are allowed to dry in a desiccator with silica gel for a six to eight week period at room temperature. This may have produced more accurate moisture results. Because of this error all results are listed on a fresh weight basis but also provided on a dry weight basis in Appendices F and G. The discussion will be focused on data from analyses done on a fresh weight basis.

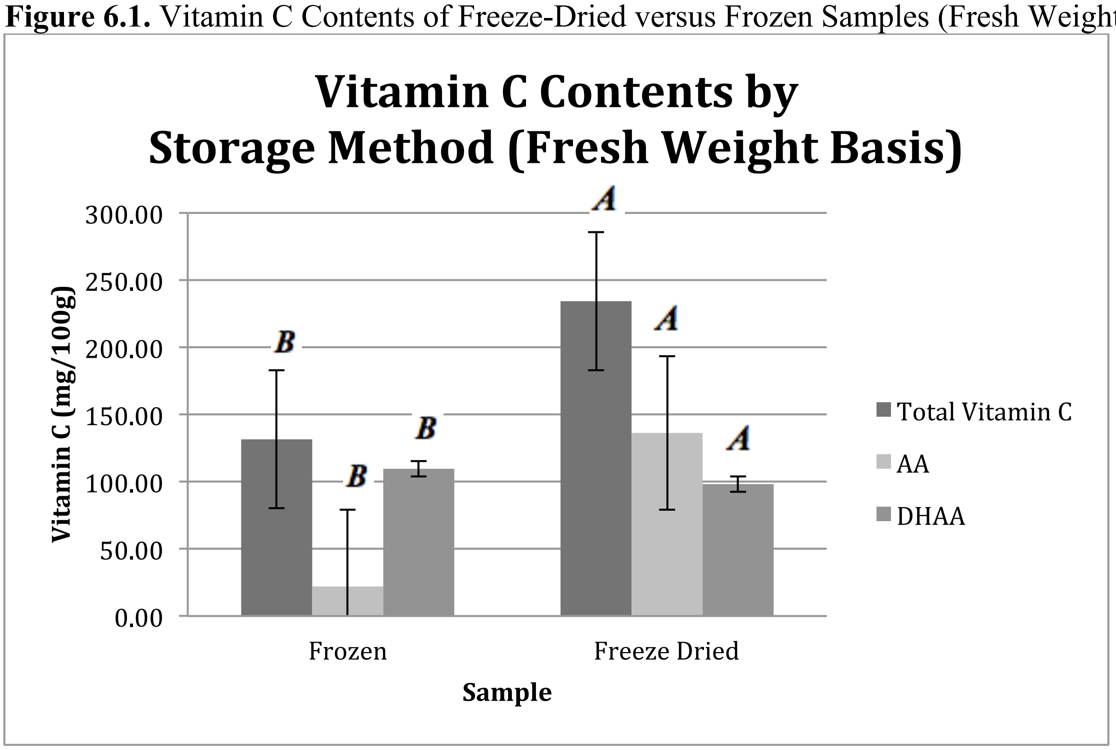
Contents of AA, DHAA, and total vitamin C were analyzed by growing location and storage method. Samples from 2014 that were frozen at-20°C had significantly lower contents of AA, DHAA, and total vitamin C as compared to the 2013 samples that were freeze dried and stored at -80°C. Theses differences are depicted in Figure 6.1 on a fresh weight basis.

Between the frozen samples grown in different locations throughout Northern and Central Maine there were significant differences between all vitamin C contents. Bradford samples had a significantly higher AA content as compared to all other growing locations, except Barnard. Barnard had a significantly higher DHAA and total vitamin C contents as compared to all other growing locations. Theses differences are depicted in Figure 6.2 on a fresh weight basis.

Between the freeze-dried samples growing in different locations throughout Maine there were significant differences between all vitamin C contents. Again, Bradford samples had a significantly higher AA and total vitamin C content as compared to all other growing locations. In this study, Barnard samples also had the significantly higher DHAA content as compared to all other growing locations. These differences are depicted in Figure 6.3 on a fresh weight basis, dry weight basis charts can be found in Appendix H.

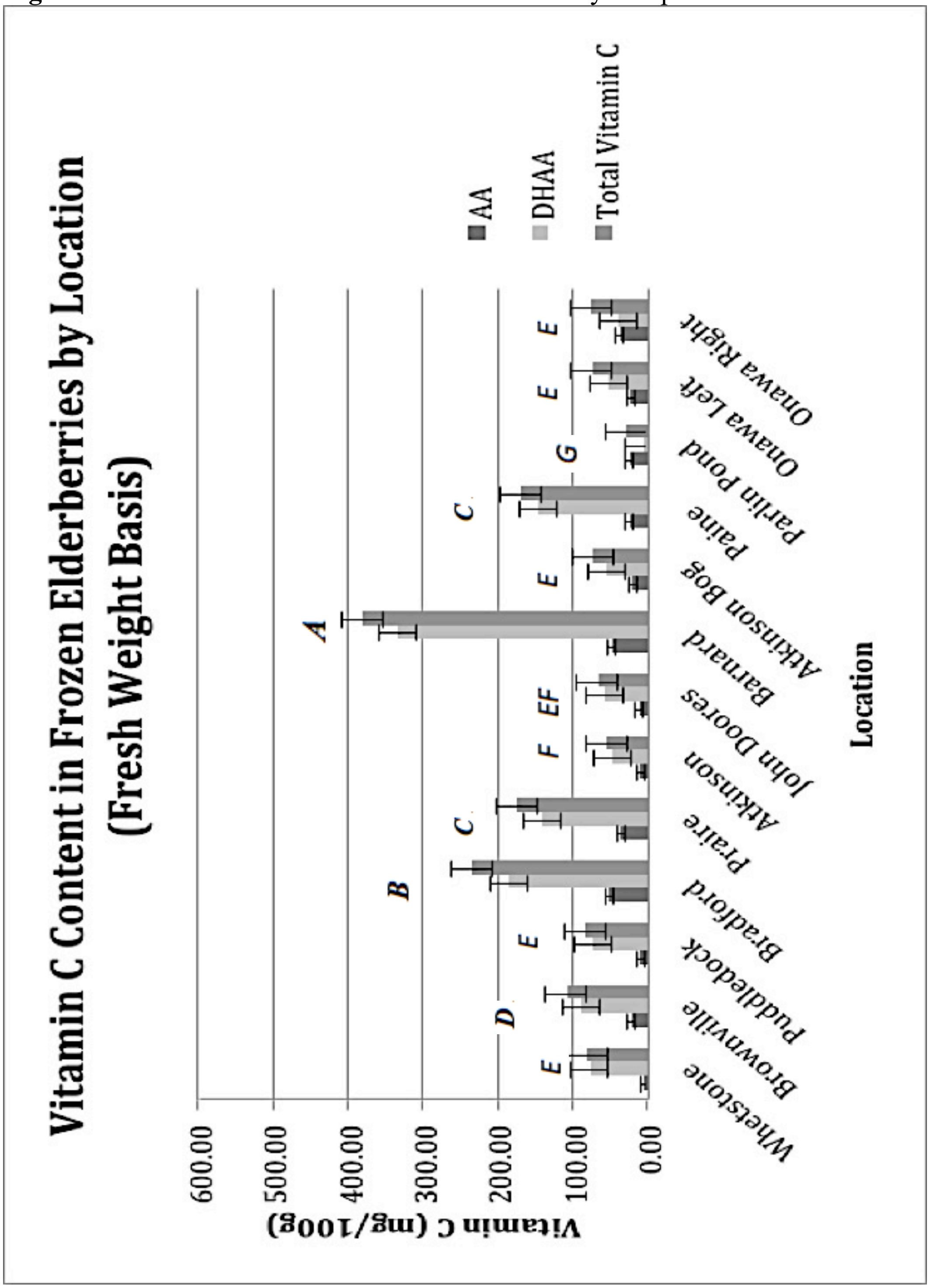
Additionally the levels of DHAA in all freeze-dried and frozen elderberry samples was significant in all growing locations and comprised of an average of half of the total vitamin C content in all samples, frozen and freeze-dried. This was a novel discovery as there are no published studies that have measured DHAA in elderberries.

Figure 6.1. Vitamin C Contents of Freeze-Dried versus Frozen Samples (Fresh Weight)



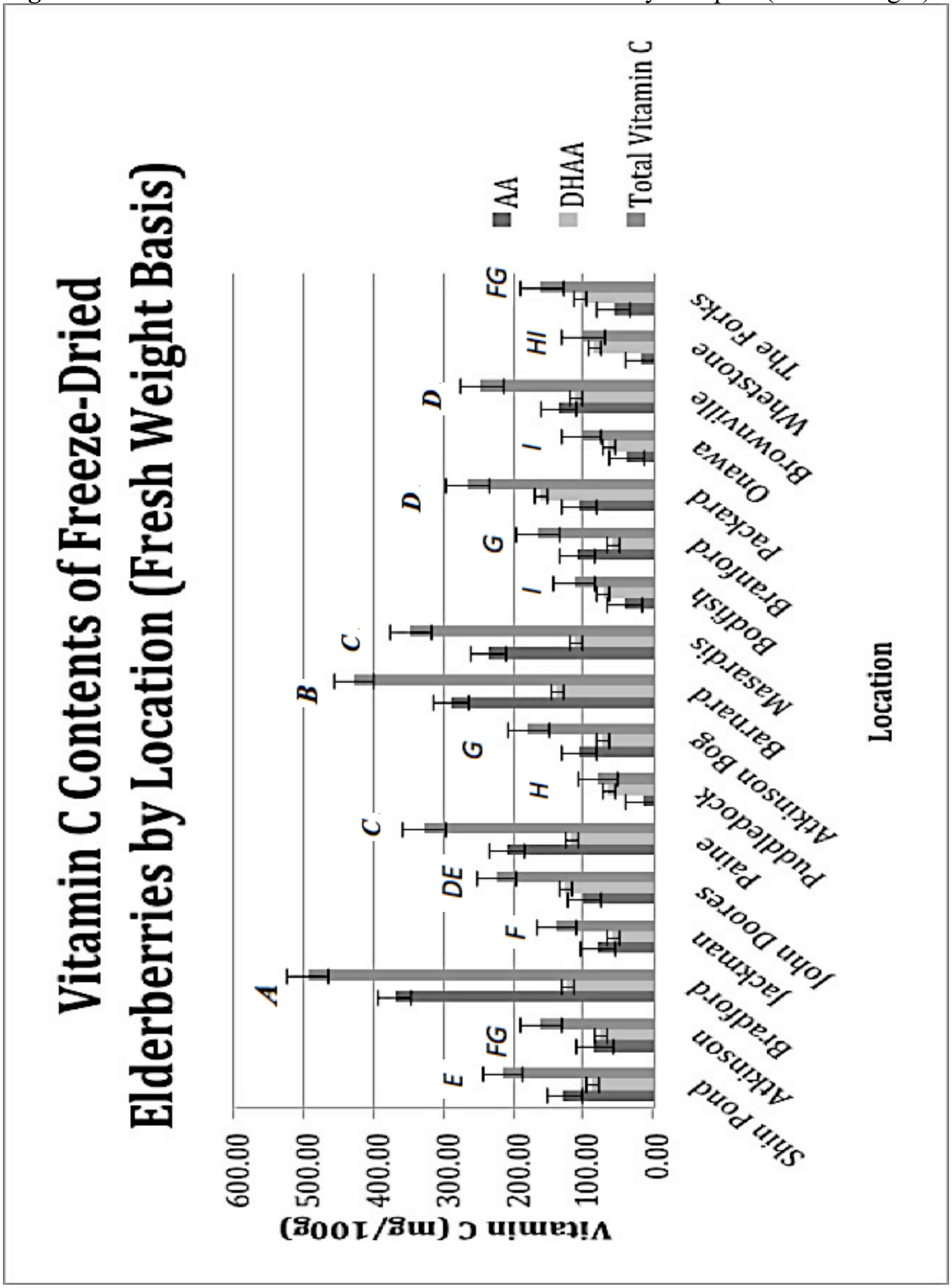
Vitamin C contents are based on averages of duplicate samples of 10 overlapping freeze-dried and frozen sample locations. Error bars were created based on standard error. Significance indicators were created Tukey's HSD calculations created using statistics run in SYSTAT.

Figure 6.2. Vitamin C Contents of Frozen Elderberry Samples



Average nutrient contents listed for each variety based on statistics run in SYSTAT. Significance indicators were created from Tukey's HSD calculations created using statistics run in SYSTAT, significance is listed for total vitamin C only. Error bars were calculated based on standard error. Average nutrient values listed for duplicate samples are listed.

Figure 6.3. Vitamin C Contents of Freeze-Dried Elderberry Samples (Fresh Weight)



Average nutrient contents listed for each variety based on statistics run in SYSTAT. Significance indicators were created from Tukey's HSD calculations created using statistics run in SYSTAT, significance is listed for total vitamin C only. Error bars were calculated based on standard error. Average nutrient values listed for duplicate samples are listed.

Conclusions

It is commonly accepted that the nutrient profile of soil has an affect on the nutrient profile of the produce grown in that soil (Mishima et al., 2013 & Adetunji et al., 1994). However, wild grown produce from a specific region of the U.S. has never been compared for vitamin C content. While total vitamin C values in frozen berries were found to be significantly lower than that of freeze-dried berries, the pattern of growing locations with the highest levels of total vitamin C to the locations with the lowest levels of total vitamin C matched. In both cases Bradford and Barnard were the locations with the highest levels, next was Paine, then Brownville, then John Doores, and finally Atkinson. This indicates that the plant itself does play a large role in the nutrient values of the fruit it produces, even when plants are growing wild and from the same region.

The significantly greater vitamin C contents in freeze-dried elderberries as compared to frozen elderberries was surprising since the harvest dates were a year apart. Frozen samples were harvested around September of 2014 and stored in standard freezer conditions until being run in April of 2015, which gives a storage time of seven months before measurement. Freeze-dried were harvested in September of 2013 and stored in standard freezer conditions until being freeze-dried between December 2013 and May of 2014 gives a storage time of between three and eight months before freeze drying and storing under -80°C conditions.

The difference in vitamin C levels between processing conditions are likely due to the difference in storage condition. At -80°C, the temperature that freeze-dried berries were stored at, the majority of enzymes are inactive (Daniel & Danson, 2013). As was discussed in the literature review, both water and enzyme activity can significantly degrade vitamin C. Similarly, freezers go through thaw cycles. The -80°C freezer went through freeze thaw cycles much less frequently than the -20°C freezer which went through freeze thaw cycles daily. This cycle of repeated thawing and freezing was apparent from the ice crystals present on some of the samples. The freezing and thawing would give the enzymes naturally present in the berries a chance to degrade the vitamin C content, even under "frozen" conditions.

Another novel result of this experiment is that DHAA has never before been measured in elderberries collected found growing wild. As discussed in the review of the literature, the bioactivity of DHAA within the human body is unclear. Reports list between 100% - 10% activity as compared to AA (Elmadfa & Koenig, 1996 & Ogiri et al., 2002, respectively). The present study showed that an average of 50% of total vitamin C in elderberries as DHAA. If the bioavailability is not 100% as compared to AA then it is important that these two values be listed separately. By listing only the combined AA and DHAA, as total vitamin C, consumers are given the wrong impression regarding the amount of nutrient they are getting when they consume the food item. Again, this can be a concern for consumers who are keenly interested in tracking their nutrient intakes to ensure they match recommendations.

