The University of Maine
DigitalCommons@UMaine

Electronic Theses and Dissertations | Fogler Library

Spring 5-6-2016

A competitive assessment of commercial elderberry (Sambucus sp.) products and the evaluation of copigmentation within elderberry tinctures

Joseph A. Galetti PhD
joegaletti@msn.com

Follow this and additional works at: http://digitalcommons.library.umaine.edu/etd

Part of the Dietetics and Clinical Nutrition Commons, Food Chemistry Commons, Food Processing Commons, and the Other Food Science Commons

Recommended Citation
Galetti, Joseph A. PhD, "A competitive assessment of commercial elderberry (Sambucus sp.) products and the evaluation of copigmentation within elderberry tinctures" (2016). *Electronic Theses and Dissertations*. 2693.
http://digitalcommons.library.umaine.edu/etd/2693

This Open-Access Dissertation is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of DigitalCommons@UMaine.

A COMPETITIVE ASSESSMENT COMMERCIAL ELDERBERRY (*SAMBUCUS* SP.) PRODUCTS

AND THE EVALUATION OF COPIGMENTATION WITHIN ELDERBERRY TINCTURES

By

Joseph A. Galetti

M.S. The University of Maine, 2010

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Food and Nutrition Sciences)

The Graduate School

The University of Maine

May 2016

Advisory Committee:

L. Brian Perkins, Research Assistant Professor of Food Science and Human Nutrition, Advisor
Rodney Bushway, Professor of Food Science and Human Nutrition
Denise Skonberg, Associate Professor of Food Science and Human Nutrition
Beth Calder, Associate Professor of Food Science and Human Nutrition
Christina Khoo, Senior Manager of Research Sciences, Ocean Spray Cranberries, Inc.

THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Joseph A. Galetti, 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, Research Assistant Professor of Food Science and Human Nutrition, School of Food and Agriculture, University of Maine

© 2016 Joseph A. Galetti
All Rights Reserved

LIBRARY RIGHTS STATEMENT

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at The University of Maine, I agree that the Library shall make it freely available for inspection. I further agree that permission for “fair use” copying of this thesis for scholarly purposes may be granted by the Librarian. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature:

Date:

A COMPETITIVE ASSESSMENT COMMERCIAL ELDERBERRY (*SAMBUCUS* SP.) PRODUCTS AND THE EVALUATION OF COPIGMENTATION WITHIN ELDERBERRY TINCTURES

By Joseph A. Galetti

Thesis Advisor: Dr. L. Brian Perkins

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
(in Food and Nutrition Sciences)
May 2016

Elderberry (*Sambucus* sp.) fruit is a healthful food with a variety of reported curative properties and is among the richest sources of anthocyanic pigmentation, which are the primary factors for its commercial use. Although a variety of value-added elderberry products are available to consumers, it is questionable as to which product form contains the highest nutrient levels and color stability characteristics, and represents the best value to consumers. It was the objectives of this research to evaluate a variety of commercial elderberry products, and to develop a value-added elderberry product with enhanced nutrient and color stability.

The first part of this research evaluated the nutrient and color stability of several value-added elderberry products (syrups, tinctures, concentrates, capsules, lozenges, dried fruit, powder) throughout 10 weeks of accelerated temperature (32° C) storage. Most of the products contained appreciable amounts of anthocyanins and other nutrients, which generally exceeded the values observed within raw elderberry fruit.

However, the elderberry tinctures contained low levels of anthocyanins, proanthocyanidins, and sugars; high levels of moisture/alcohol, and displayed poor nutrient and color stability throughout storage. The elderberry syrups, capsules, and lozenges generally displayed favorable phytochemical and color stability characteristics. Kerr Elderberry Concentrate and NP Nutra® Elderberry P.E. 10:1 powder contained substantial amounts of phytochemicals and pigmentation, which demonstrates their value within wholesale food markets.

The second part of this research determined the nutrient and color stability effects of copigment additives (rosemary extract, tannic acid, black carrot color, purple sweet potato color, enzymatically modified isoquercitrin) within elderberry tinctures throughout 6 weeks storage at 21° C. The results did not demonstrate effective copigmentation among any of the tinctures with copigment additives, which was likely due to the high ethanol content of the tinctures. All of the copigment additives contributed to increased phenolic contents and antioxidant activity within the tinctures, and black carrot and purple sweet potato color additives caused significant ($p \leq 0.05$) effects to the L*a*b* color values, monomeric anthocyanins, color density, and polymeric color of the tinctures. The results demonstrated that elderberry anthocyanins degraded into colorless products prior to converting into brown colored anthocyanin-tannin products throughout storage.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my parents and grandparents for a life-time of support and care, and for giving me the strength to accomplish my goals.

Secondly, I would like to thank Dr. Brian Perkins for inviting me into the analytical lab to continue my studies as a PhD student, and for your generous research support. I am greatly appreciative for the opportunity to complete my 'grand mission'.

I would like to thank Drs. Denise Skonberg, Beth Calder, Rodney Bushway, and Christina Khoo for your participation as part of my committee and for your expert tutelage. You're all obviously well qualified in your fields, and I feel well prepared to embark on future food science endeavors.

Special thanks to Dr. Jannie Marais, Dr. Geoff Woolford, and the entire research sciences department at Ocean Spray Cranberries, Inc. for inviting me into the team, and for your generous research support.

Additional thanks to Heath Hill Farm for your generous donation of elderberry fruit, which was utilized within this research to produce elderberry tinctures.

I dedicate this thesis to my mother, Deborah Galetti, who sacrificed her life so I could have a better one, and will always be my greatest hero.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..iv

LIST OF TABLES...ix

LIST OF FIGURES..xiii

CHAPTER 1. INTRODUCTION..1

Elderberry Ecology...1

Medicinal Uses for Elderberry..4

Elderberry Composition and Phytochemicals..7

Proanthocyanidin Analytical Methods...14

Elderberry Product Development and Marketing......................................17

Anthocyanin Copigmentation..25

Objectives..30

CHAPTER 2. A COMPETITIVE ASSESSMENT OF COMMERCIAL ELDERBERRY

(*SAMBUCUS* SP.) PRODUCTS...31

Objectives..31

Materials and Methods...31

Elderberry Products..31

Moisture...35

Water Activity...35

Soluble Solids..36

Total Proanthocyanidins...36

Proanthocyanidins Purification...36

Response Factor....39
Proanthocyanidins Recovery....40
Proanthocyanidins Assay....40
Titratable Acidity and Vitamin C....42
Spectrophotometric Color....43
L*a*b* Color....43
Sugars Profile....44
Anthocyanins Profile....45
Organic Acids Profile....47
Statistical Analyses....48
Results and Discussion....49
Syrups....49
Compositional Analyses at Week 0....49
Color Analyses at Week 0....56
Effects of Accelerated Storage....58
Tinctures....64
Compositional Analyses at Week 0....64
Color Analyses at Week 0....68
Effects of Accelerated Storage....69
Concentrates....72
Compositional Analyses at Week 0....72
Color Analyses at Week 0....75

Effects of Accelerated Storage..76

Capsules..78

Compositional Analyses at Week 0..78

Effects of Accelerated Storage..82

Lozenges..83

Compositional Analyses at Week 0..83

Effects of Accelerated Storage..87

Dried Fruit..88

Compositional Analyses at Week 0..88

Effects of Accelerated Storage..91

Powder..93

Compositional Analyses at Week 0..93

Effects of Accelerated Storage..95

Conclusions..96

CHAPTER 3. COPIGMENTATION OF ELDERBERRY (*SAMBUCUS NIGRA*) TINCTURES

TO ENHANCE NUTRIENT AND COLOR STABILITY THROUGHOUT STORAGE.......101

Objectives..101

Materials and Methods..101

Experimental Design..101

Preparation of Elderberry Tinctures..102

pH and Titratable Acidity..106

L*a*b* Color..107

Monomeric Anthocyanins..107

Color Density and Polymeric Color..108

Total Phenolics..109

Antioxidants..110

Statistical Analyses..111

Results and Discussion...112

pH and Titratable Acidity..112

L*a*b* Color..115

Monomeric Anthocyanins..122

Color Density and Polymeric Color..126

Total Phenolics..139

Antioxidants..144

Conclusions..154

CHAPTER 4. OVERALL CONCLUSIONS..159

REFERENCES...163

BIOGRAPHY OF THE AUTHOR..175

LIST OF TABLES

Table 1 Commercial Elderberry Products Analyzed in this Study....................................32