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APPENDIX A: NUTRIENT CONTENTS OF POTATOES AND COMMON PROCESSED POTATO PRODUCTS

Table A.1. Macronutrient Contents of Potatoes and Common Processed Potato Products

Nutrient	Raw	Canned	French fried	Baked, no skin	Microwaved	Baked, with skin	Fried Chips
				Macronutrients			
Water (g)	79.34	84.3	62.36	74.89	72.04	76.98	2.54
Energy (Kcal)	77	62	193	93	105	87	559
Protein (g)	2.02	1.4	2.7	2.5	2.44	1.87	4.45
Total lipid (fat) (g)	0.09	0.2	10	0.13	0.1	0.1	38.41
Carbohydrate, by difference (g)	17.47	13.6	22.95	21.15	24.24	20.13	52.02
Fiber, total dietary (g)	2.2	2.4	2.3	2.2	2.3	1.8	3.1
Sugars, total (g)	0.78	0.59	0.49	1.18	.	0.87	1.14
Fatty acids, total saturated (g)	0.026	0.051	2.59	0.035	0.026	0.026	9.492
Fatty acids, total monounsaturated (g)	0.002	0.005	.	0.003	0.002	0.002	7.048
Fatty acids, total polyunsaturated (g)	0.043	0.085	.	0.058	0.043	0.043	13.594
Fatty acids, total trans (g)	.	.	0.2	.	.	.	0.2

Table A.2. Vitamin Contents of Potatoes and Common Processed Potato Products

Nutrient	Raw	Canned	French fried	Baked, no skin	Microwaved	Baked, with skin	Fried Chips
Vitamins							
Vitamin C, total ascorbic acid (mg)	19.7	5.1	5.7	9.6	15.1	13	8.2
Thiamin (mg)	0.08	0.07	.	0.064	0.12	0.106	0.115
Riboflavin (mg)	0.032	0.01	.	0.048	0.032	0.02	0.016
Niacin (mg)	1.054	0.92	.	1.41	1.714	1.439	3.240
Vitamin B-6 (mg)	0.295	0.19	.	0.311	0.344	0.299	0.407
Folate, DFE (µg)	16	6	.	28	12	10	7
Vitamin B-12 (µg)	0	0	.	0	0	0	0
Vitamin A, RAE (µg)	0	0	.	1	0	0	0
Vitamin A (IU)	2	2	0	10	0	3	0
Vitamin E (alpha-tocopherol) (mg)	0.01	0.05	.	0.04	.	0.01	11.40
Vitamin D (D2 + D3) (µg)	0	0	.	0	0	0	0
Vitamin D (IU)	0	0	.	0	0	0	0
Vitamin K (phylloquinone) (µg)	1.9	1.5	.	2	.	2.1	7.2

Table A.3. Mineral Contents of Potatoes and Common Processed Potato Products

Nutrient	Raw	Canned	French fried	Baked, no skin	Microwaved	Baked, with skin	Fried Chips
Minerals							
Calcium	12	5	13	15	11	5	27
Iron (mg)	0.78	1.26	0.87	1.08	1.24	0.31	0.82
Magnesium (mg)	23	14	.	28	27	22	43
Phosphorus (mg)	57	28	.	70	105	44	125
Potassium (mg)	421	229	310	535	447	379	751
Sodium (mg)	6	5	393	10	8	4	529
Zinc (mg)	0.29	0.28	.	0.36	0.36	0.3	0.48

Values were summarized from USDA National Nutrient Database for Standard Reference, release 27 (USDA, 2011). Nutrient content of potatoes based on processing method per 100g sample, no salt added options.

APPENDIX B: NUTRIENT CONTENTS OF ELDERBERRIES

Table B.1. Macronutrient Contents of Elderberries

Nutrient	Content
Macronutrients	
Water (g)	79.80
Energy (Kcal)	73
Protein (g)	0.66
Total lipid (fat) (g)	0.50
Carbohydrate, by difference (g)	18.40
Fiber, total dietary (g)	7.0
Sugars, total (g)	-
Fatty acids, total saturated (g)	0.023
Fatty acids, total monounsaturated (g)	0.080
Fatty acids, total polyunsaturated (g)	0.247

Table B.2. Vitamin Contents of Elderberries

Nutrient	Content
Vitamins	
Vitamin C, total ascorbic acid (mg)	36.0
Thiamin (mg)	0.70
Riboflavin (mg)	0.60
Niacin (mg)	0.500
Vitamin B-6 (mg)	0.230
Folate, DFE (µg)	6
Vitamin B-12 (µg)	0.00
Vitamin A, RAE (µg)	30
Vitamin A (IU)	600
Vitamin E (alpha-tocopherol) (mg)	-
Vitamin D (D2 + D3) (µg)	-
Vitamin D (IU)	-
Vitamin K (phylloquinone) (µg)	-

Table B.3. Mineral Contents of Elderberries

Nutrient	Content
Minerals	
Calcium (mg)	38
Iron (mg)	1.60
Magnesium (mg)	5
Phosphorus (mg)	39
Potassium (mg)	280
Sodium (mg)	6
Zinc (mg)	0.11

Values were summarized from USDA National Nutrient Database for Standard Reference, release 27 (USDA, 2011). Nutrient content of elderberries were based on a raw, 100g sample.

APPENDIX C: POTATO VARIETIES

Table C.1. List of Potato Varieties

Brand	Type	Size	Location Grown	Package Size	Potatoes per Package	Vitamin C Content Listed
Autumn Gold	U.S. NO. 1 Yellow Potatoes	Full	Monticello, ME Foster Farms	4lbs, 1.81kg	5	45% DV
Fruit of the Earth	U.S. NO. 1 Organic Blue Gold Potatoes	Full	Littleton, ME Campbell Family Farms	5lbs, 2.27kg	18	45% DV
Hannaford Brand	U.S. NO. 1 Russet Potatoes	Full	Canada	5lbs, 2.27kg	12	45% DV
Unlisted	U.S. NO. 1 Red Potatoes	Full	Mapleton, ME Braley Family Farms	4lbs, 1.81kg	12	45% DV
Harvest Gold	U.S. NO. 1 White potatoes	Full	Corinth, ME Thomas Farms	5lbs, 2.26kg	20	45% DV
Gourmet Red	U.S. NO. 1 Red New Potatoes	New	Chelsea, MA Gold Bell Inc.	24oz	18	40% DV
Yukon Gold	U.S. NO. 1 Yukon Gold New Potatoes	New	Canada	24oz	22	40% DV
Gourmet Assorted	U.S. NO. 1 Assorted Fingerling Potatoes	Fingerling	Chelsea, MA Gold Bell Inc.	16oz	16	40% DV

APPENDIX D: POTATO PAIRWISE COMPARISON CHARTS

Table D.1. List of Pairwise Comparisons of Ascorbic Acid Content of Potato Varieties

Tukey's Honestly-Significant-Difference Test					
VARIETY$(i)	**VARIETY$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Autumn Gold	Blue Skinned	-3.304	0.005	-6.015	-0.594
Autumn Gold	Gourmet Assorted	2.031	0.338	-0.738	4.799
Autumn Gold	Harvest Gold	-1.253	0.850	-3.933	1.428
Autumn Gold	Red Skinned	3.619	0.003	0.766	6.473
Autumn Gold	Red Skinned New	4.152	0.000	1.441	6.862
Autumn Gold	Russet	-2.746	0.069	-5.600	0.108
Autumn Gold	Yukon Gold New	2.462	0.093	-0.194	5.118
Blue Skinned	Gourmet Assorted	5.335	0.000	3.461	7.209
Blue Skinned	Harvest Gold	2.052	0.009	0.310	3.794
Blue Skinned	Red Skinned	6.924	0.000	4.926	8.922
Blue Skinned	Red Skinned New	7.456	0.000	5.669	9.243
Blue Skinned	Russet	0.558	0.990	-1.440	2.557
Blue Skinned	Yukon Gold New	5.766	0.000	4.062	7.470
Gourmet Assorted	Harvest Gold	-3.283	0.000	-5.115	-1.452
Gourmet Assorted	Red Skinned	1.589	0.283	-0.488	3.665
Gourmet Assorted	Red Skinned New	2.121	0.014	0.247	3.995
Gourmet Assorted	Russet	-4.777	0.000	-6.853	-2.700
Gourmet Assorted	Yukon Gold New	0.431	0.996	-1.364	2.226
Harvest Gold	Red Skinned	4.872	0.000	2.915	6.830
Harvest Gold	Red Skinned New	5.404	0.000	3.663	7.146
Harvest Gold	Russet	-1.493	0.287	-3.451	0.465
Harvest Gold	Yukon Gold New	3.715	0.000	2.058	5.371
Red Skinned	Red Skinned New	0.532	0.993	-1.466	2.530
Red Skinned	Russet	-6.365	0.000	-8.554	-4.177
Red Skinned	Yukon Gold New	-1.158	0.604	-3.082	0.766
Red Skinned New	Russet	-6.898	0.000	-8.896	-4.900
Red Skinned New	Yukon Gold New	-1.690	0.054	-3.394	0.014
Russet	Yukon Gold New	5.208	0.000	3.284	7.132

Tukey's HSD calculations created using statistics run in SYSTAT.

Table D.2. List of Pairwise Comparisons of Dehydroascorbic Acid Content of Potato Varieties

Tukey's Honestly-Significant-Difference Test					
VARIETY$(i)	**VARIETY$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Autumn Gold	Blue Skinned	-2.361	0.000	-3.280	-1.443
Autumn Gold	Gourmet Assorted	-0.527	0.686	-1.465	0.411
Autumn Gold	Harvest Gold	-0.298	0.975	-1.207	0.610
Autumn Gold	Red Skinned	-0.239	0.995	-1.206	0.728
Autumn Gold	Red Skinned New	-0.469	0.781	-1.388	0.449
Autumn Gold	Russet	-0.113	1.000	-1.080	0.855
Autumn Gold	Yukon Gold New	-0.955	0.028	-1.856	-0.055
Blue Skinned	Gourmet Assorted	1.834	0.000	1.199	2.470
Blue Skinned	Harvest Gold	2.063	0.000	1.473	2.653
Blue Skinned	Red Skinned	2.123	0.000	1.446	2.800
Blue Skinned	Red Skinned New	1.892	0.000	1.287	2.498
Blue Skinned	Russet	2.249	0.000	1.572	2.926
Blue Skinned	Yukon Gold New	1.406	0.000	0.829	1.983
Gourmet Assorted	Harvest Gold	0.229	0.953	-0.392	0.849
Gourmet Assorted	Red Skinned	0.288	0.919	-0.415	0.992
Gourmet Assorted	Red Skinned New	0.058	1.000	-0.577	0.693
Gourmet Assorted	Russet	0.414	0.630	-0.289	1.118
Gourmet Assorted	Yukon Gold New	-0.428	0.392	-1.037	0.180
Harvest Gold	Red Skinned	0.059	1.000	-0.604	0.723
Harvest Gold	Red Skinned New	-0.171	0.988	-0.761	0.419
Harvest Gold	Russet	0.186	0.990	-0.478	0.849
Harvest Gold	Yukon Gold New	-0.657	0.009	-1.218	-0.096
Red Skinned	Red Skinned New	-0.230	0.970	-0.907	0.447
Red Skinned	Russet	0.126	1.000	-0.615	0.868
Red Skinned	Yukon Gold New	-0.717	0.020	-1.369	-0.065
Red Skinned New	Russet	0.357	0.753	-0.320	1.034
Red Skinned New	Yukon Gold New	-0.486	0.174	-1.064	0.091
Russet	Yukon Gold New	-0.843	0.002	-1.495	-0.191

Tukey's HSD calculations created using statistics run in SYSTAT.

Table D.3. List of Pairwise Comparisons of Total Vitamin C Content of Potato Varieties

Tukey's Honestly-Significant-Difference Test					
VARIETY$(i)	VARIETY$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Autumn Gold	Blue Skinned	-5.666	0.000	-8.528	-2.803
Autumn Gold	Gourmet Assorted	1.462	0.800	-1.463	4.386
Autumn Gold	Harvest Gold	-1.612	0.670	-4.443	1.219
Autumn Gold	Red Skinned	3.278	0.022	0.264	6.292
Autumn Gold	Red Skinned New	3.616	0.003	0.753	6.478
Autumn Gold	Russet	-2.858	0.078	-5.873	0.156
Autumn Gold	Yukon Gold New	1.496	0.741	-1.310	4.301
Blue Skinned	Gourmet Assorted	7.127	0.000	5.148	9.107
Blue Skinned	Harvest Gold	4.054	0.000	2.214	5.894
Blue Skinned	Red Skinned	8.944	0.000	6.834	11.054
Blue Skinned	Red Skinned New	9.282	0.000	7.394	11.169
Blue Skinned	Russet	2.807	0.001	0.697	4.918
Blue Skinned	Yukon Gold New	7.162	0.000	5.362	8.961
Gourmet Assorted	Harvest Gold	-3.074	0.000	-5.008	-1.140
Gourmet Assorted	Red Skinned	1.817	0.191	-0.377	4.010
Gourmet Assorted	Red Skinned New	2.154	0.022	0.175	4.134
Gourmet Assorted	Russet	-4.320	0.000	-6.513	-2.127
Gourmet Assorted	Yukon Gold New	0.034	1.000	-1.862	1.930
Harvest Gold	Red Skinned	4.890	0.000	2.823	6.958
Harvest Gold	Red Skinned New	5.228	0.000	3.388	7.068
Harvest Gold	Russet	-1.246	0.601	-3.314	0.821
Harvest Gold	Yukon Gold New	3.108	0.000	1.358	4.857
Red Skinned	Red Skinned New	0.338	1.000	-1.773	2.448
Red Skinned	Russet	-6.137	0.000	-8.448	-3.825
Red Skinned	Yukon Gold New	-1.782	0.136	-3.815	0.250
Red Skinned New	Russet	-6.474	0.000	-8.585	-4.364
Red Skinned New	Yukon Gold New	-2.120	0.009	-3.920	-0.320
Russet	Yukon Gold New	4.354	0.000	2.322	6.386

Tukey's HSD calculations created using statistics run in SYSTAT.

APPENDIX E: ELEDERBERRY GROWING LOCATIONS

Table E.1. List of Frozen Elderberry Growing Locations

Sample	City/Town Grown in	County
Whetstone	Bronville Junction	Piscataquis
Brownville KI	Brownville	Piscataquis
Puddledock	Charleston	Penobscot
Bradford	Bradford	Penobscot
Praire	*	*
Harris	Charleston	Penobscot
Atkinson	Atkinson	Piscataquis
John Doores	Atkinson	Piscataquis
Barnard	Barnard	Piscataquis
Atkinson Bog	Atkinson	Piscataquis
Paine	Charleston	Penobscot
Parlin Pond	Parlin Pond	Somerset
Onawa Left	Elliostville	Piscataquis
Onawa Right	Elliostville	Piscataquis

Harris samples were not used in data analysis due to insufficient sample material
** Specific origin of these samples was unlisted*

Table E.2. List of Freeze-Dried Elderberry Growing Locations

Sample	City/Town Grown in	County
Shin Pond	Mount Chase	Penobscot
Atkinson	Atkinson	Piscataquis
Bradford	Bradford	Penobscot
Jackman	Jackman	Somerset
John Doores	Atkinson	Piscataquis
Paine	Charleston	Penobscot
Puddledock	Charleston	Penobscot
Atkinson Bog	Atkinson	Piscataquis
Barnard	Barnard	Piscataquis
Masardis	Masardis	Aroostook
Bodfish	Bodfish	Piscataquis
Branford	*	*
Packard	West Sebois	Penobscot
Onawa	Elliostville	Piscataquis
Brownville	Brownville	Piscataquis
Whetstone	Bronville Junction	Piscataquis
The Forks	The Forks	Somerset

** Specific origin of these samples was unlisted*

APPENDIX F: ELDERBERRY MOISTURE ANALYSIS RESULTS

Table F.1. List of Elderberry Moisture Analysis Results

Location	% Moisture	% Dry Matter
Whetstone	31.93	68.07
Brownville	27.70	72.30
Puddledock	29.43	70.57
Bradford	26.38	73.62
Praire	27.48	72.52
Atkinson	28.11	71.89
John Doores	27.14	72.86
Barnard	27.31	72.69
Atkinson Bog	28.14	71.86
Paine	27.30	72.70
Parlin Pond	30.96	69.04
Onawa Left	27.30	72.70
Onawa Right	29.05	70.95
-Freeze Dried Composite-	4.04	95.96

Moisture amounts are based on averages of duplicate samples.