Table 2 Labeled Ingredients and Price of Elderberry Syrups..51

Table 3 Compositional Profiles of Elderberry Syrups at Week 0......................................52

Table 4 Sugar Profiles of Elderberry Syrups at Week 0...54

Table 5 Anthocyanin Profiles of Elderberry Syrups at Week 0..55

Table 6 Organic Acid Profiles of Elderberry Syrups at Week 0..56

Table 7 Labeled Ingredients and Price of Elderberry Tinctures.......................................66

Table 8 Compositional Profiles of Elderberry Tinctures at Week 0.................................67

Table 9 Sugar Profiles of Elderberry Tinctures at Week 0..67

Table 10 Anthocyanin Profiles of Elderberry Tinctures at Week 0..................................68

Table 11 Organic Acid Profiles of Elderberry Tinctures at Week 0..................................68

Table 12 Labeled Ingredients and Price of Elderberry Concentrates...............................73

Table 13 Compositional Profiles of Elderberry Concentrates at Week 0..........................74

Table 14 Sugar Profiles of Elderberry Concentrates at Week 0.......................................74

Table 15 Anthocyanin Profiles of Elderberry Concentrates at Week 0............................74

Table 16 Organic Acid Profiles of Elderberry Concentrates at Week 0............................75

Table 17 Labeled Ingredients and Price of Elderberry Capsules.....................................80

Table 18 Compositional Profiles of Elderberry Capsules at Week 0................................80

Table 19 Sugar Profiles of Elderberry Capsules at Week 0..81

Table 20 Anthocyanin Profiles of Elderberry Capsules at Week 0..................................81

Table 21 Organic Acid Profiles of Elderberry Capsules at Week 0..................................82

Table 22 Labeled Ingredients and Price Information of Elderberry Lozenges..................85

Table 23 Compositional Profiles of Elderberry Lozenges at Week 0................................86

Table 24 Sugar Profiles of Elderberry Lozenges at Week 0...86

Table 25 Anthocyanin Profiles of Elderberry Lozenges at Week 0..................................86

Table 26 Organic Acid Profiles of Elderberry Lozenges at Week 0..................................87

Table 27 Labeled Ingredients and Price of Dried Elderberry Fruit...................................89

Table 28 Compositional Profiles of Dried Elderberry Fruit at Week 0.............................90

Table 29 Sugar Profiles of Dried Elderberry Fruit at Week 0...90

Table 30 Anthocyanin Profiles of Dried Elderberry Fruit at Week 0................................90

Table 31 Organic Acid Profiles of Dried Elderberry Fruit at Week 0................................91

Table 32 Compositional Profile of Elderberry Powder at Week 0...................................94

Table 33 Sugar Profile of Elderberry Powder at Week 0..94

Table 34 Anthocyanin Profile of Elderberry Powder at Week 0......................................95

Table 35 Organic Acid Profile of Elderberry Powder at Week 0......................................95

Table 36 Concentration of Each Additive Used for the Copigmentation of
Elderberry Tinctures..106

Table 37 pH Values of Elderberry Tinctures by Copigment Treatment Over
Storage Time...113

Table 38 Titratable Acidity Values (% Citric Acid) of Elderberry Tinctures by Copigment
Treatment Over Storage Time...115

Table 39 Mean L*a*b* Color Values of Elderberry Tinctures by Copigment
Treatment..116

Table 40 L* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time......117

Table 41 a* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time......119

Table 42 b* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time......121

Table 43 Mean Monomeric Anthocyanin Values (mg/L) of Elderberry Tinctures by Copigment Treatment......124

Table 44 Mean Monomeric Anthocyanin Values (mg/L) of Elderberry Tinctures by Copigment Treatment Over Storage Time......125

Table 45 Mean Color Density Values of Elderberry Tinctures by Copigment Treatment......127

Table 46 Color Density Values of Elderberry Tinctures by Copigment Treatment Over Storage Time......129

Table 47 Mean Polymeric Color Values of Elderberry Tinctures by Copigment Treatment......132

Table 48 Polymeric Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time......134

Table 49 Mean Polymeric Color Values (%) of Elderberry Tinctures by Copigment Treatment......137

Table 50 Percent Polymeric Color of Elderberry Tinctures by Copigment Treatment Over Storage Time......138

Table 51 Mean Total Phenolic Values (mg GAE/L) of Elderberry Tinctures by Copigment Treatment....141

Table 52 Total Phenolic Values (mg GAE/L) of Elderberry Tinctures by Copigment Treatment Over Storage Time....143

Table 53 Mean Inhibition of DPPH Radical per 20 mg of Elderberry Tinctures (%) by Copigment Treatment....146

Table 54 Percent Inhibition of DPPH Radical per 20 mg of Elderberry Tinctures by Copigment Treatment Over Storage Time....147

Table 55 Mean IC_{50} (mg) of DPPH Radical Values of Elderberry Tinctures by Copigment Treatment....149

Table 56 IC_{50} (mg) of DPPH Radical Values of Elderberry Tinctures by Copigment Treatment Over Storage Time....150

Table 57 Mean Total Antioxidant Values (µg TE/100 g) of Elderberry Tinctures by Copigment Treatment....152

Table 58 Total Antioxidant Values (µg TE/100 g) of Elderberry Tinctures by Copigment Treatment Over Storage Time....153

LIST OF FIGURES

Figure 1 Illustration of elderberry (*Sambucus nigra*) stem..3

Figure 2 Illustration of anthocyanin structure...10

Figure 3 Value-added elderberry products analyzed in this study..................................34

Figure 4 Isolation of elderberry proanthocyanidins using Sephadex® LH-20 gel chromatography..38

Figure 5 L*a*b* color values of elderberry syrups at Week 0..58

Figure 6 Vitamin C values (mg/100 g) of elderberry syrups throughout weeks of accelerated temperature (32° C) storage...60

Figure 7 520/430 ratios of elderberry syrups throughout weeks of accelerated temperature (32° C) storage..62

Figure 8 Total anthocyanin values (mg/L) of elderberry syrups throughout weeks of accelerated temperature (32° C) storage...63

Figure 9 520/430 ratios of elderberry tinctures throughout weeks of accelerated temperature (32° C) storage...71

Figure 10 Total anthocyanin values (mg/L) of elderberry tinctures throughout weeks of accelerated temperature (32° C) storage...72

Figure 11 Spectrophotometric color values of elderberry concentrates at Week 0.........76

Figure 12 520/430 ratios of elderberry concentrates throughout weeks of accelerated temperature (32° C) storage...77

Figure 13 Total proanthocyanidin values (mg/L) of elderberry concentrates throughout weeks of accelerated temperature (32° C) storage..........................78

Figure 14 Total anthocyanin values (ppm) of elderberry capsules throughout weeks of accelerated temperature (32° C) storage..83

Figure 15 Total anthocyanins (ppm) of elderberry lozenges throughout weeks of accelerated temperature (32° C) storage..88

Figure 16 Total proanthocyanidins (ppm) of dried elderberry fruit throughout weeks of accelerated temperature (32° C) storage..92

Figure 17 Total anthocyanins (ppm) of dried elderberry fruit throughout weeks of accelerated temperature (32° C) storage..93

Figure 18 Total proanthocyanidins (ppm) of NP Nutra® Elderberry P.E. 10:1 powder throughout weeks of accelerated temperature (32° C) storage..96

Figure 19 Flow chart of the preparation of elderberry tinctures and treatment groups..105

Figure 20 Mean L* color values among copigment treatments overstorage time..118

Figure 21 Mean a* color values among copigment treatments over storage time..119

Figure 22 Mean b* color values among copigment treatments over storage time..121

Figure 23 Mean total monomeric anthocyanin values (mg/L) among copigment treatments over storage time..126

Figure 24 Mean color density values among copigment treatments over storage time....129

Figure 25 Mean polymeric color values among copigment treatments over storage time....134

Figure 26 Mean polymeric color values (%) among copigment treatments over storage time....139

Figure 27 Mean total phenolic values (mg GAE/L) among copigment treatments over storage time....144

Figure 28 Mean inhibition of DPPH radical per 20 mg of elderberry tinctures (%) among copigment treatments over storage time....148

Figure 29 Mean IC_{50} (mg) of DPPH radical values among copigment treatments over storage time....151

Figure 30 Mean total antioxidant values (µg TE/100 g) among copigment treatments over storage time....154

CHAPTER 1. INTRODUCTION

Elderberry Ecology

Elderberry (*Sambucus* sp.) is a genus of deciduous shrubs consisting of approximately thirty different sub-species, which typically grow in temperate and sub-tropical regions of both the Northern and Southern Hemispheres. Elderberry is found throughout the majority of Canada, Europe, and the United States, as well as regions of Asia, Australia, and South America (Byers and others 2012). Regionally it grows in and around the Sonoran Desert within Mexico, in Western and Northwestern South America, in Eastern and Northeastern Asia, in Eastern and Southeastern Australia, throughout Australasia, and within the Northern Mideast (Martin and Mott 1997). The two most prevalent varieties of elderberry are the American elderberry (*Sambucus canadensis*) and the European elderberry (*Sambucus nigra*), which are both categorized within the black elderberry (*Sambucus nigra*) species. Red elderberries (*Sambucus racemosa*) are also common within the United States and Asia, however, its fruit is not utilized for human consumption due to high levels of cyanogenic glycoside toxins (Losey and others 2003).