APPENDIX G: ELDERBERRY PAIRWISE COMPARISON CHARTS

Table G.1. List of Pairwise Comparisons of Ascorbic Acid Content in Frozen Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-10.455	0.000	-15.369	-5.541
Atkinson	Barnard	-37.735	0.000	-42.649	-32.821
Atkinson	Bradford	-41.730	0.000	-46.644	-36.816
Atkinson	Brownville	-11.765	0.000	-16.679	-6.851
Atkinson	John Doores	-1.100	0.999	-6.014	3.814
Atkinson	Onawa Left	-27.995	0.000	-32.909	-23.081
Atkinson	Onawa Right	-12.890	0.000	-17.804	-7.976
Atkinson	Paine	-14.765	0.000	-19.679	-9.851
Atkinson	Parlin Pond	-14.900	0.000	-19.814	-9.986
Atkinson	Praire	-26.305	0.000	-31.219	-21.391
Atkinson	Puddledock	-0.430	1.000	-5.344	4.484
Atkinson	Whetstone	4.855	0.054	-0.059	9.769
Atkinson Bog	Barnard	-27.280	0.000	-32.194	-22.366
Atkinson Bog	Bradford	-31.275	0.000	-36.189	-26.361
Atkinson Bog	Brownville	-1.310	0.994	-6.224	3.604
Atkinson Bog	John Doores	9.355	0.000	4.441	14.269
Atkinson Bog	Onawa Left	-17.540	0.000	-22.454	-12.626
Atkinson Bog	Onawa Right	-2.435	0.734	-7.349	2.479
Atkinson Bog	Paine	-4.310	0.110	-9.224	0.604
Atkinson Bog	Parlin Pond	-4.445	0.092	-9.359	0.469
Atkinson Bog	Praire	-15.850	0.000	-20.764	-10.936
Atkinson Bog	Puddledock	10.025	0.000	5.111	14.939
Atkinson Bog	Whetstone	15.310	0.000	10.396	20.224
Barnard	Bradford	-3.995	0.163	-8.909	0.919
Barnard	Brownville	25.970	0.000	21.056	30.884
Barnard	John Doores	36.635	0.000	31.721	41.549
Barnard	Onawa Left	9.740	0.000	4.826	14.654
Barnard	Onawa Right	24.845	0.000	19.931	29.759
Barnard	Paine	22.970	0.000	18.056	27.884
Barnard	Parlin Pond	22.835	0.000	17.921	27.749
Barnard	Praire	11.430	0.000	6.516	16.344
Barnard	Puddledock	37.305	0.000	32.391	42.219
Barnard	Whetstone	42.590	0.000	37.676	47.504
Bradford	Brownville	29.965	0.000	25.051	34.879
Bradford	John Doores	40.630	0.000	35.716	45.544
Bradford	Onawa Left	13.735	0.000	8.821	18.649
Bradford	Onawa Right	28.840	0.000	23.926	33.754
Bradford	Paine	26.965	0.000	22.051	31.879
Bradford	Parlin Pond	26.830	0.000	21.916	31.744
Bradford	Praire	15.425	0.000	10.511	20.339
Bradford	Puddledock	41.300	0.000	36.386	46.214
Bradford	Whetstone	46.585	0.000	41.671	51.499

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	**LOCATION$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Brownville	John Doores	10.665	0.000	5.751	15.579
Brownville	Onawa Left	-16.230	0.000	-21.144	-11.316
Brownville	Onawa Right	-1.125	0.999	-6.039	3.789
Brownville	Paine	-3.000	0.480	-7.914	1.914
Brownville	Parlin Pond	-3.135	0.423	-8.049	1.779
Brownville	Praire	-14.540	0.000	-19.454	-9.626
Brownville	Puddledock	11.335	0.000	6.421	16.249
Brownville	Whetstone	16.620	0.000	11.706	21.534
John Doores	Onawa Left	-26.895	0.000	-31.809	-21.981
John Doores	Onawa Right	-11.790	0.000	-16.704	-6.876
John Doores	Paine	-13.665	0.000	-18.579	-8.751
John Doores	Parlin Pond	-13.800	0.000	-18.714	-8.886
John Doores	Praire	-25.205	0.000	-30.119	-20.291
John Doores	Puddledock	0.670	1.000	-4.244	5.584
John Doores	Whetstone	5.955	0.012	1.041	10.869
Onawa Left	Onawa Right	15.105	0.000	10.191	20.019
Onawa Left	Paine	13.230	0.000	8.316	18.144
Onawa Left	Parlin Pond	13.095	0.000	8.181	18.009
Onawa Left	Praire	1.690	0.962	-3.224	6.604
Onawa Left	Puddledock	27.565	0.000	22.651	32.479
Onawa Left	Whetstone	32.850	0.000	27.936	37.764
Onawa Right	Paine	-1.875	0.927	-6.789	3.039
Onawa Right	Parlin Pond	-2.010	0.892	-6.924	2.904
Onawa Right	Praire	-13.415	0.000	-18.329	-8.501
Onawa Right	Puddledock	12.460	0.000	7.546	17.374
Onawa Right	Whetstone	17.745	0.000	12.831	22.659
Paine	Parlin Pond	-0.135	1.000	-5.049	4.779
Paine	Praire	-11.540	0.000	-16.454	-6.626
Paine	Puddledock	14.335	0.000	9.421	19.249
Paine	Whetstone	19.620	0.000	14.706	24.534
Parlin Pond	Praire	-11.405	0.000	-16.319	-6.491
Parlin Pond	Puddledock	14.470	0.000	9.556	19.384
Parlin Pond	Whetstone	19.755	0.000	14.841	24.669
Praire	Puddledock	25.875	0.000	20.961	30.789
Praire	Whetstone	31.160	0.000	26.246	36.074
Puddledock	Whetstone	5.285	0.030	0.371	10.199

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.2. List of Pairwise Comparisons of Ascorbic Acid Content in Frozen Elderberries (Dry Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	**LOCATION$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Atkinson	Atkinson Bog	-12.120	0.000	-17.784	-6.456
Atkinson	Barnard	-43.530	0.000	-49.194	-37.866
Atkinson	Bradford	-47.755	0.000	-53.419	-42.091
Atkinson	Brownville	-13.690	0.000	-19.354	-8.026
Atkinson	John Doores	-1.200	0.999	-6.864	4.464
Atkinson	Onawa Left	-32.875	0.000	-38.539	-27.211
Atkinson	Onawa Right	-14.820	0.000	-20.484	-9.156
Atkinson	Paine	-17.010	0.000	-22.674	-11.346
Atkinson	Parlin Pond	-17.965	0.000	-23.629	-12.301
Atkinson	Praire	-30.315	0.000	-35.979	-24.651
Atkinson	Puddledock	-0.575	1.000	-6.239	5.089
Atkinson	Whetstone	5.520	0.059	-0.144	11.184
Atkinson Bog	Barnard	-31.410	0.000	-37.074	-25.746
Atkinson Bog	Bradford	-35.635	0.000	-41.299	-29.971
Atkinson Bog	Brownville	-1.570	0.992	-7.234	4.094
Atkinson Bog	John Doores	10.920	0.000	5.256	16.584
Atkinson Bog	Onawa Left	-20.755	0.000	-26.419	-15.091
Atkinson Bog	Onawa Right	-2.700	0.774	-8.364	2.964
Atkinson Bog	Paine	-4.890	0.120	-10.554	0.774
Atkinson Bog	Parlin Pond	-5.845	0.041	-11.509	-0.181
Atkinson Bog	Praire	-18.195	0.000	-23.859	-12.531
Atkinson Bog	Puddledock	11.545	0.000	5.881	17.209
Atkinson Bog	Whetstone	17.640	0.000	11.976	23.304
Barnard	Bradford	-4.225	0.241	-9.889	1.439
Barnard	Brownville	29.840	0.000	24.176	35.504
Barnard	John Doores	42.330	0.000	36.666	47.994
Barnard	Onawa Left	10.655	0.000	4.991	16.319
Barnard	Onawa Right	28.710	0.000	23.046	34.374
Barnard	Paine	26.520	0.000	20.856	32.184
Barnard	Parlin Pond	25.565	0.000	19.901	31.229
Barnard	Praire	13.215	0.000	7.551	18.879
Barnard	Puddledock	42.955	0.000	37.291	48.619
Barnard	Whetstone	49.050	0.000	43.386	54.714
Bradford	Brownville	34.065	0.000	28.401	39.729
Bradford	John Doores	46.555	0.000	40.891	52.219
Bradford	Onawa Left	14.880	0.000	9.216	20.544
Bradford	Onawa Right	32.935	0.000	27.271	38.599
Bradford	Paine	30.745	0.000	25.081	36.409
Bradford	Parlin Pond	29.790	0.000	24.126	35.454
Bradford	Praire	17.440	0.000	11.776	23.104
Bradford	Puddledock	47.180	0.000	41.516	52.844
Bradford	Whetstone	53.275	0.000	47.611	58.939
Brownville	John Doores	12.490	0.000	6.826	18.154
Brownville	Onawa Left	-19.185	0.000	-24.849	-13.521

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	**LOCATION$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Brownville	Onawa Right	-1.130	1.000	-6.794	4.534
Brownville	Paine	-3.320	0.532	-8.984	2.344
Brownville	Parlin Pond	-4.275	0.229	-9.939	1.389
Brownville	Praire	-16.625	0.000	-22.289	-10.961
Brownville	Puddledock	13.115	0.000	7.451	18.779
Brownville	Whetstone	19.210	0.000	13.546	24.874
John Doores	Onawa Left	-31.675	0.000	-37.339	-26.011
John Doores	Onawa Right	-13.620	0.000	-19.284	-7.956
John Doores	Paine	-15.810	0.000	-21.474	-10.146
John Doores	Parlin Pond	-16.765	0.000	-22.429	-11.101
John Doores	Praire	-29.115	0.000	-34.779	-23.451
John Doores	Puddledock	0.625	1.000	-5.039	6.289
John Doores	Whetstone	6.720	0.015	1.056	12.384
Onawa Left	Onawa Right	18.055	0.000	12.391	23.719
Onawa Left	Paine	15.865	0.000	10.201	21.529
Onawa Left	Parlin Pond	14.910	0.000	9.246	20.574
Onawa Left	Praire	2.560	0.822	-3.104	8.224
Onawa Left	Puddledock	32.300	0.000	26.636	37.964
Onawa Left	Whetstone	38.395	0.000	32.731	44.059
Onawa Right	Paine	-2.190	0.921	-7.854	3.474
Onawa Right	Parlin Pond	-3.145	0.602	-8.809	2.519
Onawa Right	Praire	-15.495	0.000	-21.159	-9.831
Onawa Right	Puddledock	14.245	0.000	8.581	19.909
Onawa Right	Whetstone	20.340	0.000	14.676	26.004
Paine	Parlin Pond	-0.955	1.000	-6.619	4.709
Paine	Praire	-13.305	0.000	-18.969	-7.641
Paine	Puddledock	16.435	0.000	10.771	22.099
Paine	Whetstone	22.530	0.000	16.866	28.194
Parlin Pond	Praire	-12.350	0.000	-18.014	-6.686
Parlin Pond	Puddledock	17.390	0.000	11.726	23.054
Parlin Pond	Whetstone	23.485	0.000	17.821	29.149
Praire	Puddledock	29.740	0.000	24.076	35.404
Praire	Whetstone	35.835	0.000	30.171	41.499
Puddledock	Whetstone	6.095	0.030	0.431	11.759

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.3. List of Pairwise Comparisons of Dehhdroascorbic Acid Content in Frozen Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-8.370	0.327	-20.509	3.769
Atkinson	Barnard	-288.130	0.000	-300.269	-275.991
Atkinson	Bradford	-139.110	0.000	-151.249	-126.971
Atkinson	Brownville	-41.830	0.000	-53.969	-29.691
Atkinson	John Doores	-11.005	0.091	-23.144	1.134
Atkinson	Onawa Left	6.330	0.678	-5.809	18.469
Atkinson	Onawa Right	-6.940	0.565	-19.079	5.199
Atkinson	Paine	-100.065	0.000	-112.204	-87.926
Atkinson	Parlin Pond	40.820	0.000	28.681	52.959
Atkinson	Praire	-94.620	0.000	-106.759	-82.481
Atkinson	Puddledock	-27.395	0.000	-39.534	-15.256
Atkinson	Whetstone	-30.915	0.000	-43.054	-18.776
Atkinson Bog	Barnard	-279.760	0.000	-291.899	-267.621
Atkinson Bog	Bradford	-130.740	0.000	-142.879	-118.601
Atkinson Bog	Brownville	-33.460	0.000	-45.599	-21.321
Atkinson Bog	John Doores	-2.635	0.999	-14.774	9.504
Atkinson Bog	Onawa Left	14.700	0.013	2.561	26.839
Atkinson Bog	Onawa Right	1.430	1.000	-10.709	13.569
Atkinson Bog	Paine	-91.695	0.000	-103.834	-79.556
Atkinson Bog	Parlin Pond	49.190	0.000	37.051	61.329
Atkinson Bog	Praire	-86.250	0.000	-98.389	-74.111
Atkinson Bog	Puddledock	-19.025	0.001	-31.164	-6.886
Atkinson Bog	Whetstone	-22.545	0.000	-34.684	-10.406
Barnard	Bradford	149.020	0.000	136.881	161.159
Barnard	Brownville	246.300	0.000	234.161	258.439
Barnard	John Doores	277.125	0.000	264.986	289.264
Barnard	Onawa Left	294.460	0.000	282.321	306.599
Barnard	Onawa Right	281.190	0.000	269.051	293.329
Barnard	Paine	188.065	0.000	175.926	200.204
Barnard	Parlin Pond	328.950	0.000	316.811	341.089
Barnard	Praire	193.510	0.000	181.371	205.649
Barnard	Puddledock	260.735	0.000	248.596	272.874
Barnard	Whetstone	257.215	0.000	245.076	269.354
Bradford	Brownville	97.280	0.000	85.141	109.419
Bradford	John Doores	128.105	0.000	115.966	140.244
Bradford	Onawa Left	145.440	0.000	133.301	157.579
Bradford	Onawa Right	132.170	0.000	120.031	144.309
Bradford	Paine	39.045	0.000	26.906	51.184
Bradford	Parlin Pond	179.930	0.000	167.791	192.069
Bradford	Praire	44.490	0.000	32.351	56.629
Bradford	Puddledock	111.715	0.000	99.576	123.854
Bradford	Whetstone	108.195	0.000	96.056	120.334
Brownville	John Doores	30.825	0.000	18.686	42.964
Brownville	Onawa Left	48.160	0.000	36.021	60.299

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Onawa Right	34.890	0.000	22.751	47.029
Brownville	Paine	-58.235	0.000	-70.374	-46.096
Brownville	Parlin Pond	82.650	0.000	70.511	94.789
Brownville	Praire	-52.790	0.000	-64.929	-40.651
Brownville	Puddledock	14.435	0.014	2.296	26.574
Brownville	Whetstone	10.915	0.096	-1.224	23.054
John Doores	Onawa Left	17.335	0.003	5.196	29.474
John Doores	Onawa Right	4.065	0.968	-8.074	16.204
John Doores	Paine	-89.060	0.000	-101.199	-76.921
John Doores	Parlin Pond	51.825	0.000	39.686	63.964
John Doores	Praire	-83.615	0.000	-95.754	-71.476
John Doores	Puddledock	-16.390	0.005	-28.529	-4.251
John Doores	Whetstone	-19.910	0.001	-32.049	-7.771
Onawa Left	Onawa Right	-13.270	0.027	-25.409	-1.131
Onawa Left	Paine	-106.395	0.000	-118.534	-94.256
Onawa Left	Parlin Pond	34.490	0.000	22.351	46.629
Onawa Left	Praire	-100.950	0.000	-113.089	-88.811
Onawa Left	Puddledock	-33.725	0.000	-45.864	-21.586
Onawa Left	Whetstone	-37.245	0.000	-49.384	-25.106
Onawa Right	Paine	-93.125	0.000	-105.264	-80.986
Onawa Right	Parlin Pond	47.760	0.000	35.621	59.899
Onawa Right	Praire	-87.680	0.000	-99.819	-75.541
Onawa Right	Puddledock	-20.455	0.001	-32.594	-8.316
Onawa Right	Whetstone	-23.975	0.000	-36.114	-11.836
Paine	Parlin Pond	140.885	0.000	128.746	153.024
Paine	Praire	5.445	0.828	-6.694	17.584
Paine	Puddledock	72.670	0.000	60.531	84.809
Paine	Whetstone	69.150	0.000	57.011	81.289
Parlin Pond	Praire	-135.440	0.000	-147.579	-123.301
Parlin Pond	Puddledock	-68.215	0.000	-80.354	-56.076
Parlin Pond	Whetstone	-71.735	0.000	-83.874	-59.596
Praire	Puddledock	67.225	0.000	55.086	79.364
Praire	Whetstone	63.705	0.000	51.566	75.844
Puddledock	Whetstone	-3.520	0.989	-15.659	8.619