Elderberry leaves are structured pinnately, are typically between five and twenty centimeters in length, and generally contain between five and eleven leaflets, with singly toothed serrated edges (Martin and Mott 1997, Charlebois 2007) (Figure 1). Elderberry shrubs grow between six and twelve feet tall in fertile semi-dry, wet sandy, or bottomland soils, and prefer a soil pH between 5.5 and 7.5 (Martin and Mott 1997,

Byers and others 2012). It thrives in partially sunny or sunny areas (Byers and others 2012). Elderberry fruit grows in large umbrella-shaped clusters from showy white flowers, and ripens from July to September (Martin and Mott 1997, Byers and others 2012). The harvest of elderberry fruit is a labor intensive process due to the lack of mechanized harvesting equipment. Strang (2012) estimates for every ⅕ acre of elderberry shrubs, 130 hours for harvesting/freezing and 20 hours for processing are required, which equates to approximately $2,965 of total expenses per ⅕ acre. Mature elderberry plants yield between two to four tons of fruit per acre, or eight kilograms per plant, respectively (Charlebois 2007, Byers and others 2012, Strang 2012). Elderberry fruit can be separated from the stem by freezing and shaking off, or by stripping the fruit (Strang 2012).

Figure 1 Illustration of elderberry (*Sambucus nigra*) stem.
Used with permission from the Southwest School of Botanical Medicine (www.swsbm.com).

The fruit is considered a very desirable food source for a wide variety of animals such as songbirds, game birds, mice, rats, opossums, raccoons, squirrels, rabbits, woodchucks, foxes, bears, livestock, and deer (Martin and Mott 1997). Elderberry flowers attract butterflies and bees, and are considered a valuable resource for nectar.

The traditional breeding of elderberry has yielded several commercial cultivars which produce large flower and fruit clusters. Among these cultivars are York, Kent, Scotia, Johns, Nova, Victoria and Adams, which can often be purchased at commercial nurseries (Martin and Mott 1997, Charlebois 2007). Commercial elderberries are tolerant to insects and disease, however, infrequent tomato ringspot, elderberry sawfly, twig canker, verticillium wilt, powdery mildew, leaf spot, thread blight, borers, flea beetles, grape mealybugs, and thrips can cause deleterious effects to elderberry crops (Martin and Mott 1997, Byers and others 2012, Strang 2012).

Medicinal Uses for Elderberry

Early medicinal uses for elderberries have been documented back to indigenous Native American tribes and Europeans during the Middle Ages (French 1651, Blochwitz 1677, Borchers and others 2000, Barak and others 2002, Losey and others 2003). It has been used for centuries to treat a variety of illnesses, such as rheumatism, fever, plague and edema, and has long been used as a diaphoretic and natural antibiotic (Charlebois 2007). The fruit and flowers of elderberry contain tannins, flavonoids, and rutin, which improve immune function, as well as reduce bleeding, congestion, and diarrhea (Stevens 2001, Vespalcova and others 2011).

Current research has evaluated the effects of black elderberry syrup formulations on the production of inflammatory cytokines, and has shown that the syrup had a strong stimulatory effect on cytokine production by human monocytes (Barak and others 2001, Barak and others 2002, Waknine-Grinberg 2009). In addition, it

was reported that the elderberry extract formulation which contained the highest level of elderberry syrup resulted in the greatest production of cytokines, thusly increasing inflammatory response and overall immune function (Barak and others 2001, Barak and others 2002). It was also suggested that elderberry syrup may increase the production of hematopoietic growth factor GM-CSF and lymphocytes, which may have positive implications for people with decreased immune functions (Barak and others 2001, Barak and others 2002). Children, elderly, and immunocompromised people are at greatest risk from flu related symptoms due to low cytotoxic T-lymphocyte activity, which is responsible for viral recovery (Cox and others 2004).

Burge and others (1999) demonstrated the effect of the black elderberry extract Sambucol® (Razei Bar, Jerusalem, Israel) on chimpanzees infected with influenza over a period of approximately six months. Chimpanzees in the control group exhibited flu-like symptoms throughout thirty-nine days, whereas chimpanzees in the Sambucol® group exhibited flu-like symptoms throughout only twelve days of the study (Burge and others 1999). In a similar study, Zakay-Rones and others (2004) standardized an elderberry extract based on flavonoid content and investigated its effect on sixty patients suffering from influenza-like symptoms (coughing, mucus discharge, poor quality of sleep, nasal congestion) in a randomized, double-blind, placebo-controlled experiment. Patients recorded their symptoms using an analogue scale of 0=‘no improvement' to 10=‘pronounced improvement’. The researchers reported scores near ‘pronounced improvement’ after 3.1 days in the elderberry group compared to 7.1 days in the placebo group (Zakay-Rones and others 2004). In addition, the use of rescue

medications (antipyretic/analgesic paracetamol, acetylsalicylic pain killer, nasal sprays) was significantly ($p<0.001$) less in the elderberry group compared to the placebo group (Zakay-Rones and others 2004). Interestingly, these results may indicate that elderberry fruit is a better antiviral agent than common antiviral drugs, due to good efficacy and the lack of harmful side effects. Whereas, neuraminidase inhibitors and vaccinations do not universally protect against the wide array of ever-changing influenza strains, and vaccinations are only 60%-90% effective (Cox and others 2004).

Elderberry fruit is a rich source of anthocyanins, which serve as natural antioxidants and are considered to provide a wide range of curative properties. Antioxidants neutralize free radicals, which assists in the reduction in age-associated oxidative stress and cellular damage, as well as improve cognitive brain function, maintain normal vascular permeability, improve endothelial function, and prevent inflammation and cancer (Youdim and others 2000, Bagchi and others 2004, Thole and others 2006, Elisia and others 2007, Jing and others 2008). In addition, antioxidants have been shown to have a protective effect from damage caused by irradiation (Youdim and others 2000).

In addition to elderberry fruit, research shows that elderberry flowers have medicinal applications. Mild teas prepared from elderberry flowers have been used to break fevers, reduce headaches, promote perspiration, relieve indigestion, reduce the effects of edema, alleviate rheumatism, as well as fight the effects of colds, influenza, tuberculosis, and bladder or kidney infections (Stevens 2001, Merica and others 2006, Charlebois 2007).

Elderberry Composition and Phytochemicals

The proximate composition of fresh elderberry fruit has been reported to be 79.8% moisture, 11.4% sugars, 7.0% fiber (28% daily value/100 g), 0.7% protein, 0.6% ash and 0.5% polyunsaturated fatty acids, respectively (Anonymous 2009). Fresh elderberry fruit also contains appreciable amounts of vitamin C (29.0 mg/100 g or 48% daily value/100 g), vitamin A (27.1 µg RE/100 g or 1.8% daily value/100 g), vitamin B6 (0.18 g/100 g or 10% daily value/100 g), iron (1.6 mg/100 g or 9% daily value/100 g), potassium (321.0 mg/100 g or 6.8% daily value/100 g), and calcium (47.4 mg/100 g or 4.7% daily value/100 g) (Anonymous 2009). Polyphenol oxidase and peroxidase, which cause the rapid polymerization of red pigments and other polyphenols, have been detected within raw elderberry fruit at 0.51 and 0.66 units of activity/g (Galić and others 2009). For elderberry juice, Byers and others (2012) reported total soluble solids levels of 11-12° brix, pH of 4.5-5.0, and malic acid equivalent titratable acidity of 0.60-0.70 g/100 ml.

Elderberry fruit has been shown to contain a variety of biologically active phenolic compounds within the flavonoid and non-flavonoid classes. Within the flavonoid group, elderberries contain the flavonols quercetin, kaempferol and rutin; the anthocyanidins cyanidin and its anthocyanin derivatives; as well as the flavanol proanthocyanidins are found within elderberry fruit. Of the non-flavonoids, hydroxycinnamic acid derivates (cinnamic, chlorogenic, neochlorogenic, and caffeic acids), as well as malic, benzoic and palmitic acids are most prevalent. Of all of the phenolic compounds found in elderberry fruit, anthocyanins are most abundant,

followed by flavonols (tannins, proanthocyanidins), flavan-3-ols, flavones, and non-flavonoids (Han and others 2007, Jakobek and others 2007). The major phenolic compounds in elderberry must and wine have been identified as chlorogenic acid, neochlorogenic acid, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-rutinoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside and cyanidin-3-rutinoside (Johansen and others 1991, Schmitzer and others 2010). Galić and others (2009) determined the total phenolic and anthocyanin contents of wild elderberry fruit to be 32.2 mg/g and 13.1 mg/g on a dry weight basis, whereas Wu and others (2006) reported an average anthocyanin content of 11.3 mg/g on a dry weight basis for *Sambucus nigra* cultivars. Özgen and others (2010) reported that anthocyanins comprised between 41.4% and 87.9% of the total phenolics identified among fourteen American elderberry accessions. The major flavanol in elderberry fruit is quercetin, with an average of 17.1 mg/100 g on a fresh weight basis (Kyle and Duthie 2006). The healthful benefits of these compounds advocate the growth of elderberry cultivars that are high in anthocyanins and other polyphenolic compounds. Among all plants found in nature, catechin and epicatechin account for the largest amount of flavonoids, of which are present in elderberry fruit (Marais and others 2006).

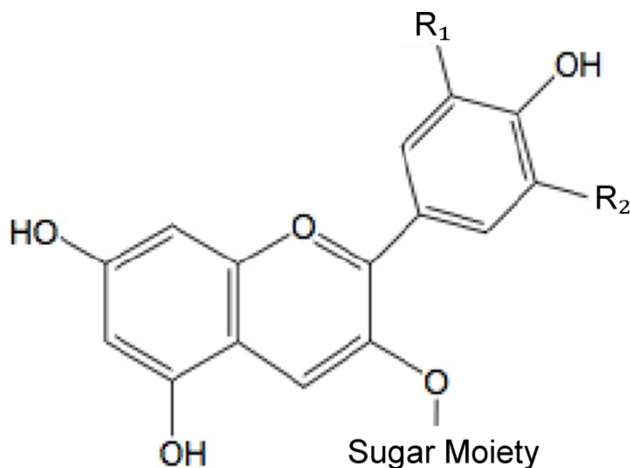
Due to the significant variability of anthocyanin and phenolic contents among elderberry cultivars, research shows that traditional breeding techniques may be useful in developing elderberries with high or low phytonutrient contents (Lee and Finn 2007, Özgen and others 2010). Özgen and others (2010) suggest that the breeding of wild

germplasm into existing cultivars could result in elderberries with enhanced horticultural, post-harvest or processing traits and could contain superior phytochemical profiles. A variety of factors can influence the phenolic content of elderberry fruit including cultivar, species, temperature, ripeness, yield, growing season, plot management, post-harvest storage, and processing (Moyer and others 2002, Lee and Finn 2007).

In a study investigating a population of women (*n*=38,445), the average flavonoid (flavonols, flavones) intake from all food sources, omitting flavan-3-ols, was estimated to be 24.6 mg/day/person, with quercetin as the major flavonoid contributor (70.2%) (Sesso and others 2003). In another study investigating the flavonoid intake of Scottish people (*n*=81), the flavonoid intake was estimated to be 18.8 (flavonols), 0.1 (flavones), 22.5 (proanthocyanidins), 59.0 (catechins), and 1.2 (flavanones) mg/day/person, with major dietary sources which included black tea, apple, sweet pepper, red wine, onion, and lettuce (Kyle and Duthie 2006). It is theorized that flavonoids are absorbed well in the oral cavity but under-absorbed in the stomach and intestine, due to the presence of microflora which cleave sugar moieties and are required for efficient absorption (Wu and others 2002).

Anthocyanins are a variety of flavonoid compounds found in all parts of higher order plants and are responsible for the attractive colors of most fruits and vegetables. They are water-soluble pigments which are stored in the vacuoles of plant tissues and can range from red, purple to blue depending on chemical structure and pH (Anderson and Jordheim 2006). The ecological purpose of anthocyanins is to attract animals and

pollinators to the reproductive organs of the plants, which helps disperse the seeds and genes of the plants, subsequently ensuring future growth. In addition, anthocyanins have been shown to provide a protective effect from the sun's ultra-violet rays. If plants are exposed to too much sunlight, they undergo photoinhibition, or light-induced reduction of photosynthetic activity (Steyn and others 2002). When isolated, anthocyanins have been described as having no odor and little to no flavor, however, they contribute to an astringent sensation. In their natural from, anthocyanins are often attached to sugar molecules (i.e. glycosides), and their aglycones are referred to as anthocyanidins (Charlebois 2007) (Figure 2). Glycosylation often results in increased anthocyanin stability and solubility (Stintzing and Carle 2004). The six common anthocyanidins are cyanidin, delphinidin, pelargonidin, malvidin, peonidin, and petunidin; with cyanidin accounting for approximately 45% of total anthocyanidin intake among people within the United States (Clifford 2000, Anderson and Jordheim 2006, Wu and others 2006, Charlebois 2007).



Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H
Peonidin	OCH_3	H
Petunidin	OCH_3	OH

Figure 2 Illustration of anthocyanin structure.

The total number of anthocyanins isolated from plants has been reported to be 539 (Anderson and Jordheim 2006). The anthocyanin content of elderberry fruit is between 11.3 mg/g and 13.1 mg/g on a dry weight basis, which contributes to 41.4% to 87.9% of total phenolics (Wu and others 2006, Galić and others 2009, Özgen and others 2010). Elderberry fruit contains four major anthocyanins within the cyanidin group (cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside), of which cyanidin-3-sambubioside and cyanidin-3-glucoside account for more than 90% of total anthocyanins (Johansen and others 1991, Wu and others 2002, Seabra and others 2010a). In comparison, cyanidin-3-glucoside accounts for 87.5% of total anthocyanins in crude blackberry extract (Elisia and others 2007). Anthocyanins pelargonidin-3-glucoside and pelargonidin-3-sambubioside have also been identified within elderberry fruit, to a lesser extent (Wu and others 2004). Additionally, due to the wide array of anthocyanins present in foods, anthocyanins serve as important chemical markers for identification of fruits and vegetables within value-added food products (Clifford 2000, Borges and others 2010).

Anthocyanins can occur naturally in acylated or non-acylated forms. Acylated anthocyanins are anthocyanins that are covalently linked to organic or phenolic acid acyl group, which have greater stability compared to their non-acylated anthocyanin glycoside counterparts (Clifford 2000). Greater than sixty-five percent of anthocyanins occur naturally in the acylated form (Anderson and Jordheim 2006). The acyl groups of anthocyanins are organic or phenolic acids, such as cinnamic (*p*-coumaric, caffeic, ferulic, sinapic), aliphatic (acetic, malonic, succinic), hydroxybenzoic (*p*-hydroxybenzoic,

gallic), hydroxycinnamic, malic, oxalic, or tartaric acids (Anderson and Jordheim 2006). Malonic acid is the most common acyl group, followed by *p*-coumaric, caffeic, ferulic, acetic, and sinapic acids (Anderson and Jordheim 2006). Anthocyanin acylation improves elderberry anthocyanin stability to heat and light (Inami and others 1996, Malien-Aubert and others 2001, Turker and others 2004, Charlebois 2007, Lee and Finn 2007). In addition, acylated anthocyanins have increased antioxidant activity compared to their non-acylated counterparts (Stintzing and Carle 2004, Charlebois 2007).