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.4. List of Pairwise Comparisons of Dehydroascorbic Acid Content in Frozen Elderberries (Dry Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	6.685	0.000	2.908	10.462
Atkinson	Barnard	-8.250	0.000	-12.027	-4.473
Atkinson	Bradford	-1.245	0.972	-5.022	2.532
Atkinson	Brownville	-0.625	1.000	-4.402	3.152
Atkinson	John Doores	-0.640	1.000	-4.417	3.137
Atkinson	Onawa Left	-1.890	0.724	-5.667	1.887
Atkinson	Onawa Right	8.275	0.000	4.498	12.052
Atkinson	Paine	-6.665	0.000	-10.442	-2.888
Atkinson	Parlin Pond	9.630	0.000	5.853	13.407
Atkinson	Praire	-14.315	0.000	-18.092	-10.538
Atkinson	Puddledock	-2.165	0.561	-5.942	1.612
Atkinson	Whetstone	-1.660	0.844	-5.437	2.117
Atkinson Bog	Barnard	-14.935	0.000	-18.712	-11.158
Atkinson Bog	Bradford	-7.930	0.000	-11.707	-4.153
Atkinson Bog	Brownville	-7.310	0.000	-11.087	-3.533
Atkinson Bog	John Doores	-7.325	0.000	-11.102	-3.548
Atkinson Bog	Onawa Left	-8.575	0.000	-12.352	-4.798
Atkinson Bog	Onawa Right	1.590	0.874	-2.187	5.367
Atkinson Bog	Paine	-13.350	0.000	-17.127	-9.573
Atkinson Bog	Parlin Pond	2.945	0.198	-0.832	6.722
Atkinson Bog	Praire	-21.000	0.000	-24.777	-17.223
Atkinson Bog	Puddledock	-8.850	0.000	-12.627	-5.073
Atkinson Bog	Whetstone	-8.345	0.000	-12.122	-4.568
Barnard	Bradford	7.005	0.000	3.228	10.782
Barnard	Brownville	7.625	0.000	3.848	11.402
Barnard	John Doores	7.610	0.000	3.833	11.387
Barnard	Onawa Left	6.360	0.001	2.583	10.137
Barnard	Onawa Right	16.525	0.000	12.748	20.302
Barnard	Paine	1.585	0.876	-2.192	5.362
Barnard	Parlin Pond	17.880	0.000	14.103	21.657
Barnard	Praire	-6.065	0.001	-9.842	-2.288
Barnard	Puddledock	6.085	0.001	2.308	9.862
Barnard	Whetstone	6.590	0.000	2.813	10.367
Bradford	Brownville	0.620	1.000	-3.157	4.397
Bradford	John Doores	0.605	1.000	-3.172	4.382
Bradford	Onawa Left	-0.645	1.000	-4.422	3.132
Bradford	Onawa Right	9.520	0.000	5.743	13.297
Bradford	Paine	-5.420	0.003	-9.197	-1.643
Bradford	Parlin Pond	10.875	0.000	7.098	14.652
Bradford	Praire	-13.070	0.000	-16.847	-9.293
Bradford	Puddledock	-0.920	0.997	-4.697	2.857
Bradford	Whetstone	-0.415	1.000	-4.192	3.362
Brownville	John Doores	-0.015	1.000	-3.792	3.762
Brownville	Onawa Left	-1.265	0.968	-5.042	2.512

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Onawa Right	8.900	0.000	5.123	12.677
Brownville	Paine	-6.040	0.001	-9.817	-2.263
Brownville	Parlin Pond	10.255	0.000	6.478	14.032
Brownville	Praire	-13.690	0.000	-17.467	-9.913
Brownville	Puddledock	-1.540	0.894	-5.317	2.237
Brownville	Whetstone	-1.035	0.993	-4.812	2.742
John Doores	Onawa Left	-1.250	0.971	-5.027	2.527
John Doores	Onawa Right	8.915	0.000	5.138	12.692
John Doores	Paine	-6.025	0.001	-9.802	-2.248
John Doores	Parlin Pond	10.270	0.000	6.493	14.047
John Doores	Praire	-13.675	0.000	-17.452	-9.898
John Doores	Puddledock	-1.525	0.900	-5.302	2.252
John Doores	Whetstone	-1.020	0.994	-4.797	2.757
Onawa Left	Onawa Right	10.165	0.000	6.388	13.942
Onawa Left	Paine	-4.775	0.009	-8.552	-0.998
Onawa Left	Parlin Pond	11.520	0.000	7.743	15.297
Onawa Left	Praire	-12.425	0.000	-16.202	-8.648
Onawa Left	Puddledock	-0.275	1.000	-4.052	3.502
Onawa Left	Whetstone	0.230	1.000	-3.547	4.007
Onawa Right	Paine	-14.940	0.000	-18.717	-11.163
Onawa Right	Parlin Pond	1.355	0.950	-2.422	5.132
Onawa Right	Praire	-22.590	0.000	-26.367	-18.813
Onawa Right	Puddledock	-10.440	0.000	-14.217	-6.663
Onawa Right	Whetstone	-9.935	0.000	-13.712	-6.158
Paine	Parlin Pond	16.295	0.000	12.518	20.072
Paine	Praire	-7.650	0.000	-11.427	-3.873
Paine	Puddledock	4.500	0.014	0.723	8.277
Paine	Whetstone	5.005	0.006	1.228	8.782
Parlin Pond	Praire	-23.945	0.000	-27.722	-20.168
Parlin Pond	Puddledock	-11.795	0.000	-15.572	-8.018
Parlin Pond	Whetstone	-11.290	0.000	-15.067	-7.513
Praire	Puddledock	12.150	0.000	8.373	15.927
Praire	Whetstone	12.655	0.000	8.878	16.432
Puddledock	Whetstone	0.505	1.000	-3.272	4.282

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.5. List of Pairwise Comparisons of Total Vitamin C Content in Frozen Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	**LOCATION$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Atkinson	Atkinson Bog	-18.825	0.014	-34.646	-3.004
Atkinson	Barnard	-325.865	0.000	-341.686	-310.044
Atkinson	Bradford	-180.835	0.000	-196.656	-165.014
Atkinson	Brownville	-53.600	0.000	-69.421	-37.779
Atkinson	John Doores	-12.105	0.216	-27.926	3.716
Atkinson	Onawa Left	-21.665	0.004	-37.486	-5.844
Atkinson	Onawa Right	-19.830	0.009	-35.651	-4.009
Atkinson	Paine	-114.835	0.000	-130.656	-99.014
Atkinson	Parlin Pond	25.925	0.001	10.104	41.746
Atkinson	Praire	-120.925	0.000	-136.746	-105.104
Atkinson	Puddledock	-27.830	0.000	-43.651	-12.009
Atkinson	Whetstone	-26.060	0.001	-41.881	-10.239
Atkinson Bog	Barnard	-307.040	0.000	-322.861	-291.219
Atkinson Bog	Bradford	-162.010	0.000	-177.831	-146.189
Atkinson Bog	Brownville	-34.775	0.000	-50.596	-18.954
Atkinson Bog	John Doores	6.720	0.868	-9.101	22.541
Atkinson Bog	Onawa Left	-2.840	1.000	-18.661	12.981
Atkinson Bog	Onawa Right	-1.005	1.000	-16.826	14.816
Atkinson Bog	Paine	-96.010	0.000	-111.831	-80.189
Atkinson Bog	Parlin Pond	44.750	0.000	28.929	60.571
Atkinson Bog	Praire	-102.100	0.000	-117.921	-86.279
Atkinson Bog	Puddledock	-9.005	0.570	-24.826	6.816
Atkinson Bog	Whetstone	-7.235	0.812	-23.056	8.586
Barnard	Bradford	145.030	0.000	129.209	160.851
Barnard	Brownville	272.265	0.000	256.444	288.086
Barnard	John Doores	313.760	0.000	297.939	329.581
Barnard	Onawa Left	304.200	0.000	288.379	320.021
Barnard	Onawa Right	306.035	0.000	290.214	321.856
Barnard	Paine	211.030	0.000	195.209	226.851
Barnard	Parlin Pond	351.790	0.000	335.969	367.611
Barnard	Praire	204.940	0.000	189.119	220.761
Barnard	Puddledock	298.035	0.000	282.214	313.856
Barnard	Whetstone	299.805	0.000	283.984	315.626
Bradford	Brownville	127.235	0.000	111.414	143.056
Bradford	John Doores	168.730	0.000	152.909	184.551
Bradford	Onawa Left	159.170	0.000	143.349	174.991
Bradford	Onawa Right	161.005	0.000	145.184	176.826
Bradford	Paine	66.000	0.000	50.179	81.821
Bradford	Parlin Pond	206.760	0.000	190.939	222.581
Bradford	Praire	59.910	0.000	44.089	75.731
Bradford	Puddledock	153.005	0.000	137.184	168.826
Bradford	Whetstone	154.775	0.000	138.954	170.596
Brownville	John Doores	41.495	0.000	25.674	57.316
Brownville	Onawa Left	31.935	0.000	16.114	47.756

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Onawa Right	33.770	0.000	17.949	49.591
Brownville	Paine	-61.235	0.000	-77.056	-45.414
Brownville	Parlin Pond	79.525	0.000	63.704	95.346
Brownville	Praire	-67.325	0.000	-83.146	-51.504
Brownville	Puddledock	25.770	0.001	9.949	41.591
Brownville	Whetstone	27.540	0.000	11.719	43.361
John Doores	Onawa Left	-9.560	0.493	-25.381	6.261
John Doores	Onawa Right	-7.725	0.750	-23.546	8.096
John Doores	Paine	-102.730	0.000	-118.551	-86.909
John Doores	Parlin Pond	38.030	0.000	22.209	53.851
John Doores	Praire	-108.820	0.000	-124.641	-92.999
John Doores	Puddledock	-15.725	0.052	-31.546	0.096
John Doores	Whetstone	-13.955	0.107	-29.776	1.866
Onawa Left	Onawa Right	1.835	1.000	-13.986	17.656
Onawa Left	Paine	-93.170	0.000	-108.991	-77.349
Onawa Left	Parlin Pond	47.590	0.000	31.769	63.411
Onawa Left	Praire	-99.260	0.000	-115.081	-83.439
Onawa Left	Puddledock	-6.165	0.918	-21.986	9.656
Onawa Left	Whetstone	-4.395	0.992	-20.216	11.426
Onawa Right	Paine	-95.005	0.000	-110.826	-79.184
Onawa Right	Parlin Pond	45.755	0.000	29.934	61.576
Onawa Right	Praire	-101.095	0.000	-116.916	-85.274
Onawa Right	Puddledock	-8.000	0.712	-23.821	7.821
Onawa Right	Whetstone	-6.230	0.913	-22.051	9.591
Paine	Parlin Pond	140.760	0.000	124.939	156.581
Paine	Praire	-6.090	0.923	-21.911	9.731
Paine	Puddledock	87.005	0.000	71.184	102.826
Paine	Whetstone	88.775	0.000	72.954	104.596
Parlin Pond	Praire	-146.850	0.000	-162.671	-131.029
Parlin Pond	Puddledock	-53.755	0.000	-69.576	-37.934
Parlin Pond	Whetstone	-51.985	0.000	-67.806	-36.164
Praire	Puddledock	93.095	0.000	77.274	108.916
Praire	Whetstone	94.865	0.000	79.044	110.686
Puddledock	Whetstone	1.770	1.000	-14.051	17.591

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.6. List of Pairwise Comparisons of Total Vitamin C Content in Frozen Elderberries (Dry Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-5.435	0.083	-11.336	0.466
Atkinson	Barnard	-51.785	0.000	-57.686	-45.884
Atkinson	Bradford	-48.995	0.000	-54.896	-43.094
Atkinson	Brownville	-14.315	0.000	-20.216	-8.414
Atkinson	John Doores	-1.830	0.982	-7.731	4.071
Atkinson	Onawa Left	-34.765	0.000	-40.666	-28.864
Atkinson	Onawa Right	-6.545	0.024	-12.446	-0.644
Atkinson	Paine	-23.675	0.000	-29.576	-17.774
Atkinson	Parlin Pond	-8.335	0.003	-14.236	-2.434
Atkinson	Praire	-44.630	0.000	-50.531	-38.729
Atkinson	Puddledock	-2.740	0.798	-8.641	3.161
Atkinson	Whetstone	3.860	0.391	-2.041	9.761
Atkinson Bog	Barnard	-46.350	0.000	-52.251	-40.449
Atkinson Bog	Bradford	-43.560	0.000	-49.461	-37.659
Atkinson Bog	Brownville	-8.880	0.002	-14.781	-2.979
Atkinson Bog	John Doores	3.605	0.479	-2.296	9.506
Atkinson Bog	Onawa Left	-29.330	0.000	-35.231	-23.429
Atkinson Bog	Onawa Right	-1.110	1.000	-7.011	4.791
Atkinson Bog	Paine	-18.240	0.000	-24.141	-12.339
Atkinson Bog	Parlin Pond	-2.900	0.743	-8.801	3.001
Atkinson Bog	Praire	-39.195	0.000	-45.096	-33.294
Atkinson Bog	Puddledock	2.695	0.813	-3.206	8.596
Atkinson Bog	Whetstone	9.295	0.001	3.394	15.196
Barnard	Bradford	2.790	0.781	-3.111	8.691
Barnard	Brownville	37.470	0.000	31.569	43.371
Barnard	John Doores	49.955	0.000	44.054	55.856
Barnard	Onawa Left	17.020	0.000	11.119	22.921
Barnard	Onawa Right	45.240	0.000	39.339	51.141
Barnard	Paine	28.110	0.000	22.209	34.011
Barnard	Parlin Pond	43.450	0.000	37.549	49.351
Barnard	Praire	7.155	0.012	1.254	13.056
Barnard	Puddledock	49.045	0.000	43.144	54.946
Barnard	Whetstone	55.645	0.000	49.744	61.546
Bradford	Brownville	34.680	0.000	28.779	40.581
Bradford	John Doores	47.165	0.000	41.264	53.066
Bradford	Onawa Left	14.230	0.000	8.329	20.131
Bradford	Onawa Right	42.450	0.000	36.549	48.351
Bradford	Paine	25.320	0.000	19.419	31.221
Bradford	Parlin Pond	40.660	0.000	34.759	46.561
Bradford	Praire	4.365	0.249	-1.536	10.266
Bradford	Puddledock	46.255	0.000	40.354	52.156
Bradford	Whetstone	52.855	0.000	46.954	58.756
Brownville	John Doores	12.485	0.000	6.584	18.386
Brownville	Onawa Left	-20.450	0.000	-26.351	-14.549

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Onawa Right	7.770	0.006	1.869	13.671
Brownville	Paine	-9.360	0.001	-15.261	-3.459
Brownville	Parlin Pond	5.980	0.046	0.079	11.881
Brownville	Praire	-30.315	0.000	-36.216	-24.414
Brownville	Puddledock	11.575	0.000	5.674	17.476
Brownville	Whetstone	18.175	0.000	12.274	24.076
John Doores	Onawa Left	-32.935	0.000	-38.836	-27.034
John Doores	Onawa Right	-4.715	0.177	-10.616	1.186
John Doores	Paine	-21.845	0.000	-27.746	-15.944
John Doores	Parlin Pond	-6.505	0.026	-12.406	-0.604
John Doores	Praire	-42.800	0.000	-48.701	-36.899
John Doores	Puddledock	-0.910	1.000	-6.811	4.991
John Doores	Whetstone	5.690	0.063	-0.211	11.591
Onawa Left	Onawa Right	28.220	0.000	22.319	34.121
Onawa Left	Paine	11.090	0.000	5.189	16.991
Onawa Left	Parlin Pond	26.430	0.000	20.529	32.331
Onawa Left	Praire	-9.865	0.001	-15.766	-3.964
Onawa Left	Puddledock	32.025	0.000	26.124	37.926
Onawa Left	Whetstone	38.625	0.000	32.724	44.526
Onawa Right	Paine	-17.130	0.000	-23.031	-11.229
Onawa Right	Parlin Pond	-1.790	0.985	-7.691	4.111
Onawa Right	Praire	-38.085	0.000	-43.986	-32.184
Onawa Right	Puddledock	3.805	0.409	-2.096	9.706
Onawa Right	Whetstone	10.405	0.000	4.504	16.306
Paine	Parlin Pond	15.340	0.000	9.439	21.241
Paine	Praire	-20.955	0.000	-26.856	-15.054
Paine	Puddledock	20.935	0.000	15.034	26.836
Paine	Whetstone	27.535	0.000	21.634	33.436
Parlin Pond	Praire	-36.295	0.000	-42.196	-30.394
Parlin Pond	Puddledock	5.595	0.070	-0.306	11.496
Parlin Pond	Whetstone	12.195	0.000	6.294	18.096
Praire	Puddledock	41.890	0.000	35.989	47.791
Praire	Whetstone	48.490	0.000	42.589	54.391
Puddledock	Whetstone	6.600	0.023	0.699	12.501

Tukey's HSD calculations created using statistics run in SYSTAT.