Using data from the National Health and Nutrition Examination Survey (NHANES) from 2001-2002, Wu and others (2006) estimated that the average intake of anthocyanin levels are 12.5 mg/day/person in the United States, with cyanidin, delphinidin, and malvidin contributing to 45%, 21%, and 15% of total intake, respectively. Other reports suggest that anthocyanin intake may be as high as 215 mg/day/person (Kühnau 1976, Cao and Prior 1999, Kyle and Duthie 2006). It was observed that non-acylated anthocyanins contributed to 77% of total intake, whereas acylated anthocyanins contributed to 23% (Wu and others 2006).

Proanthocyanidins, also known as ‘condensed tannins’, are an important subclass of flavonoids found in elderberry fruit and are comprised of oligomeric and polymeric flavan-3-ols, specifically catechin and epicatechin isomers. Their size, or molecular weight, is often described by their degree of polymerization (Gu and others 2002). Proanthocyanidins can occur with A- or B-type interflavanyl bonds. A-type proanthocyanidins are less common than their B-type counterparts and contain an atypical second ether interflavanyl linkage between C2’O7. B-type proanthocyanidins

possess only a single interflavanyl linkage which occurs primarily between C4’C8 or C4’C6 (Cunningham and others 2002, Prior and Gu 2005, Ferreira and others 2006). As a result, the conformation of A-type proanthocyanidins is more stable than B-type proanthocyanidins, which results in unmistakable nuclear magnetic resonance (NMR) spectra (Ferreira and others 2006). Due to the effects of dynamic rotational isomerism, B-type proanthocyanidins are rotational isomers which lead to equivocal NMR spectra (Kolodziej 1992, Ferreira and others 2006).

A-type proanthocyanidins have gained particular attention as important phytochemicals due to their protective effect on urinary tract health. A-type cranberry proanthocyanidins have been shown to prevent the adhesion of P-fimbriated uropathogenic *Escherichia coli* to uroepithelial cells (Howell and others 2005). Foods which do not contain A-type, but do contain B-type proanthocyanidins (purple grape juice, apple juice, green tea, dark chocolate), showed no significant in-vitro urinary bacterial anti-adhesion activity (Howell and others 2005). It has been previously theorized that the low acidity of cranberry juice, which contains A-type proanthocyanidins, caused the acidification of urine and subsequently resulted in an antibiotic effect, which is now known to be false (Prior and Gu 2005).

The estimated daily consumption of proanthocyanidins is 57.7 mg/person within the United States, with apples (32.0%), chocolate (17.9%), and grapes (17.8%) accounting for 67.7% of the total intake (Gu and others 2004). Proanthocyanidin monomers (catechin, epicatechin), dimers, trimers, and oligomers/polymers are estimated to contribute 7.1%, 11.2%, 7.8% and 73.9% of total proanthocyanidins,

respectively (Gu and others 2004). Although these values are believed to be good estimates, the complexities of proanthocyanidin molecules and the lack of appropriate analytical standards result in somewhat empirical quantifications (Pérez-Jiménez and others 2009, Wallace and Giusti 2010).

Proanthocyanidin Analytical Methods

There are a number of analytical techniques currently employed for the determination of proanthocyanidins in foods, including gravimetric, colorimetric, thiolytic, chromatographic, and mass spectrometric methods (Cunningham and others 2002, Gu and others 2003, Kelm and others 2005, Prior and others 2010). Gravimetric techniques involve the purification of proanthocyanidins, which often employs flash and/or gel chromatography. The final weight of purified proanthocyanidins is compared to the mass of the initial product and can be reported on a dry weight or percent basis. This technique is expensive, time consuming, and not environmentally friendly due to the usage of large solvent volumes.

The vanillin assay is another quantification technique, which involves the extraction of proanthocyanidins, followed by depolymerization in a weak acid solution and colorimetric analysis at 500 nm. This assay is most effective when analyzing proanthocyanidins that have been previously isolated based on degree of polymerization due to varying effects of mineral acid on differing proanthocyanidin chain lengths. It should be noted that vitamin C, acetone, and naturally present anthocyanins, which have maximum absorption at 520 nm, can cause interference at

500 nm (Cao and Prior 1999, Cunningham and others 2002, Prior and Gu 2005, Prior and others 2010).

The 4-(Dimethylamino)cinnamaldehyde (DMAC) assay is a commonly employed colorimetric technique which is faster, more accurate, and more sensitive for the determination of proanthocyanidins than the vanillin assay. DMAC reacts with flavan-3-ols (catechin, epicatechin) or the terminal monomer of proanthocyanidins at the C8 position of the A-ring flavonoid, which produces a green chromophore with maximum absorption at wavelength 640 nm (Cunningham and others 2002, Prior and Gu 2005, Prior and others 2010, Feliciano and others 2012). Performing spectrophotometric analysis at 640 nm is advantageous compared to the vanillin assay due to the lack of interference by anthocyanins and other compounds (Feliciano and others 2012). Due to the fact that DMAC reacts with a single flavan-3-ol monomer, proanthocyanidins are often underestimated when they occur in polymeric forms, and estimations of proanthocyanidins are often reported as catechin equivalents (Prior and Gu 2005). The lack of appropriate standards for products containing proanthocyanidins results in data which is difficult to compare among samples (Kelm and others 2005, Prior and Gu 2005). Optimally, standards should reflect the heterogeneous nature of the proanthocyanidins within specific foods and account for proanthocyanidin subunit structure, interflavan linkage types, and degree of polymerization (Prior and Gu 2005, Feliciano and others 2012). Feliciano and others (2012) isolated cranberry proanthocyanidins from cranberry press cake for use as an analytical standard. They reported that the slope of the standard curve from cranberry proanthocyanidins was 2.5 times lower than for

procyanidins A2 and B2, and 7.1 times lower than catechin, which indicated that the contents of proanthocyanidins from cranberry press cake would be underestimated by 2.5- or 7.1-fold if these standards were used in the DMAC assay. This supports the theory proposed by Prior and others (2010) that large polymeric proanthocyanidins have a lower response than monomeric or dimeric procyanidins. DMAC reagent is also useful in histology for the localization of proanthocyanidins within plant tissue.

A number of chromatographic techniques are available for proanthocyanidin analysis. Normal-phase high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is able to isolate proanthocyanidins based on their degree of polymerization up to decamers, however quantification is underestimated due to poor resolution of highly polymeric (>decamer) proanthocyanidins (Hammerstone and others 1999, Gu and others 2002, Prior and Gu 2005). It is believed that current normal-phase HPLC-MS technology is able to separate proanthocyanidins up to tridecamers (Gu and others 2002). Reversed-phase HPLC techniques are able to separate proanthocyanidin monomers, dimers, and trimers, however, separation of proanthocyanidins with degree of polymerization beyond 4 results in a broad, unresolved peak (Lazarus and others 2001, Prior and Gu 2005). Additionally, polymeric proanthocyanidins can be depolymerized by benzyl mercaptan (i.e. thiolysis) into catechin and epicatechin monomers, which can then be analyzed using HPLC for an empirical estimation of proanthocyanidins (Gu and others 2002).

Elderberry Product Development and Marketing

Currently, elderberry (*Sambucus nigra*) is primarily cultivated for commercial production within Austria, Denmark, Poland, France, Italy, Quebec, and Chile, as well as within the U.S. states of Washington, Oregon, Missouri, and Maine. Elderberry reaches full production maturity after 3 to 4 years of growth. Often, elderberry fruit ripens at different rates among canes on the same bush, thus elderberry fruit is harvested incrementally over a period of three to four weeks. The most common method of elderberry harvest is to cut the entire fruit cluster from the bush before freezing and stripping the fruit from the branch (Strang 2012). To obtain elderberry flowers, the flower cluster is harvested and rubbed over screens (Byers and others 2012). The fruit is either frozen for long term storage or is processed immediately, and the flowers are usually frozen or dried (Byers and others 2012). Based on estimates of elderberry production costs (irrigation, harvesting, marketing), gross returns of a 2012 elderberry crop was approximately $3.00 per pound, or $400.00 per 1/5 acre (Strang 2012). During optimum growing conditions, up to four tons of fruit can be harvested per acre (Strang 2012). Frozen storage of elderberry fruit is not recommended beyond a few months. Freezer cycling, repeated freezing and thawing and/or over-processing will lead to a loss of anthocyanins, which will result in brown products with lowered antioxidant activity (Byers and others 2012). Slow or repeated freezing of elderberry fruit will cause the rupture of cellular membranes, which increases anthocyanin hydrolysis and the formation of blown color anthocyanin-tannin pigments (Muldrew and McGann 1994).