Table G.7. List of Pairwise Comparisons of Ascorbic Acid Content in Freeze-Dried Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	**LOCATION$(j)**	**Difference**	**p-value**	**95.0% Confidence Interval**	
				Lower	**Upper**
Atkinson	Atkinson Bog	-22.215	0.000	-32.714	-11.716
Atkinson	Barnard	-204.735	0.000	-215.234	-194.236
Atkinson	Bodfish	43.530	0.000	33.031	54.029
Atkinson	Bradford	-286.075	0.000	-296.574	-275.576
Atkinson	Branford	-24.785	0.000	-35.284	-14.286
Atkinson	Brownville	-50.850	0.000	-61.349	-40.351
Atkinson	Jackman	5.235	0.817	-5.264	15.734
Atkinson	John Doores	-14.075	0.004	-24.574	-3.576
Atkinson	Masardis	-152.280	0.000	-162.779	-141.781
Atkinson	Onawa	46.500	0.000	36.001	56.999
Atkinson	Packard	-21.265	0.000	-31.764	-10.766
Atkinson	Paine	-126.165	0.000	-136.664	-115.666
Atkinson	Puddledock	69.395	0.000	58.896	79.894
Atkinson	Shin Pond	-43.945	0.000	-54.444	-33.446
Atkinson	The Forks	27.710	0.000	17.211	38.209
Atkinson	Whetstone	68.630	0.000	58.131	79.129
Atkinson Bog	Barnard	-182.520	0.000	-193.019	-172.021
Atkinson Bog	Bodfish	65.745	0.000	55.246	76.244
Atkinson Bog	Bradford	-263.860	0.000	-274.359	-253.361
Atkinson Bog	Branford	-2.570	1.000	-13.069	7.929
Atkinson Bog	Brownville	-28.635	0.000	-39.134	-18.136
Atkinson Bog	Jackman	27.450	0.000	16.951	37.949
Atkinson Bog	John Doores	8.140	0.236	-2.359	18.639
Atkinson Bog	Masardis	-130.065	0.000	-140.564	-119.566
Atkinson Bog	Onawa	68.715	0.000	58.216	79.214
Atkinson Bog	Packard	0.950	1.000	-9.549	11.449
Atkinson Bog	Paine	-103.950	0.000	-114.449	-93.451
Atkinson Bog	Puddledock	91.610	0.000	81.111	102.109
Atkinson Bog	Shin Pond	-21.730	0.000	-32.229	-11.231
Atkinson Bog	The Forks	49.925	0.000	39.426	60.424
Atkinson Bog	Whetstone	90.845	0.000	80.346	101.344
Barnard	Bodfish	248.265	0.000	237.766	258.764
Barnard	Bradford	-81.340	0.000	-91.839	-70.841
Barnard	Branford	179.950	0.000	169.451	190.449
Barnard	Brownville	153.885	0.000	143.386	164.384
Barnard	Jackman	209.970	0.000	199.471	220.469
Barnard	John Doores	190.660	0.000	180.161	201.159
Barnard	Masardis	52.455	0.000	41.956	62.954
Barnard	Onawa	251.235	0.000	240.736	261.734
Barnard	Packard	183.470	0.000	172.971	193.969
Barnard	Paine	78.570	0.000	68.071	89.069
Barnard	Puddledock	274.130	0.000	263.631	284.629
Barnard	Shin Pond	160.790	0.000	150.291	171.289
Barnard	The Forks	232.445	0.000	221.946	242.944

Tukey's Honestly-Significant-Difference Test

LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	273.365	0.000	262.866	283.864
Bodfish	Bradford	-329.605	0.000	-340.104	-319.106
Bodfish	Branford	-68.315	0.000	-78.814	-57.816
Bodfish	Brownville	-94.380	0.000	-104.879	-83.881
Bodfish	Jackman	-38.295	0.000	-48.794	-27.796
Bodfish	John Doores	-57.605	0.000	-68.104	-47.106
Bodfish	Masardis	-195.810	0.000	-206.309	-185.311
Bodfish	Onawa	2.970	0.998	-7.529	13.469
Bodfish	Packard	-64.795	0.000	-75.294	-54.296
Bodfish	Paine	-169.695	0.000	-180.194	-159.196
Bodfish	Puddledock	25.865	0.000	15.366	36.364
Bodfish	Shin Pond	-87.475	0.000	-97.974	-76.976
Bodfish	The Forks	-15.820	0.001	-26.319	-5.321
Bodfish	Whetstone	25.100	0.000	14.601	35.599
Bradford	Branford	261.290	0.000	250.791	271.789
Bradford	Brownville	235.225	0.000	224.726	245.724
Bradford	Jackman	291.310	0.000	280.811	301.809
Bradford	John Doores	272.000	0.000	261.501	282.499
Bradford	Masardis	133.795	0.000	123.296	144.294
Bradford	Onawa	332.575	0.000	322.076	343.074
Bradford	Packard	264.810	0.000	254.311	275.309
Bradford	Paine	159.910	0.000	149.411	170.409
Bradford	Puddledock	355.470	0.000	344.971	365.969
Bradford	Shin Pond	242.130	0.000	231.631	252.629
Bradford	The Forks	313.785	0.000	303.286	324.284
Bradford	Whetstone	354.705	0.000	344.206	365.204
Branford	Brownville	-26.065	0.000	-36.564	-15.566
Branford	Jackman	30.020	0.000	19.521	40.519
Branford	John Doores	10.710	0.043	0.211	21.209
Branford	Masardis	-127.495	0.000	-137.994	-116.996
Branford	Onawa	71.285	0.000	60.786	81.784
Branford	Packard	3.520	0.990	-6.979	14.019
Branford	Paine	-101.380	0.000	-111.879	-90.881
Branford	Puddledock	94.180	0.000	83.681	104.679
Branford	Shin Pond	-19.160	0.000	-29.659	-8.661
Branford	The Forks	52.495	0.000	41.996	62.994
Branford	Whetstone	93.415	0.000	82.916	103.914
Brownville	Jackman	56.085	0.000	45.586	66.584
Brownville	John Doores	36.775	0.000	26.276	47.274
Brownville	Masardis	-101.430	0.000	-111.929	-90.931
Brownville	Onawa	97.350	0.000	86.851	107.849
Brownville	Packard	29.585	0.000	19.086	40.084
Brownville	Paine	-75.315	0.000	-85.814	-64.816
Brownville	Puddledock	120.245	0.000	109.746	130.744
Brownville	Shin Pond	6.905	0.455	-3.594	17.404
Brownville	The Forks	78.560	0.000	68.061	89.059

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	119.480	0.000	108.981	129.979
Jackman	John Doores	-19.310	0.000	-29.809	-8.811
Jackman	Masardis	-157.515	0.000	-168.014	-147.016
Jackman	Onawa	41.265	0.000	30.766	51.764
Jackman	Packard	-26.500	0.000	-36.999	-16.001
Jackman	Paine	-131.400	0.000	-141.899	-120.901
Jackman	Puddledock	64.160	0.000	53.661	74.659
Jackman	Shin Pond	-49.180	0.000	-59.679	-38.681
Jackman	The Forks	22.475	0.000	11.976	32.974
Jackman	Whetstone	63.395	0.000	52.896	73.894
John Doores	Masardis	-138.205	0.000	-148.704	-127.706
John Doores	Onawa	60.575	0.000	50.076	71.074
John Doores	Packard	-7.190	0.397	-17.689	3.309
John Doores	Paine	-112.090	0.000	-122.589	-101.591
John Doores	Puddledock	83.470	0.000	72.971	93.969
John Doores	Shin Pond	-29.870	0.000	-40.369	-19.371
John Doores	The Forks	41.785	0.000	31.286	52.284
John Doores	Whetstone	82.705	0.000	72.206	93.204
Masardis	Onawa	198.780	0.000	188.281	209.279
Masardis	Packard	131.015	0.000	120.516	141.514
Masardis	Paine	26.115	0.000	15.616	36.614
Masardis	Puddledock	221.675	0.000	211.176	232.174
Masardis	Shin Pond	108.335	0.000	97.836	118.834
Masardis	The Forks	179.990	0.000	169.491	190.489
Masardis	Whetstone	220.910	0.000	210.411	231.409
Onawa	Packard	-67.765	0.000	-78.264	-57.266
Onawa	Paine	-172.665	0.000	-183.164	-162.166
Onawa	Puddledock	22.895	0.000	12.396	33.394
Onawa	Shin Pond	-90.445	0.000	-100.944	-79.946
Onawa	The Forks	-18.790	0.000	-29.289	-8.291
Onawa	Whetstone	22.130	0.000	11.631	32.629
Packard	Paine	-104.900	0.000	-115.399	-94.401
Packard	Puddledock	90.660	0.000	80.161	101.159
Packard	Shin Pond	-22.680	0.000	-33.179	-12.181
Packard	The Forks	48.975	0.000	38.476	59.474
Packard	Whetstone	89.895	0.000	79.396	100.394
Paine	Puddledock	195.560	0.000	185.061	206.059
Paine	Shin Pond	82.220	0.000	71.721	92.719
Paine	The Forks	153.875	0.000	143.376	164.374
Paine	Whetstone	194.795	0.000	184.296	205.294
Puddledock	Shin Pond	-113.340	0.000	-123.839	-102.841
Puddledock	The Forks	-41.685	0.000	-52.184	-31.186
Puddledock	Whetstone	-0.765	1.000	-11.264	9.734
Shin Pond	The Forks	71.655	0.000	61.156	82.154
Shin Pond	Whetstone	112.575	0.000	102.076	123.074
The Forks	Whetstone	40.920	0.000	30.421	51.419

Table G.8. List of Pairwise Comparisons of Ascorbic Acid Content in Freeze-Dried Elderberries (Dry Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-23.015	0.000	-33.853	-12.177
Atkinson	Barnard	-212.090	0.000	-222.928	-201.252
Atkinson	Bodfish	45.085	0.000	34.247	55.923
Atkinson	Bradford	-296.345	0.000	-307.183	-285.507
Atkinson	Branford	-25.680	0.000	-36.518	-14.842
Atkinson	Brownville	-52.680	0.000	-63.518	-41.842
Atkinson	Jackman	5.420	0.814	-5.418	16.258
Atkinson	John Doores	-14.585	0.004	-25.423	-3.747
Atkinson	Masardis	-157.755	0.000	-168.593	-146.917
Atkinson	Onawa	48.165	0.000	37.327	59.003
Atkinson	Packard	-22.230	0.000	-33.068	-11.392
Atkinson	Paine	-130.690	0.000	-141.528	-119.852
Atkinson	Puddledock	71.880	0.000	61.042	82.718
Atkinson	Shin Pond	-45.530	0.000	-56.368	-34.692
Atkinson	The Forks	28.700	0.000	17.862	39.538
Atkinson	Whetstone	71.090	0.000	60.252	81.928
Atkinson Bog	Barnard	-189.075	0.000	-199.913	-178.237
Atkinson Bog	Bodfish	68.100	0.000	57.262	78.938
Atkinson Bog	Bradford	-273.330	0.000	-284.168	-262.492
Atkinson Bog	Branford	-2.665	1.000	-13.503	8.173
Atkinson Bog	Brownville	-29.665	0.000	-40.503	-18.827
Atkinson Bog	Jackman	28.435	0.000	17.597	39.273
Atkinson Bog	John Doores	8.430	0.233	-2.408	19.268
Atkinson Bog	Masardis	-134.740	0.000	-145.578	-123.902
Atkinson Bog	Onawa	71.180	0.000	60.342	82.018
Atkinson Bog	Packard	0.785	1.000	-10.053	11.623
Atkinson Bog	Paine	-107.675	0.000	-118.513	-96.837
Atkinson Bog	Puddledock	94.895	0.000	84.057	105.733
Atkinson Bog	Shin Pond	-22.515	0.000	-33.353	-11.677
Atkinson Bog	The Forks	51.715	0.000	40.877	62.553
Atkinson Bog	Whetstone	94.105	0.000	83.267	104.943
Barnard	Bodfish	257.175	0.000	246.337	268.013
Barnard	Bradford	-84.255	0.000	-95.093	-73.417
Barnard	Branford	186.410	0.000	175.572	197.248
Barnard	Brownville	159.410	0.000	148.572	170.248
Barnard	Jackman	217.510	0.000	206.672	228.348
Barnard	John Doores	197.505	0.000	186.667	208.343
Barnard	Masardis	54.335	0.000	43.497	65.173
Barnard	Onawa	260.255	0.000	249.417	271.093
Barnard	Packard	189.860	0.000	179.022	200.698
Barnard	Paine	81.400	0.000	70.562	92.238
Barnard	Puddledock	283.970	0.000	273.132	294.808
Barnard	Shin Pond	166.560	0.000	155.722	177.398
Barnard	The Forks	240.790	0.000	229.952	251.628

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	283.180	0.000	272.342	294.018
Bodfish	Bradford	-341.430	0.000	-352.268	-330.592
Bodfish	Branford	-70.765	0.000	-81.603	-59.927
Bodfish	Brownville	-97.765	0.000	-108.603	-86.927
Bodfish	Jackman	-39.665	0.000	-50.503	-28.827
Bodfish	John Doores	-59.670	0.000	-70.508	-48.832
Bodfish	Masardis	-202.840	0.000	-213.678	-192.002
Bodfish	Onawa	3.080	0.998	-7.758	13.918
Bodfish	Packard	-67.315	0.000	-78.153	-56.477
Bodfish	Paine	-175.775	0.000	-186.613	-164.937
Bodfish	Puddledock	26.795	0.000	15.957	37.633
Bodfish	Shin Pond	-90.615	0.000	-101.453	-79.777
Bodfish	The Forks	-16.385	0.001	-27.223	-5.547
Bodfish	Whetstone	26.005	0.000	15.167	36.843
Bradford	Branford	270.665	0.000	259.827	281.503
Bradford	Brownville	243.665	0.000	232.827	254.503
Bradford	Jackman	301.765	0.000	290.927	312.603
Bradford	John Doores	281.760	0.000	270.922	292.598
Bradford	Masardis	138.590	0.000	127.752	149.428
Bradford	Onawa	344.510	0.000	333.672	355.348
Bradford	Packard	274.115	0.000	263.277	284.953
Bradford	Paine	165.655	0.000	154.817	176.493
Bradford	Puddledock	368.225	0.000	357.387	379.063
Bradford	Shin Pond	250.815	0.000	239.977	261.653
Bradford	The Forks	325.045	0.000	314.207	335.883
Bradford	Whetstone	367.435	0.000	356.597	378.273
Branford	Brownville	-27.000	0.000	-37.838	-16.162
Branford	Jackman	31.100	0.000	20.262	41.938
Branford	John Doores	11.095	0.042	0.257	21.933
Branford	Masardis	-132.075	0.000	-142.913	-121.237
Branford	Onawa	73.845	0.000	63.007	84.683
Branford	Packard	3.450	0.994	-7.388	14.288
Branford	Paine	-105.010	0.000	-115.848	-94.172
Branford	Puddledock	97.560	0.000	86.722	108.398
Branford	Shin Pond	-19.850	0.000	-30.688	-9.012
Branford	The Forks	54.380	0.000	43.542	65.218
Branford	Whetstone	96.770	0.000	85.932	107.608
Brownville	Jackman	58.100	0.000	47.262	68.938
Brownville	John Doores	38.095	0.000	27.257	48.933
Brownville	Masardis	-105.075	0.000	-115.913	-94.237
Brownville	Onawa	100.845	0.000	90.007	111.683
Brownville	Packard	30.450	0.000	19.612	41.288
Brownville	Paine	-78.010	0.000	-88.848	-67.172
Brownville	Puddledock	124.560	0.000	113.722	135.398
Brownville	Shin Pond	7.150	0.450	-3.688	17.988
Brownville	The Forks	81.380	0.000	70.542	92.218

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	123.770	0.000	112.932	134.608
Jackman	John Doores	-20.005	0.000	-30.843	-9.167
Jackman	Masardis	-163.175	0.000	-174.013	-152.337
Jackman	Onawa	42.745	0.000	31.907	53.583
Jackman	Packard	-27.650	0.000	-38.488	-16.812
Jackman	Paine	-136.110	0.000	-146.948	-125.272
Jackman	Puddledock	66.460	0.000	55.622	77.298
Jackman	Shin Pond	-50.950	0.000	-61.788	-40.112
Jackman	The Forks	23.280	0.000	12.442	34.118
Jackman	Whetstone	65.670	0.000	54.832	76.508
John Doores	Masardis	-143.170	0.000	-154.008	-132.332
John Doores	Onawa	62.750	0.000	51.912	73.588
John Doores	Packard	-7.645	0.355	-18.483	3.193
John Doores	Paine	-116.105	0.000	-126.943	-105.267
John Doores	Puddledock	86.465	0.000	75.627	97.303
John Doores	Shin Pond	-30.945	0.000	-41.783	-20.107
John Doores	The Forks	43.285	0.000	32.447	54.123
John Doores	Whetstone	85.675	0.000	74.837	96.513
Masardis	Onawa	205.920	0.000	195.082	216.758
Masardis	Packard	135.525	0.000	124.687	146.363
Masardis	Paine	27.065	0.000	16.227	37.903
Masardis	Puddledock	229.635	0.000	218.797	240.473
Masardis	Shin Pond	112.225	0.000	101.387	123.063
Masardis	The Forks	186.455	0.000	175.617	197.293
Masardis	Whetstone	228.845	0.000	218.007	239.683
Onawa	Packard	-70.395	0.000	-81.233	-59.557
Onawa	Paine	-178.855	0.000	-189.693	-168.017
Onawa	Puddledock	23.715	0.000	12.877	34.553
Onawa	Shin Pond	-93.695	0.000	-104.533	-82.857
Onawa	The Forks	-19.465	0.000	-30.303	-8.627
Onawa	Whetstone	22.925	0.000	12.087	33.763
Packard	Paine	-108.460	0.000	-119.298	-97.622
Packard	Puddledock	94.110	0.000	83.272	104.948
Packard	Shin Pond	-23.300	0.000	-34.138	-12.462
Packard	The Forks	50.930	0.000	40.092	61.768
Packard	Whetstone	93.320	0.000	82.482	104.158
Paine	Puddledock	202.570	0.000	191.732	213.408
Paine	Shin Pond	85.160	0.000	74.322	95.998
Paine	The Forks	159.390	0.000	148.552	170.228
Paine	Whetstone	201.780	0.000	190.942	212.618
Puddledock	Shin Pond	-117.410	0.000	-128.248	-106.572
Puddledock	The Forks	-43.180	0.000	-54.018	-32.342
Puddledock	Whetstone	-0.790	1.000	-11.628	10.048
Shin Pond	The Forks	74.230	0.000	63.392	85.068
Shin Pond	Whetstone	116.620	0.000	105.782	127.458
The Forks	Whetstone	42.390	0.000	31.552	53.228

Table G.9. List of Pairwise Comparisons of Dehhdroascorbic Acid Content in Freeze-Dried Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	4.495	1.000	-16.431	25.421
Atkinson	Barnard	-62.230	0.000	-83.156	-41.304
Atkinson	Bodfish	5.190	1.000	-15.736	26.116
Atkinson	Bradford	-47.280	0.000	-68.206	-26.354
Atkinson	Branford	19.360	0.086	-1.566	40.286
Atkinson	Brownville	-34.745	0.000	-55.671	-13.819
Atkinson	Jackman	17.910	0.140	-3.016	38.836
Atkinson	John Doores	-49.170	0.000	-70.096	-28.244
Atkinson	Masardis	-34.540	0.000	-55.466	-13.614
Atkinson	Onawa	11.595	0.697	-9.331	32.521
Atkinson	Packard	-84.275	0.000	-105.201	-63.349
Atkinson	Paine	-41.705	0.000	-62.631	-20.779
Atkinson	Puddledock	12.795	0.562	-8.131	33.721
Atkinson	Shin Pond	-10.635	0.798	-31.561	10.291
Atkinson	The Forks	-27.385	0.005	-48.311	-6.459
Atkinson	Whetstone	-9.095	0.919	-30.021	11.831
Atkinson Bog	Barnard	-66.725	0.000	-87.651	-45.799
Atkinson Bog	Bodfish	0.695	1.000	-20.231	21.621
Atkinson Bog	Bradford	-51.775	0.000	-72.701	-30.849
Atkinson Bog	Branford	14.865	0.346	-6.061	35.791
Atkinson Bog	Brownville	-39.240	0.000	-60.166	-18.314
Atkinson Bog	Jackman	13.415	0.492	-7.511	34.341
Atkinson Bog	John Doores	-53.665	0.000	-74.591	-32.739
Atkinson Bog	Masardis	-39.035	0.000	-59.961	-18.109
Atkinson Bog	Onawa	7.100	0.989	-13.826	28.026
Atkinson Bog	Packard	-88.770	0.000	-109.696	-67.844
Atkinson Bog	Paine	-46.200	0.000	-67.126	-25.274
Atkinson Bog	Puddledock	8.300	0.958	-12.626	29.226
Atkinson Bog	Shin Pond	-15.130	0.322	-36.056	5.796
Atkinson Bog	The Forks	-31.880	0.001	-52.806	-10.954
Atkinson Bog	Whetstone	-13.590	0.473	-34.516	7.336
Barnard	Bodfish	67.420	0.000	46.494	88.346
Barnard	Bradford	14.950	0.338	-5.976	35.876
Barnard	Branford	81.590	0.000	60.664	102.516
Barnard	Brownville	27.485	0.005	6.559	48.411
Barnard	Jackman	80.140	0.000	59.214	101.066
Barnard	John Doores	13.060	0.532	-7.866	33.986
Barnard	Masardis	27.690	0.004	6.764	48.616
Barnard	Onawa	73.825	0.000	52.899	94.751
Barnard	Packard	-22.045	0.033	-42.971	-1.119
Barnard	Paine	20.525	0.058	-0.401	41.451
Barnard	Puddledock	75.025	0.000	54.099	95.951
Barnard	Shin Pond	51.595	0.000	30.669	72.521
Barnard	The Forks	34.845	0.000	13.919	55.771

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	53.135	0.000	32.209	74.061
Bodfish	Bradford	-52.470	0.000	-73.396	-31.544
Bodfish	Branford	14.170	0.413	-6.756	35.096
Bodfish	Brownville	-39.935	0.000	-60.861	-19.009
Bodfish	Jackman	12.720	0.570	-8.206	33.646
Bodfish	John Doores	-54.360	0.000	-75.286	-33.434
Bodfish	Masardis	-39.730	0.000	-60.656	-18.804
Bodfish	Onawa	6.405	0.996	-14.521	27.331
Bodfish	Packard	-89.465	0.000	-110.391	-68.539
Bodfish	Paine	-46.895	0.000	-67.821	-25.969
Bodfish	Puddledock	7.605	0.979	-13.321	28.531
Bodfish	Shin Pond	-15.825	0.265	-36.751	5.101
Bodfish	The Forks	-32.575	0.001	-53.501	-11.649
Bodfish	Whetstone	-14.285	0.401	-35.211	6.641
Bradford	Branford	66.640	0.000	45.714	87.566
Bradford	Brownville	12.535	0.591	-8.391	33.461
Bradford	Jackman	65.190	0.000	44.264	86.116
Bradford	John Doores	-1.890	1.000	-22.816	19.036
Bradford	Masardis	12.740	0.568	-8.186	33.666
Bradford	Onawa	58.875	0.000	37.949	79.801
Bradford	Packard	-36.995	0.000	-57.921	-16.069
Bradford	Paine	5.575	0.999	-15.351	26.501
Bradford	Puddledock	60.075	0.000	39.149	81.001
Bradford	Shin Pond	36.645	0.000	15.719	57.571
Bradford	The Forks	19.895	0.072	-1.031	40.821
Bradford	Whetstone	38.185	0.000	17.259	59.111
Branford	Brownville	-54.105	0.000	-75.031	-33.179
Branford	Jackman	-1.450	1.000	-22.376	19.476
Branford	John Doores	-68.530	0.000	-89.456	-47.604
Branford	Masardis	-53.900	0.000	-74.826	-32.974
Branford	Onawa	-7.765	0.975	-28.691	13.161
Branford	Packard	-103.635	0.000	-124.561	-82.709
Branford	Paine	-61.065	0.000	-81.991	-40.139
Branford	Puddledock	-6.565	0.995	-27.491	14.361
Branford	Shin Pond	-29.995	0.002	-50.921	-9.069
Branford	The Forks	-46.745	0.000	-67.671	-25.819
Branford	Whetstone	-28.455	0.003	-49.381	-7.529
Brownville	Jackman	52.655	0.000	31.729	73.581
Brownville	John Doores	-14.425	0.387	-35.351	6.501
Brownville	Masardis	0.205	1.000	-20.721	21.131
Brownville	Onawa	46.340	0.000	25.414	67.266
Brownville	Packard	-49.530	0.000	-70.456	-28.604
Brownville	Paine	-6.960	0.990	-27.886	13.966
Brownville	Puddledock	47.540	0.000	26.614	68.466
Brownville	Shin Pond	24.110	0.016	3.184	45.036
Brownville	The Forks	7.360	0.984	-13.566	28.286

Tukey's Honestly-Significant-Difference Test

LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	25.650	0.009	4.724	46.576
Jackman	John Doores	-67.080	0.000	-88.006	-46.154
Jackman	Masardis	-52.450	0.000	-73.376	-31.524
Jackman	Onawa	-6.315	0.996	-27.241	14.611
Jackman	Packard	-102.185	0.000	-123.111	-81.259
Jackman	Paine	-59.615	0.000	-80.541	-38.689
Jackman	Puddledock	-5.115	1.000	-26.041	15.811
Jackman	Shin Pond	-28.545	0.003	-49.471	-7.619
Jackman	The Forks	-45.295	0.000	-66.221	-24.369
Jackman	Whetstone	-27.005	0.005	-47.931	-6.079
John Doores	Masardis	14.630	0.368	-6.296	35.556
John Doores	Onawa	60.765	0.000	39.839	81.691
John Doores	Packard	-35.105	0.000	-56.031	-14.179
John Doores	Paine	7.465	0.982	-13.461	28.391
John Doores	Puddledock	61.965	0.000	41.039	82.891
John Doores	Shin Pond	38.535	0.000	17.609	59.461
John Doores	The Forks	21.785	0.037	0.859	42.711
John Doores	Whetstone	40.075	0.000	19.149	61.001
Masardis	Onawa	46.135	0.000	25.209	67.061
Masardis	Packard	-49.735	0.000	-70.661	-28.809
Masardis	Paine	-7.165	0.988	-28.091	13.761
Masardis	Puddledock	47.335	0.000	26.409	68.261
Masardis	Shin Pond	23.905	0.017	2.979	44.831
Masardis	The Forks	7.155	0.988	-13.771	28.081
Masardis	Whetstone	25.445	0.010	4.519	46.371
Onawa	Packard	-95.870	0.000	-116.796	-74.944
Onawa	Paine	-53.300	0.000	-74.226	-32.374
Onawa	Puddledock	1.200	1.000	-19.726	22.126
Onawa	Shin Pond	-22.230	0.031	-43.156	-1.304
Onawa	The Forks	-38.980	0.000	-59.906	-18.054
Onawa	Whetstone	-20.690	0.054	-41.616	0.236
Packard	Paine	42.570	0.000	21.644	63.496
Packard	Puddledock	97.070	0.000	76.144	117.996
Packard	Shin Pond	73.640	0.000	52.714	94.566
Packard	The Forks	56.890	0.000	35.964	77.816
Packard	Whetstone	75.180	0.000	54.254	96.106
Paine	Puddledock	54.500	0.000	33.574	75.426
Paine	Shin Pond	31.070	0.001	10.144	51.996
Paine	The Forks	14.320	0.398	-6.606	35.246
Paine	Whetstone	32.610	0.001	11.684	53.536
Puddledock	Shin Pond	-23.430	0.020	-44.356	-2.504
Puddledock	The Forks	-40.180	0.000	-61.106	-19.254
Puddledock	Whetstone	-21.890	0.035	-42.816	-0.964
Shin Pond	The Forks	-16.750	0.202	-37.676	4.176
Shin Pond	Whetstone	1.540	1.000	-19.386	22.466
The Forks	Whetstone	18.290	0.124	-2.636	39.216

Table G.10. List of Pairwise Comparisons of Dehydroascorbic Acid Content in Freeze-Dried Elderberries (Dry Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	4.655	1.000	-16.997	26.307
Atkinson	Barnard	-64.465	0.000	-86.117	-42.813
Atkinson	Bodfish	5.380	1.000	-16.272	27.032
Atkinson	Bradford	-48.975	0.000	-70.627	-27.323
Atkinson	Branford	20.055	0.086	-1.597	41.707
Atkinson	Brownville	-35.995	0.000	-57.647	-14.343
Atkinson	Jackman	18.555	0.139	-3.097	40.207
Atkinson	John Doores	-50.935	0.000	-72.587	-29.283
Atkinson	Masardis	-35.785	0.000	-57.437	-14.133
Atkinson	Onawa	12.010	0.696	-9.642	33.662
Atkinson	Packard	-87.595	0.000	-109.247	-65.943
Atkinson	Paine	-43.200	0.000	-64.852	-21.548
Atkinson	Puddledock	13.255	0.560	-8.397	34.907
Atkinson	Shin Pond	-11.020	0.796	-32.672	10.632
Atkinson	The Forks	-28.370	0.005	-50.022	-6.718
Atkinson	Whetstone	-9.420	0.919	-31.072	12.232
Atkinson Bog	Barnard	-69.120	0.000	-90.772	-47.468
Atkinson Bog	Bodfish	0.725	1.000	-20.927	22.377
Atkinson Bog	Bradford	-53.630	0.000	-75.282	-31.978
Atkinson Bog	Branford	15.400	0.344	-6.252	37.052
Atkinson Bog	Brownville	-40.650	0.000	-62.302	-18.998
Atkinson Bog	Jackman	13.900	0.490	-7.752	35.552
Atkinson Bog	John Doores	-55.590	0.000	-77.242	-33.938
Atkinson Bog	Masardis	-40.440	0.000	-62.092	-18.788
Atkinson Bog	Onawa	7.355	0.988	-14.297	29.007
Atkinson Bog	Packard	-92.250	0.000	-113.902	-70.598
Atkinson Bog	Paine	-47.855	0.000	-69.507	-26.203
Atkinson Bog	Puddledock	8.600	0.957	-13.052	30.252
Atkinson Bog	Shin Pond	-15.675	0.320	-37.327	5.977
Atkinson Bog	The Forks	-33.025	0.001	-54.677	-11.373
Atkinson Bog	Whetstone	-14.075	0.472	-35.727	7.577
Barnard	Bodfish	69.845	0.000	48.193	91.497
Barnard	Bradford	15.490	0.336	-6.162	37.142
Barnard	Branford	84.520	0.000	62.868	106.172
Barnard	Brownville	28.470	0.004	6.818	50.122
Barnard	Jackman	83.020	0.000	61.368	104.672
Barnard	John Doores	13.530	0.530	-8.122	35.182
Barnard	Masardis	28.680	0.004	7.028	50.332
Barnard	Onawa	76.475	0.000	54.823	98.127
Barnard	Packard	-23.130	0.030	-44.782	-1.478
Barnard	Paine	21.265	0.057	-0.387	42.917
Barnard	Puddledock	77.720	0.000	56.068	99.372
Barnard	Shin Pond	53.445	0.000	31.793	75.097
Barnard	The Forks	36.095	0.000	14.443	57.747

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	55.045	0.000	33.393	76.697
Bodfish	Bradford	-54.355	0.000	-76.007	-32.703
Bodfish	Branford	14.675	0.411	-6.977	36.327
Bodfish	Brownville	-41.375	0.000	-63.027	-19.723
Bodfish	Jackman	13.175	0.569	-8.477	34.827
Bodfish	John Doores	-56.315	0.000	-77.967	-34.663
Bodfish	Masardis	-41.165	0.000	-62.817	-19.513
Bodfish	Onawa	6.630	0.996	-15.022	28.282
Bodfish	Packard	-92.975	0.000	-114.627	-71.323
Bodfish	Paine	-48.580	0.000	-70.232	-26.928
Bodfish	Puddledock	7.875	0.979	-13.777	29.527
Bodfish	Shin Pond	-16.400	0.263	-38.052	5.252
Bodfish	The Forks	-33.750	0.001	-55.402	-12.098
Bodfish	Whetstone	-14.800	0.399	-36.452	6.852
Bradford	Branford	69.030	0.000	47.378	90.682
Bradford	Brownville	12.980	0.590	-8.672	34.632
Bradford	Jackman	67.530	0.000	45.878	89.182
Bradford	John Doores	-1.960	1.000	-23.612	19.692
Bradford	Masardis	13.190	0.567	-8.462	34.842
Bradford	Onawa	60.985	0.000	39.333	82.637
Bradford	Packard	-38.620	0.000	-60.272	-16.968
Bradford	Paine	5.775	0.999	-15.877	27.427
Bradford	Puddledock	62.230	0.000	40.578	83.882
Bradford	Shin Pond	37.955	0.000	16.303	59.607
Bradford	The Forks	20.605	0.071	-1.047	42.257
Bradford	Whetstone	39.555	0.000	17.903	61.207
Branford	Brownville	-56.050	0.000	-77.702	-34.398
Branford	Jackman	-1.500	1.000	-23.152	20.152
Branford	John Doores	-70.990	0.000	-92.642	-49.338
Branford	Masardis	-55.840	0.000	-77.492	-34.188
Branford	Onawa	-8.045	0.975	-29.697	13.607
Branford	Packard	-107.650	0.000	-129.302	-85.998
Branford	Paine	-63.255	0.000	-84.907	-41.603
Branford	Puddledock	-6.800	0.994	-28.452	14.852
Branford	Shin Pond	-31.075	0.002	-52.727	-9.423
Branford	The Forks	-48.425	0.000	-70.077	-26.773
Branford	Whetstone	-29.475	0.003	-51.127	-7.823
Brownville	Jackman	54.550	0.000	32.898	76.202
Brownville	John Doores	-14.940	0.386	-36.592	6.712
Brownville	Masardis	0.210	1.000	-21.442	21.862
Brownville	Onawa	48.005	0.000	26.353	69.657
Brownville	Packard	-51.600	0.000	-73.252	-29.948
Brownville	Paine	-7.205	0.990	-28.857	14.447
Brownville	Puddledock	49.250	0.000	27.598	70.902
Brownville	Shin Pond	24.975	0.016	3.323	46.627
Brownville	The Forks	7.625	0.984	-14.027	29.277