Currently, the majority of the world's elderberries are cultivated on a hobbyist scale and are not produced commercially (Cernusca and others 2011). Elderberry wines, cordials, jams, jellies, pies, beers, dressings, sauces, and teas are produced and sold in limited quantities, whereas dried elderberries, syrups, powders, concentrates, juices, capsules, lozenges, and tinctures are commercially sold within niche markets (Cernusca and others 2011, Byers and others 2012). Tight regulations of alcoholic beverages limit the sale of elderberry wines, cordials, and beers to within U.S. state borders (Byers and others 2012). Syrups and extracts currently command the highest prices, with an estimated commercial value of $50-200 per kilogram (King and others 2003).

In a survey of 74 elderberry producers within the United States, respondents reported that they most commonly sell plants (42%), followed by fruit (22%), wine (18%), juice (11%), flowers (8%), concentrate (8%), and nutraceuticals (4%); with 43% of respondents selling nationally, 40% selling regionally, and 57% selling locally (Cernusca and others 2011). Fifty-nine percent of the same respondents declared that elderberry demand will increase within the next five years, and 41% of respondents stated that the elderberry industry is non-competitive (Cernusca and others 2011). Market competitiveness is defined as potential profitability, which is controlled by market forces such as: customers, suppliers, potential entrants, and substitute products (Cernusca and others 2011). In a separate survey of 508 respondents representative of the general U.S. population, 31% 'had heard of elderberry' while 69% were 'not familiar' (Mohebalian and others 2012). Seventeen percent of total respondents had sampled elderberry products and 14% purchased products made with elderberry (Mohebalian

and others 2012). Respondents also reported that elderberry juice, elderberry jelly, and elderberry wine were the three most commonly purchased elderberry products (35%, 31%, 24%), respectively (Mohebalian and others 2012).

The appearance of food products is one of the most important quality characteristics which determines consumer acceptability, after flavor and economic factors (Casati and others 2012). Currently, consumer trends are moving towards the consumption of natural colorants as a substitute for synthetic dye or lake pigments. Juice from black elderberry fruit (*Sambucus nigra*) has been investigated as a natural colorant due to its high anthocyanin content, and elderberry concentrate can be produced by membrane concentration or filtration technologies (Carlsen and Stapelfeldt 1997, Malien-Aubert and others 2001, Lee and Finn 2007). Anthocyanins are a beneficial natural food colorant due to its potential health benefits, although poor stability is their major drawback. Anthocyanin stability depends on structure, degree of acylation, storage temperature, presence of copigments, extraction method, pH, presence of oxygen, exposure to light, and presence of complexing agents (Rodríguez-Saona and others 1999, Malien-Aubert and others 2001, Jing and others 2008). Schmitzer and others (2010) reported that 94% of anthocyanic pigments were lost in elderberry wine during three years of storage, and there was a 5-fold decrease after 16 months. During storage, the wine gained brown hues which were likely the result of formations of polymeric pigments from degradation reactions (Schmitzer and others 2010). Carlsen and Stapelfeldt (1997) investigated the sensitivity of an elderberry anthocyanin colorant to visible and ultra-violet light and concluded that irradiation wavelengths had a strong

effect on color stability, indicating that a uv-barrier is needed within elderberry packaging material. Interestingly, it was also concluded that the pH of the solution had no effect on the photochemical breakdown of the elderberry extract and only affected color intensity (Carlsen and Stapelfeldt 1997).

Among all of the fruits and vegetables, elderberry fruit has one of the highest anthocyanin contents, which makes it suitable for solvent extraction on a commercial scale. Anthocyanins are highly polar molecules and are soluble in a variety of solvents, which are typically acidified (Galić and others 2009). Crude elderberry extracts can be obtained using methanol, ethanol, acetone, or water as extragents, which can be further utilized for the production of elderberry syrups or tinctures (King and others 2003, Gourdin and others 2008, Denev and others 2010). Additionally, crude elderberry extracts contains by-products, such as sugars, pectin, sugar alcohols, amino acids, organic acids, and/or proteins, which may cause problems during subsequent elderberry processing or cause a reduction in overall shelf-life (Denev and others 2010). Solid-phase extraction or gel filtration techniques can be often employed for subsequent anthocyanin purification after a crude extract is obtained (Denev and others 2010).

Carbon dioxide is another useful extractive and has advantages over organic solvents. Carbon dioxide has the ability to extract food chemicals, such as anthocyanins, without adding toxicity to the food or the environment, unlike organic solvents (Fitzgerald and others 1999). It is extremely environmentally friendly and is most effective as a solvent at its critical point. Critical point is defined as the point in which two phases of any pure substance become indistinguishable as a result of modifications

of temperature and pressure, and it most commonly pertains to the liquid-gas phase boundary (Fitzgerald and others 1999, Waibel and others 2008). A supercritical fluid is any substance at a pressure and temperature above its critical point in which no liquid or gas phases are clearly evident (Waibel and others 2008). Supercritical carbon dioxide has the advantage of diffusing through elderberry matrices, like a gas, and extracting anthocyanins and other phytochemicals, like a liquid, due to its lack of surface tension. In addition, small modifications of temperature or pressure can cause substantial changes in fluid density, which grants the ability of the fluid to be adjusted accordingly (Fitzgerald and others 1999). During supercritical carbon dioxide extraction, adjusting fluid density allows for selectivity during the extraction process and simple depressurization recovers the extracted material (Fitzgerald and others 1999). Although supercritical carbon dioxide is advantageous compared to the use of organic solvents in many ways, it has limited extraction performance because of its non-polar nature, which may limit the extraction of high molecular weight compounds (Seabra and others 2010a, Seabra and others 2010b). The addition of organic co-solvents is commonly employed to enhance the performance of supercritical carbon dioxide extraction (Seabra and others 2010a).

Subcritical water also has a useful role in phytochemical extractions. King and others (2003) tested the anthocyanin extraction efficiency of acidified (2.3 pH) subcritical water on elderberry pomace using an accelerated solvent extractor, and determined that 0.724 mg of anthocyanins were extracted per gram of pomace when using a water extraction temperature of 120° C. A water temperature of 120° C may

seem high due to the thermal vulnerability of anthocyanins, however, rapid transport of anthocyanins from the elderberry pomace to the outside of the extraction cells preserved their conformation and antioxidant activity (King and others 2003). Additionally, it is theorized that rapid sterilization of the resultant elderberry product could be achieved using the hot subcritical water extraction process (King and others 2003).

Within the United States, drying is the second most common processing technique for fruits, after winemaking, and is often a prerequisite for the extraction of nutraceuticals (Anonymous 2008). Drying reduces shipping costs, increases shelf-life, and typically preserves fruit nutrients. The moisture content of dried fruits can range from 3% to 21%. California is responsible for the largest majority of dried fruit production within the United States, accounting for the production of 99% of raisins, 99% of dried plums, 98% of dried figs, 96% of dried peaches, 92% of dried apricots, and over 90% of dried dates (Anonymous 2008). Apples, cherries, cranberries, pineapples, strawberries, blueberries, papayas, mangos, and coconuts are other notable fruits which are consumed dried within the United States and abroad. Interestingly, cranberries, blueberries, and Concord grapes are the only three fruits native to North America. Fruits which contain low amounts of sugar (cranberries, cherries, blueberries, strawberries) typically require the addition of a sweetener prior to drying for microbial control and consumer acceptability. Elderberry fruit is unique because it is sold on a limited commercial scale as an unsweetened dried fruit, and is generally not considered ‘sweet’ by consumers in its native form. Commercial opportunities may exist for the creation,

production, and sale of sweetened dried elderberries. Sugar, organic acids, sorbates, and/or glycerol are commonly employed within dried fruits and fruit extracts to reduce water activity, and to control spoilage and pathogenic microorganisms.