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	26.575	0.009	4.923	48.227
Jackman	John Doores	-69.490	0.000	-91.142	-47.838
Jackman	Masardis	-54.340	0.000	-75.992	-32.688
Jackman	Onawa	-6.545	0.996	-28.197	15.107
Jackman	Packard	-106.150	0.000	-127.802	-84.498
Jackman	Paine	-61.755	0.000	-83.407	-40.103
Jackman	Puddledock	-5.300	1.000	-26.952	16.352
Jackman	Shin Pond	-29.575	0.003	-51.227	-7.923
Jackman	The Forks	-46.925	0.000	-68.577	-25.273
Jackman	Whetstone	-27.975	0.005	-49.627	-6.323
John Doores	Masardis	15.150	0.366	-6.502	36.802
John Doores	Onawa	62.945	0.000	41.293	84.597
John Doores	Packard	-36.660	0.000	-58.312	-15.008
John Doores	Paine	7.735	0.982	-13.917	29.387
John Doores	Puddledock	64.190	0.000	42.538	85.842
John Doores	Shin Pond	39.915	0.000	18.263	61.567
John Doores	The Forks	22.565	0.036	0.913	44.217
John Doores	Whetstone	41.515	0.000	19.863	63.167
Masardis	Onawa	47.795	0.000	26.143	69.447
Masardis	Packard	-51.810	0.000	-73.462	-30.158
Masardis	Paine	-7.415	0.988	-29.067	14.237
Masardis	Puddledock	49.040	0.000	27.388	70.692
Masardis	Shin Pond	24.765	0.017	3.113	46.417
Masardis	The Forks	7.415	0.988	-14.237	29.067
Masardis	Whetstone	26.365	0.010	4.713	48.017
Onawa	Packard	-99.605	0.000	-121.257	-77.953
Onawa	Paine	-55.210	0.000	-76.862	-33.558
Onawa	Puddledock	1.245	1.000	-20.407	22.897
Onawa	Shin Pond	-23.030	0.031	-44.682	-1.378
Onawa	The Forks	-40.380	0.000	-62.032	-18.728
Onawa	Whetstone	-21.430	0.054	-43.082	0.222
Packard	Paine	44.395	0.000	22.743	66.047
Packard	Puddledock	100.850	0.000	79.198	122.502
Packard	Shin Pond	76.575	0.000	54.923	98.227
Packard	The Forks	59.225	0.000	37.573	80.877
Packard	Whetstone	78.175	0.000	56.523	99.827
Paine	Puddledock	56.455	0.000	34.803	78.107
Paine	Shin Pond	32.180	0.001	10.528	53.832
Paine	The Forks	14.830	0.396	-6.822	36.482
Paine	Whetstone	33.780	0.001	12.128	55.432
Puddledock	Shin Pond	-24.275	0.020	-45.927	-2.623
Puddledock	The Forks	-41.625	0.000	-63.277	-19.973
Puddledock	Whetstone	-22.675	0.035	-44.327	-1.023
Shin Pond	The Forks	-17.350	0.201	-39.002	4.302
Shin Pond	Whetstone	1.600	1.000	-20.052	23.252
The Forks	Whetstone	18.950	0.123	-2.702	40.602

Table G.11. List of Pairwise Comparisons of Total Vitamin C Content in Freeze-Dried Elderberries (Fresh Weight)

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-17.715	0.285	-41.524	6.094
Atkinson	Barnard	-266.965	0.000	-290.774	-243.156
Atkinson	Bodfish	48.720	0.000	24.911	72.529
Atkinson	Bradford	-333.355	0.000	-357.164	-309.546
Atkinson	Branford	-5.425	1.000	-29.234	18.384
Atkinson	Brownville	-85.590	0.000	-109.399	-61.781
Atkinson	Jackman	23.145	0.061	-0.664	46.954
Atkinson	John Doores	-63.245	0.000	-87.054	-39.436
Atkinson	Masardis	-186.820	0.000	-210.629	-163.011
Atkinson	Onawa	58.095	0.000	34.286	81.904
Atkinson	Packard	-105.540	0.000	-129.349	-81.731
Atkinson	Paine	-167.870	0.000	-191.679	-144.061
Atkinson	Puddledock	82.190	0.000	58.381	105.999
Atkinson	Shin Pond	-54.580	0.000	-78.389	-30.771
Atkinson	The Forks	0.325	1.000	-23.484	24.134
Atkinson	Whetstone	59.535	0.000	35.726	83.344
Atkinson Bog	Barnard	-249.250	0.000	-273.059	-225.441
Atkinson Bog	Bodfish	66.435	0.000	42.626	90.244
Atkinson Bog	Bradford	-315.640	0.000	-339.449	-291.831
Atkinson Bog	Branford	12.290	0.781	-11.519	36.099
Atkinson Bog	Brownville	-67.875	0.000	-91.684	-44.066
Atkinson Bog	Jackman	40.860	0.000	17.051	64.669
Atkinson Bog	John Doores	-45.530	0.000	-69.339	-21.721
Atkinson Bog	Masardis	-169.105	0.000	-192.914	-145.296
Atkinson Bog	Onawa	75.810	0.000	52.001	99.619
Atkinson Bog	Packard	-87.825	0.000	-111.634	-64.016
Atkinson Bog	Paine	-150.155	0.000	-173.964	-126.346
Atkinson Bog	Puddledock	99.905	0.000	76.096	123.714
Atkinson Bog	Shin Pond	-36.865	0.001	-60.674	-13.056
Atkinson Bog	The Forks	18.040	0.263	-5.769	41.849
Atkinson Bog	Whetstone	77.250	0.000	53.441	101.059
Barnard	Bodfish	315.685	0.000	291.876	339.494
Barnard	Bradford	-66.390	0.000	-90.199	-42.581
Barnard	Branford	261.540	0.000	237.731	285.349
Barnard	Brownville	181.375	0.000	157.566	205.184
Barnard	Jackman	290.110	0.000	266.301	313.919
Barnard	John Doores	203.720	0.000	179.911	227.529
Barnard	Masardis	80.145	0.000	56.336	103.954
Barnard	Onawa	325.060	0.000	301.251	348.869
Barnard	Packard	161.425	0.000	137.616	185.234
Barnard	Paine	99.095	0.000	75.286	122.904
Barnard	Puddledock	349.155	0.000	325.346	372.964
Barnard	Shin Pond	212.385	0.000	188.576	236.194
Barnard	The Forks	267.290	0.000	243.481	291.099

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	326.500	0.000	302.691	350.309
Bodfish	Bradford	-382.075	0.000	-405.884	-358.266
Bodfish	Branford	-54.145	0.000	-77.954	-30.336
Bodfish	Brownville	-134.310	0.000	-158.119	-110.501
Bodfish	Jackman	-25.575	0.029	-49.384	-1.766
Bodfish	John Doores	-111.965	0.000	-135.774	-88.156
Bodfish	Masardis	-235.540	0.000	-259.349	-211.731
Bodfish	Onawa	9.375	0.960	-14.434	33.184
Bodfish	Packard	-154.260	0.000	-178.069	-130.451
Bodfish	Paine	-216.590	0.000	-240.399	-192.781
Bodfish	Puddledock	33.470	0.002	9.661	57.279
Bodfish	Shin Pond	-103.300	0.000	-127.109	-79.491
Bodfish	The Forks	-48.395	0.000	-72.204	-24.586
Bodfish	Whetstone	10.815	0.893	-12.994	34.624
Bradford	Branford	327.930	0.000	304.121	351.739
Bradford	Brownville	247.765	0.000	223.956	271.574
Bradford	Jackman	356.500	0.000	332.691	380.309
Bradford	John Doores	270.110	0.000	246.301	293.919
Bradford	Masardis	146.535	0.000	122.726	170.344
Bradford	Onawa	391.450	0.000	367.641	415.259
Bradford	Packard	227.815	0.000	204.006	251.624
Bradford	Paine	165.485	0.000	141.676	189.294
Bradford	Puddledock	415.545	0.000	391.736	439.354
Bradford	Shin Pond	278.775	0.000	254.966	302.584
Bradford	The Forks	333.680	0.000	309.871	357.489
Bradford	Whetstone	392.890	0.000	369.081	416.699
Branford	Brownville	-80.165	0.000	-103.974	-56.356
Branford	Jackman	28.570	0.011	4.761	52.379
Branford	John Doores	-57.820	0.000	-81.629	-34.011
Branford	Masardis	-181.395	0.000	-205.204	-157.586
Branford	Onawa	63.520	0.000	39.711	87.329
Branford	Packard	-100.115	0.000	-123.924	-76.306
Branford	Paine	-162.445	0.000	-186.254	-138.636
Branford	Puddledock	87.615	0.000	63.806	111.424
Branford	Shin Pond	-49.155	0.000	-72.964	-25.346
Branford	The Forks	5.750	1.000	-18.059	29.559
Branford	Whetstone	64.960	0.000	41.151	88.769
Brownville	Jackman	108.735	0.000	84.926	132.544
Brownville	John Doores	22.345	0.078	-1.464	46.154
Brownville	Masardis	-101.230	0.000	-125.039	-77.421
Brownville	Onawa	143.685	0.000	119.876	167.494
Brownville	Packard	-19.950	0.158	-43.759	3.859
Brownville	Paine	-82.280	0.000	-106.089	-58.471
Brownville	Puddledock	167.780	0.000	143.971	191.589
Brownville	Shin Pond	31.010	0.005	7.201	54.819
Brownville	The Forks	85.915	0.000	62.106	109.724

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	145.125	0.000	121.316	168.934
Jackman	John Doores	-86.390	0.000	-110.199	-62.581
Jackman	Masardis	-209.965	0.000	-233.774	-186.156
Jackman	Onawa	34.950	0.001	11.141	58.759
Jackman	Packard	-128.685	0.000	-152.494	-104.876
Jackman	Paine	-191.015	0.000	-214.824	-167.206
Jackman	Puddledock	59.045	0.000	35.236	82.854
Jackman	Shin Pond	-77.725	0.000	-101.534	-53.916
Jackman	The Forks	-22.820	0.068	-46.629	0.989
Jackman	Whetstone	36.390	0.001	12.581	60.199
John Doores	Masardis	-123.575	0.000	-147.384	-99.766
John Doores	Onawa	121.340	0.000	97.531	145.149
John Doores	Packard	-42.295	0.000	-66.104	-18.486
John Doores	Paine	-104.625	0.000	-128.434	-80.816
John Doores	Puddledock	145.435	0.000	121.626	169.244
John Doores	Shin Pond	8.665	0.979	-15.144	32.474
John Doores	The Forks	63.570	0.000	39.761	87.379
John Doores	Whetstone	122.780	0.000	98.971	146.589
Masardis	Onawa	244.915	0.000	221.106	268.724
Masardis	Packard	81.280	0.000	57.471	105.089
Masardis	Paine	18.950	0.208	-4.859	42.759
Masardis	Puddledock	269.010	0.000	245.201	292.819
Masardis	Shin Pond	132.240	0.000	108.431	156.049
Masardis	The Forks	187.145	0.000	163.336	210.954
Masardis	Whetstone	246.355	0.000	222.546	270.164
Onawa	Packard	-163.635	0.000	-187.444	-139.826
Onawa	Paine	-225.965	0.000	-249.774	-202.156
Onawa	Puddledock	24.095	0.046	0.286	47.904
Onawa	Shin Pond	-112.675	0.000	-136.484	-88.866
Onawa	The Forks	-57.770	0.000	-81.579	-33.961
Onawa	Whetstone	1.440	1.000	-22.369	25.249
Packard	Paine	-62.330	0.000	-86.139	-38.521
Packard	Puddledock	187.730	0.000	163.921	211.539
Packard	Shin Pond	50.960	0.000	27.151	74.769
Packard	The Forks	105.865	0.000	82.056	129.674
Packard	Whetstone	165.075	0.000	141.266	188.884
Paine	Puddledock	250.060	0.000	226.251	273.869
Paine	Shin Pond	113.290	0.000	89.481	137.099
Paine	The Forks	168.195	0.000	144.386	192.004
Paine	Whetstone	227.405	0.000	203.596	251.214
Puddledock	Shin Pond	-136.770	0.000	-160.579	-112.961
Puddledock	The Forks	-81.865	0.000	-105.674	-58.056
Puddledock	Whetstone	-22.655	0.071	-46.464	1.154
Shin Pond	The Forks	54.905	0.000	31.096	78.714
Shin Pond	Whetstone	114.115	0.000	90.306	137.924
The Forks	Whetstone	59.210	0.000	35.401	83.019

Table G.12. List of Pairwise Comparisons of Total Vitamin C Content in Freeze-Dried Elderberries (Dry Weight)

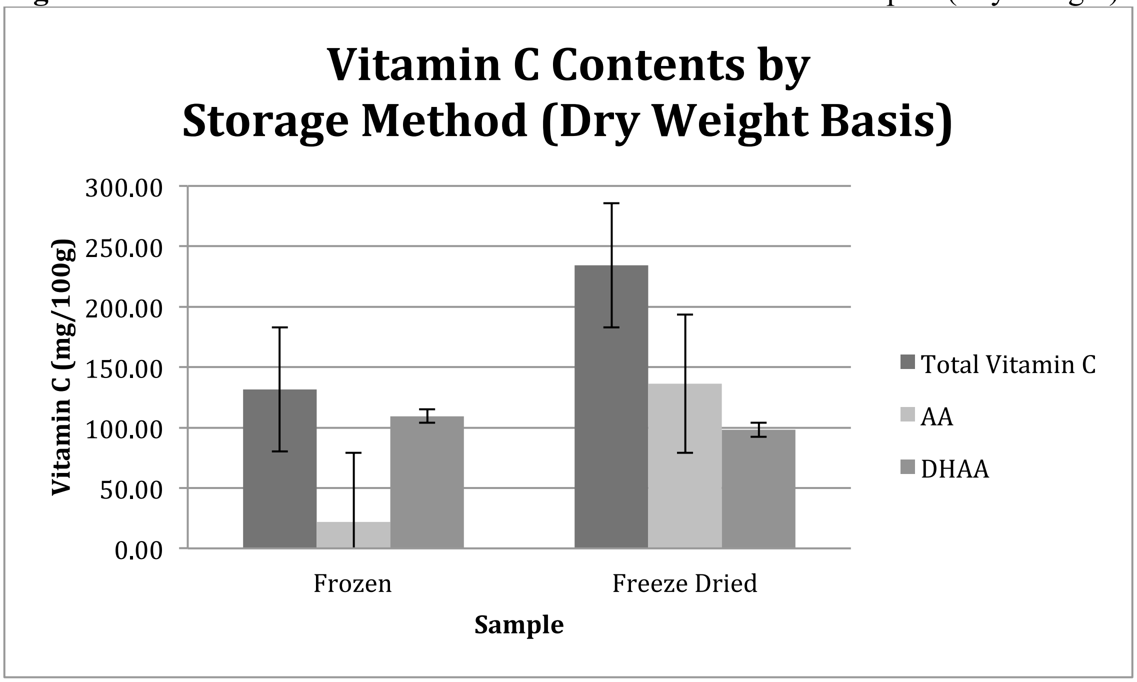
Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Atkinson	Atkinson Bog	-18.355	0.281	-42.945	6.235
Atkinson	Barnard	-276.550	0.000	-301.140	-251.960
Atkinson	Bodfish	50.470	0.000	25.880	75.060
Atkinson	Bradford	-345.315	0.000	-369.905	-320.725
Atkinson	Branford	-5.620	1.000	-30.210	18.970
Atkinson	Brownville	-88.675	0.000	-113.265	-64.085
Atkinson	Jackman	23.975	0.060	-0.615	48.565
Atkinson	John Doores	-65.515	0.000	-90.105	-40.925
Atkinson	Masardis	-193.535	0.000	-218.125	-168.945
Atkinson	Onawa	60.180	0.000	35.590	84.770
Atkinson	Packard	-109.820	0.000	-134.410	-85.230
Atkinson	Paine	-173.890	0.000	-198.480	-149.300
Atkinson	Puddledock	85.145	0.000	60.555	109.735
Atkinson	Shin Pond	-56.545	0.000	-81.135	-31.955
Atkinson	The Forks	0.335	1.000	-24.255	24.925
Atkinson	Whetstone	61.675	0.000	37.085	86.265
Atkinson Bog	Barnard	-258.195	0.000	-282.785	-233.605
Atkinson Bog	Bodfish	68.825	0.000	44.235	93.415
Atkinson Bog	Bradford	-326.960	0.000	-351.550	-302.370
Atkinson Bog	Branford	12.735	0.778	-11.855	37.325
Atkinson Bog	Brownville	-70.320	0.000	-94.910	-45.730
Atkinson Bog	Jackman	42.330	0.000	17.740	66.920
Atkinson Bog	John Doores	-47.160	0.000	-71.750	-22.570
Atkinson Bog	Masardis	-175.180	0.000	-199.770	-150.590
Atkinson Bog	Onawa	78.535	0.000	53.945	103.125
Atkinson Bog	Packard	-91.465	0.000	-116.055	-66.875
Atkinson Bog	Paine	-155.535	0.000	-180.125	-130.945
Atkinson Bog	Puddledock	103.500	0.000	78.910	128.090
Atkinson Bog	Shin Pond	-38.190	0.001	-62.780	-13.600
Atkinson Bog	The Forks	18.690	0.259	-5.900	43.280
Atkinson Bog	Whetstone	80.030	0.000	55.440	104.620
Barnard	Bodfish	327.020	0.000	302.430	351.610
Barnard	Bradford	-68.765	0.000	-93.355	-44.175
Barnard	Branford	270.930	0.000	246.340	295.520
Barnard	Brownville	187.875	0.000	163.285	212.465
Barnard	Jackman	300.525	0.000	275.935	325.115
Barnard	John Doores	211.035	0.000	186.445	235.625
Barnard	Masardis	83.015	0.000	58.425	107.605
Barnard	Onawa	336.730	0.000	312.140	361.320
Barnard	Packard	166.730	0.000	142.140	191.320
Barnard	Paine	102.660	0.000	78.070	127.250
Barnard	Puddledock	361.695	0.000	337.105	386.285
Barnard	Shin Pond	220.005	0.000	195.415	244.595
Barnard	The Forks	276.885	0.000	252.295	301.475