Two production issues can occur during fruit drying, enzymatic and non-enzymatic browning. Sulfites have traditionally been an effective treatment to control fruit browning. Non-enzymatic browning occurs as a result of the Maillard reaction, in which reducing sugars within the fruit react with amino compounds to form intermediate products, which subsequently polymerize and cause browning (Nafisi-Movaghar 1991). Sulfites interrupt non-enzymatic browning by reacting with the reducing sugars instead of the amino compounds to form a sulfonate product (Nafisi-Movaghar 1991). The sulfonate product does not polymerize to cause browning. Enzymatic browning of fruits is also controlled by the addition of sulfites. Enzymatic browning involves naturally present polyphenol oxidase, catechol oxidase, or other enzymes which react with innate phenols and oxygen, and this reaction causes browning. Sulfites inhibit enzymatic browning by scavenging available oxygen, which would otherwise participate in fruit browning (Nafisi-Movaghar 1991). Sulfites also inhibit the growth of microorganisms and are not typically used within products in which browning is considered desirable, such as in the production of dried figs or raisins. The major drawback of sulfite use is that it can cause allergic reactions in people with allergies or sensitivities to sulfites. As a result, the U.S. Food and Drug Administration requires the labeling of foods containing sulfites at greater than ten parts per million.

Spray drying of fruit juices or extracts is another useful and quick preservation technique which is commonly employed in the food industry. It has the advantage of preserving fruit phytonutrients through microencapsulation with wall materials, such as maltodextrin, gum acacia, soy protein, egg white, as well as other gums and starches (Murugesan 2010, Murugesan and others 2012). One research study identified gum acacia as the wall material of choice, compared to maltodextrin and a variety of soy preparations for the preservation of elderberry phenolics, color, and yield (Murugesan 2010, Murugesan and Orsat 2011). Spray drying is a valuable technique for the production of elderberry capsules, lozenges, and powdered extracts.

Due to the fact that the major selling factor for elderberry products is their rich source of polyphenolics and other nutraceuticals, elderberry fruit must be processed in ways to preserve these nutrients. However, common processing techniques such as solvent extraction, pasteurization, chopping, sulfur dioxide treatments, pH modification, enzymatic clarification, canning, heating, drying, shredding, irradiation, peeling, and fermentation have been shown to affect the flavonoid content of foods (Kaack and others 2008, Kyle and Duthie 2006). Winemaking has been reported to increase anthocyanin polymerization and condensation (Schmitzer and others 2010). Galić and others (2009) demonstrated that blanching of elderberry fruit did not result in significant overall polyphenol decrease, however, non-flavonoid concentration was about 25% lower in the blanched berries. It was also reported that the disintegration of elderberries resulted in an increase of polyphenols, which was likely due to the inactivation of enzymes (Galić and others 2009). Overall, there are a variety of

processing choices which may be applicable to elderberry fruit. As consumer food choice continues to trend in a more healthful direction, it is even more important that elderberry processors choose processing techniques which preserve or enhance the fruits' healthful properties.

Anthocyanin Copigmentation

In addition to flavor and cost factors, color is one of the most important characteristics that determines the acceptability of food and beverages. Anthocyanin molecules are responsible for the red, blue, and purple pigments of many fruit and vegetable products, and are of substantial importance within the food and beverage industry. Currently, there are significant demands for the replacement of synthetic food dyes with anthocyanin-rich concentrates, however their utilization is limited by their relative instability. The rapid degradation of anthocyanins during processing and storage results in substantial losses of product color, nutritional and sensory characteristics. The major factors which affect anthocyanin stability include temperature, pH, enzyme activity, light, structure, concentration, and the presence of copigments or metal ions (Mazza and Brouillard 1990, Talcott and others 2003, Rein 2005, Kammerer and others 2007, Kaack and others 2008, He and others 2012).

One of the most important factors governing the stability, hue, and chroma of anthocyanin pigments is pH. The pH of a solution controls the equilibrium of four anthocyanin chromophores (part of the molecule responsible for its color), which includes the red flavylium cation, blue or red quinonoidal base, colorless carbinol

pseudobase, and colorless chalcone (Brouillard 1982, Hubbermann 2006). In very acidic solutions (pH<2) the red flavylium cation dominates. As pH increases, protons are lost from the flavylium cation, which yields the blue or red quinonoidal base. Furthermore, hydration of the flavylium cation occurs over time, which results in the formation of colorless carbinol pseudobases, and subsequently colorless chalcones through the hydration and opening of the flavonoid ring structure (Brouillard 1982, Hubbermann 2006). Considering that the flavylium cation is the most stable form of anthocyanin chromophores and is responsible for the red anthocyanin color, it is important to process and store anthocyanin-rich products at low pH values. Anthocyanins are most stable between 2.8-3.4 pH.

The structure of anthocyanin molecules also has a substantial effect on anthocyanin stability and color. In their natural form, anthocyanins generally occur as glycosides, which have greater stability than their anthocyanidin backbone substructures, due to the presence of intramolecular hydrogen bonding networks. Furthermore, intramolecular copigmentation occurs as a result of anthocyanin acylation, which results in even greater pigment stability compared to anthocyanin glycosides. The greater stability of acylated anthocyanins is a result of organic or phenolic acid acyl groups which are covalently bonded to anthocyanin molecules, whereas anthocyanin glycosides contain weaker hydrogen bonds. Radishes, red potatoes, red cabbage, black carrots, red onions, and purple sweet potatoes primarily contain anthocyanins in the acylated form (Giusti and Wrolstad 2003), whereas strawberries, elderberries, blueberries, grapes, blackberries, cranberries, cherries,

plums, pomegranates, and black currants are examples of foods which primarily contain non-acylated anthocyanins. The biosynthesis of anthocyanins occurs within the cytosol of plants after the production of anthocyanidin synthase, and their degree of glycosylation, methylation, or acylation determines their stability (He and others 2010).

Although there are many factors which degrade anthocyanin pigmentation, the stability of anthocyanins can be improved through copigmentation. Copigmentation is defined as the molecular complexation between anthocyanins and other flavonoids, polysaccharides, amino acids, or metal ions, which results in anthocyanin molecules with enhanced color stability (Malien-Aubert and others 2001, Eiro and Heinonen 2002, Talcott and others 2003, Del Pozo-Insfran and others 2007). This molecular complexation prevents hydration of the flavylium cation to colorless pseudobases and undesirable brown degradation compounds, and can result in synergistic enhancements to the color intensity of anthocyanin based products. Colorless copigments are able to link with anthocyanin molecules in their flavylium or quinonoidal forms through covalent bonding (intramolecular copigmentation), hydrogen bonding (intermolecular copigmentation), hydrophobic interactions (intermolecular copigmentation), electrostatic interactions (intermolecular copigmentation), anthocyanin self-association (inter-/intramolecular copigmentation), or metal complexation (inter-/intramolecular copigmentation) (Talcott and others 2003, Rein 2005). Interestingly, copigmentation is unique to the anthocyanin class of pigments, and does not occur among betalains, carmine, carotenoids, chlorophylls, flavonoids, hemes, melanins, quinones, tannins, or xanthones.

Intramolecular copigmentation is loosely defined as a phenolic copigment joined with an anthocyanin molecule through covalent acylation to form a highly stable pigment (Brouillard 1983, Rein 2005). Although intramolecular copigmentation results in the greatest pigment stability among the types of copigmentation reactions, its use for the development of anthocyanin-rich food and beverages is limited since it copigmentation generally occurs as a natural process of plant growth. Intermolecular copigmentation is described as a non-acylated anthocyanin and a colorless copigment complex through hydrogen bonding, hydrophobic interactions, or electrostatic interactions, using water as an important cofactor (Talcott and others 2003, Rein 2005). Intermolecular copigmentation is of great interest to food and beverage producers, and is considered to be the most important technique for the stabilization of anthocyanin molecules.

The effect of copigmentation can be detected by bathochromic or hyperchromic shifts, where a change of the λmax of the absorption spectra can be detected (Eiro and Heinonen 2002). A bathochromic shift is defined as an increase of absorption spectra, where the λmax shifts towards a higher wavelength. When this phenomenon occurs as a result of copigmentation, it is also known as the bluing effect, because anthocyanin pigments become more blue (Brouillard and others 1989). A hyperchromic shift involves a decrease of absorption spectra, where the λmax shifts towards a lower wavelength and there is an increase of the intensity of red pigmentation.