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Barnard	Whetstone	338.225	0.000	313.635	362.815
Bodfish	Bradford	-395.785	0.000	-420.375	-371.195
Bodfish	Branford	-56.090	0.000	-80.680	-31.500
Bodfish	Brownville	-139.145	0.000	-163.735	-114.555
Bodfish	Jackman	-26.495	0.028	-51.085	-1.905
Bodfish	John Doores	-115.985	0.000	-140.575	-91.395
Bodfish	Masardis	-244.005	0.000	-268.595	-219.415
Bodfish	Onawa	9.710	0.959	-14.880	34.300
Bodfish	Packard	-160.290	0.000	-184.880	-135.700
Bodfish	Paine	-224.360	0.000	-248.950	-199.770
Bodfish	Puddledock	34.675	0.002	10.085	59.265
Bodfish	Shin Pond	-107.015	0.000	-131.605	-82.425
Bodfish	The Forks	-50.135	0.000	-74.725	-25.545
Bodfish	Whetstone	11.205	0.890	-13.385	35.795
Bradford	Branford	339.695	0.000	315.105	364.285
Bradford	Brownville	256.640	0.000	232.050	281.230
Bradford	Jackman	369.290	0.000	344.700	393.880
Bradford	John Doores	279.800	0.000	255.210	304.390
Bradford	Masardis	151.780	0.000	127.190	176.370
Bradford	Onawa	405.495	0.000	380.905	430.085
Bradford	Packard	235.495	0.000	210.905	260.085
Bradford	Paine	171.425	0.000	146.835	196.015
Bradford	Puddledock	430.460	0.000	405.870	455.050
Bradford	Shin Pond	288.770	0.000	264.180	313.360
Bradford	The Forks	345.650	0.000	321.060	370.240
Bradford	Whetstone	406.990	0.000	382.400	431.580
Branford	Brownville	-83.055	0.000	-107.645	-58.465
Branford	Jackman	29.595	0.011	5.005	54.185
Branford	John Doores	-59.895	0.000	-84.485	-35.305
Branford	Masardis	-187.915	0.000	-212.505	-163.325
Branford	Onawa	65.800	0.000	41.210	90.390
Branford	Packard	-104.200	0.000	-128.790	-79.610
Branford	Paine	-168.270	0.000	-192.860	-143.680
Branford	Puddledock	90.765	0.000	66.175	115.355
Branford	Shin Pond	-50.925	0.000	-75.515	-26.335
Branford	The Forks	5.955	1.000	-18.635	30.545
Branford	Whetstone	67.295	0.000	42.705	91.885
Brownville	Jackman	112.650	0.000	88.060	137.240
Brownville	John Doores	23.160	0.077	-1.430	47.750
Brownville	Masardis	-104.860	0.000	-129.450	-80.270
Brownville	Onawa	148.855	0.000	124.265	173.445
Brownville	Packard	-21.145	0.136	-45.735	3.445
Brownville	Paine	-85.215	0.000	-109.805	-60.625
Brownville	Puddledock	173.820	0.000	149.230	198.410
Brownville	Shin Pond	32.130	0.005	7.540	56.720
Brownville	The Forks	89.010	0.000	64.420	113.600

Tukey's Honestly-Significant-Difference Test					
LOCATION$(i)	LOCATION$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Brownville	Whetstone	150.350	0.000	125.760	174.940
Jackman	John Doores	-89.490	0.000	-114.080	-64.900
Jackman	Masardis	-217.510	0.000	-242.100	-192.920
Jackman	Onawa	36.205	0.001	11.615	60.795
Jackman	Packard	-133.795	0.000	-158.385	-109.205
Jackman	Paine	-197.865	0.000	-222.455	-173.275
Jackman	Puddledock	61.170	0.000	36.580	85.760
Jackman	Shin Pond	-80.520	0.000	-105.110	-55.930
Jackman	The Forks	-23.640	0.066	-48.230	0.950
Jackman	Whetstone	37.700	0.001	13.110	62.290
John Doores	Masardis	-128.020	0.000	-152.610	-103.430
John Doores	Onawa	125.695	0.000	101.105	150.285
John Doores	Packard	-44.305	0.000	-68.895	-19.715
John Doores	Paine	-108.375	0.000	-132.965	-83.785
John Doores	Puddledock	150.660	0.000	126.070	175.250
John Doores	Shin Pond	8.970	0.978	-15.620	33.560
John Doores	The Forks	65.850	0.000	41.260	90.440
John Doores	Whetstone	127.190	0.000	102.600	151.780
Masardis	Onawa	253.715	0.000	229.125	278.305
Masardis	Packard	83.715	0.000	59.125	108.305
Masardis	Paine	19.645	0.204	-4.945	44.235
Masardis	Puddledock	278.680	0.000	254.090	303.270
Masardis	Shin Pond	136.990	0.000	112.400	161.580
Masardis	The Forks	193.870	0.000	169.280	218.460
Masardis	Whetstone	255.210	0.000	230.620	279.800
Onawa	Packard	-170.000	0.000	-194.590	-145.410
Onawa	Paine	-234.070	0.000	-258.660	-209.480
Onawa	Puddledock	24.965	0.045	0.375	49.555
Onawa	Shin Pond	-116.725	0.000	-141.315	-92.135
Onawa	The Forks	-59.845	0.000	-84.435	-35.255
Onawa	Whetstone	1.495	1.000	-23.095	26.085
Packard	Paine	-64.070	0.000	-88.660	-39.480
Packard	Puddledock	194.965	0.000	170.375	219.555
Packard	Shin Pond	53.275	0.000	28.685	77.865
Packard	The Forks	110.155	0.000	85.565	134.745
Packard	Whetstone	171.495	0.000	146.905	196.085
Paine	Puddledock	259.035	0.000	234.445	283.625
Paine	Shin Pond	117.345	0.000	92.755	141.935
Paine	The Forks	174.225	0.000	149.635	198.815
Paine	Whetstone	235.565	0.000	210.975	260.155
Puddledock	Shin Pond	-141.690	0.000	-166.280	-117.100
Puddledock	The Forks	-84.810	0.000	-109.400	-60.220
Puddledock	Whetstone	-23.470	0.070	-48.060	1.120
Shin Pond	The Forks	56.880	0.000	32.290	81.470
Shin Pond	Whetstone	118.220	0.000	93.630	142.810
The Forks	Whetstone	61.340	0.000	36.750	85.930

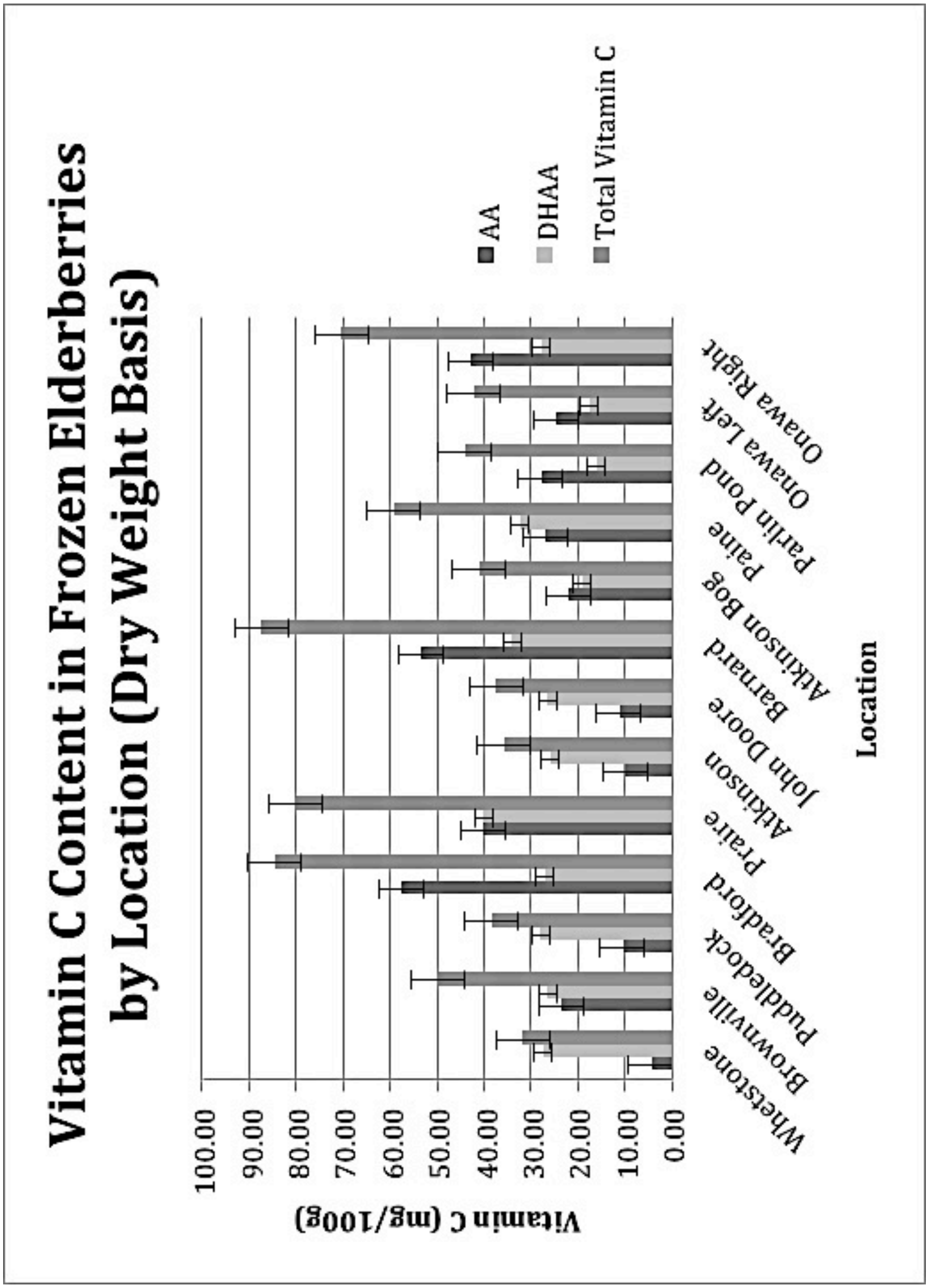
APPENDIX H: ELDERBERRY DRY WEIGHT BASIS CHARTS

Figure H.1. Vitamin C Contents of Freeze-Dried versus Frozen Samples (Dry Weight)



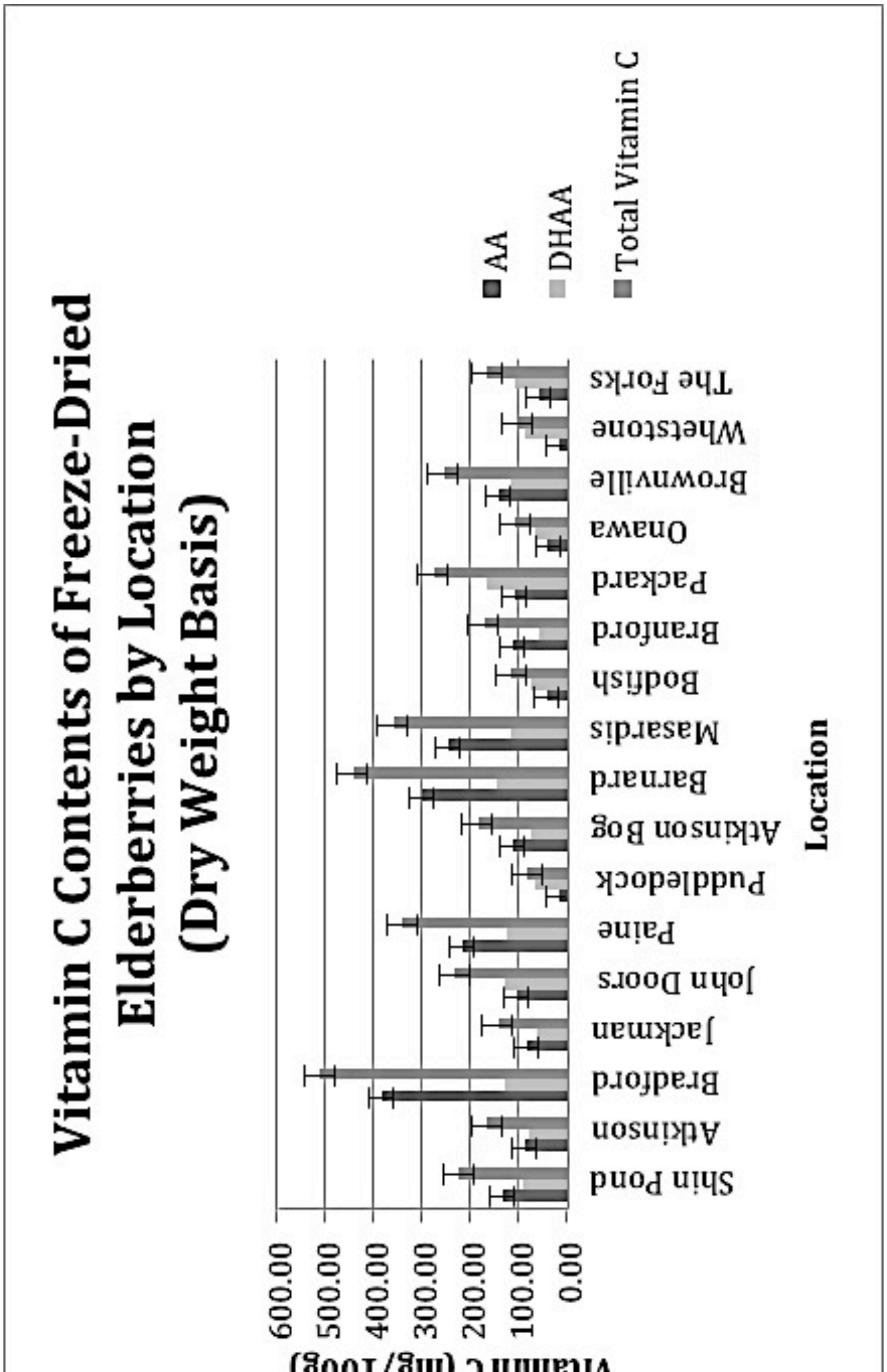
Average vitamin C contents based on 10 overlapping freeze dried and frozen samples. Error bars were created based on standard error.

Figure H.2. Vitamin C Contents of Frozen Elderberry Samples (Dry Weight)



Average nutrient contents listed for each variety based on statistics run in SYSTAT. Error bars were calculated based on standard error. Average nutrient values listed for duplicate samples are listed.

Figure H.3. Vitamin C Contents of Freeze-Dried Elderberry Samples (Dry Weight)



Average nutrient contents listed for each variety based on statistics run in SYSTAT. Error bars were calculated based on standard error. Average nutrient values listed for duplicate samples are listed.

BIOGRAPHY OF THE AUTHOR

Hannah D. Hutt was raised in Medway, Massachusetts and graduated from Medway Public High School in 2009. She attended Drexel University and graduated in 2013 with a Bachelor's of Science degree in Culinary Science with minors in Food Science, Human Nutrition, and Culinary Arts. She came to Maine and entered the Food Science and Human Nutrition graduate program at The University of Maine in the fall of 2013. Hannah is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in August 2015.