Elevated temperatures and the addition of solvents impede copigmentation reactions by preventing or dissociating intermolecular complexation (Mazza and

Brouillard 1990, Rein 2005, He and others 2012). Generally, copigmentation reactions do not occur below anthocyanin concentration of 3.5 x 10^{-5} M, and greater anthocyanin concentrations result in greater molecular self-association and improved color stability (Asen 1976, Giusti and Wrolstad 2003, Rein 2005). Previous studies have shown that wines containing 10%-21% ethanol are susceptible to copigmentation reactions, however no research has demonstrated the effectiveness of copigments within tinctures (>25% ethanol) or liqueurs (15%-55% ethanol) (Boulton 2001). Additives such as rosemary extract, black carrot color, elderberry color, thyme extract, purple sweet potato color, and tannic acid have been shown to be effective copigments within elderberry and other anthocyanin-rich systems, and can cause enhanced pigment stability and antioxidant activity (Talcott and others 2003, Del Pozo-Insfran and others, Kammerer and others 2007).

Objectives

No studies have analytically evaluated commercial elderberry products or have evaluated the effect of copigmentation within high ethanol (<25%) anthocyanin systems. Therefore, it was the overall objectives of this research to increase the market competitiveness of elderberry products through the analytical evaluation of commercial elderberry products, and to develop a value-added elderberry product with enhanced nutrient and color stability characteristics using the effect of copigmentation.

The specific objectives of these studies were:

1) To make analytical comparisons among a variety of commercial value-added elderberry products (n=33), including syrups, tinctures, concentrates, capsules, lozenges, dried fruit, and a powder at 0, 5, and 10 weeks of accelerated temperature (32° C) storage, which will allow consumers to make informed elderberry purchases and enhance the competitiveness among value-added elderberry producers.

2) To determine how additions of rosemary extract, tannic acid, black carrot color, purple sweet potato color, and enzymatically modified isoquercitrin will affect the nutrient and color stability of elderberry tinctures at 0, 2, 4, and 6 weeks storage at 21° C, with one week of accelerated temperature (32° C) storage which occurred between 5 and 6 weeks.

CHAPTER 2. A COMPETITIVE ASSESSMENT OF COMMERCIAL ELDERBERRY (*SAMBUCUS* SP.) PRODUCTS

Objectives

The objectives of this study were to evaluate the moisture, proanthocyanidins, sugars, anthocyanins, organic acids, and vitamin C contents of a variety of commercial elderberry products (*n*=33), including syrups, tinctures, concentrates, capsules, lozenges, dried fruit, and a powder at 0, 5, and 10 weeks of accelerated temperature (32° C) storage.

Materials and Methods

Elderberry Products

A total of 33 important value-added elderberry products were chosen for this study (Table 1, Figure 3). Each product was placed into one of seven categories; syrups (*n*=14), tinctures (*n*=5), concentrates (*n*=3), capsules (*n*=3), lozenges (*n*=4), dried fruit (*n*=3), or powder (*n*=1). Syrups were fluid elderberry products categorized by their high sugar contents (40-70° brix), and not labeled as concentrate. Tinctures were defined as fluid elderberry products which contained alcohol as the primary ingredient and minimally added sugar. Concentrates were categorized as liquid elderberry products of at least 50° brix and labeled as concentrate. Capsules were elderberry products which contained a shell; whereas lozenges did not contain a shell, and were either in tablet or chewable form.

Table 1 Commercial Elderberry Products Analyzed in this Study

Product Category	Product Code	Product	Product Source
Syrups	EMG	Evergreen Manufacturing Group Elderberry Syrup	(Evergreen Manufacturing Group, LLC., Madawaska, ME)
	NW SF	Nature’s Way® Sugar-Free Sambucus Syrup	(Nature’s Way® Products, Inc., Green Bay, WI)
	NW	Nature’s Way® Sambucus Syrup	(Nature’s Way® Products, Inc., Green Bay, WI)
	Sam K	Sambucol® for Kids Syrup	(PharmaCare U.S., Inc., San Diego, CA)
	NA	Nature’s Answer® Black Elderberry Extract	(Nature’s Answer®, Hauppauge, NY)
	Sam	Sambucol® Syrup	(PharmaCare U.S., Inc., San Diego, CA)
	Bio SE	Bio-Botanica® SE Elderberry Syrup	(Bio-Botanica®, Inc., Hauppauge, NY)
	Bio AF	Bio-Botanica® AF Elderberry Syrup	(Bio-Botanica®, Inc., Hauppauge, NY)
	Dyn	Dynamic Health® Black Elderberry Tonic	(Dynamic Health Laboratories, Inc.®, Brooklyn, NY)
	Inte	Integrative Therapeutics™ Sambucus Extract	(Integrative Therapeutics™, Inc., Green Bay, WI)
	Now	Now® Elderberry, Zinc and Echinacea Syrup	(NOW® Foods, Bloomingdale, IL)
	Antho	AnthoImmune™ Organic Elderberry Syrup	(Maine Medicinals, Inc.™, Dresden, ME)
	Honey	Honey Gardens™ Elderberry Syrup	(Beehive Organics™, Inc., Park City, UT)
	Planet	Planetary™ Herbals Full Spectrum™ Elderberry Syrup	(Planetary™ Herbals, LLC., Soquel, CA)
Tinctures	Quant	Quantum® Health Elderberry Liquid Extract	(Quantum®, Inc., Eugene, OR)
	Source	Source Naturals® Wellness Elderberry Liquid Extract™	(Source Naturals®, Inc., Scotts Valley, CA)
	Gaia	Gaia® Organics Black Elderberry Liquid Extract	(Gaia® Herbs, Inc., Brevard, NC)
	Herb	Herb Pharm® Black Elderberry Extract	(Herb Pharm®, LLC., Williams, OR)
	Planet	Planetary™ Herbals Full Spectrum™ Elderberry Fluid Extract	(Planetary™ Herbals, LLC., Soquel, CA)

Table 1 continued

Concentrates	Kerr	Kerr Elderberry Concentrate	(Kerr Concentrates, Inc., Oxnard, CA)
	Sambu	Sambu® Wild Grown Elderberry Concentrate	(Dr Dünner AG, Immensee, Switzerland)
	Nat	Natural Sources® Natural Elderberry Concentrate	(Natural Sources®, Inc., San Clemente, CA)
Capsules	NW	Nature's Way® Elderberry Capsules	(Nature's Way® Products, Inc., Green Bay, WI)
	Swan	Swanson® Premium Brand Elderberry Capsules	(Swanson® Health Products, Fargo, ND)
	Eclectic	Eclectic Institute™ Elderberry Capsules	(Eclectic Institute™, Inc., Sandy, OR)
Lozenges	Now	Now® Elderberry and Zinc Lozenges	(NOW® Foods, Bloomingdale, IL)
	Sam	Sambucol® Chewable Tablets	(PharmaCare U.S., Inc., San Diego, CA)
	Rub	Rubini® ProFlavon Elderberry Complex Lozenges	(East Coast International Natural Healthcare, Dublin, Ireland)
	Planet	Planetary™ Herbals Full Spectrum™ Elderberry Tablets	(Planetary™ Herbals, LLC., Soquel, CA)
Dried Fruit	Front	Frontier® Whole European Elderberries	(Frontier® Natural Products Co-Op, Norway, IA)
	Flor M	Florida Herb House Minced Dried Elderberries	(Florida Herb House, LLC., South Daytona, FL)
	Flor W	Florida Herb House Dried Elderberries	(Florida Herb House, LLC., South Daytona, FL)
Powder	NP Nutra	NP Nutra® Elderberry P.E. 10:1	(NP Nutra®, Inc., Gardena, CA)

n=2.

Figure 3 Value-added elderberry products analyzed in this study.
Refer to Table 1 for product identification. From top left: (row 1) EMG, NW SF, NW (syrup), Sam K, NA, Sam (syrup), Bio SE, (row 2) Bio AF, Dyn, Inte, Now (syrup), Antho, Quant, Source, (row 3) Honey, Gaia, Herb, Planet (tincture), Planet (syrup), Kerr, Sambu, (row 4) Nat, NW (capsule), Swan, Eclectic, Now (lozenge), Sam (lozenge), Rub, (row 5) Planet (lozenge), Front, Flor M, Flor W, NP Nutra. Capsules and lozenges not shown in their native conformations.

Each product was received within 24 months from its date of manufacture (μ=7 months) based on product investigation and was representative of product expectations. Products were analyzed initially for moisture content, water activity, °brix, total proanthocyanidins, vitamin C, spectrophotometric color, L*a*b* color, sugars profile, anthocyanins profile, and organic acids profile. After initial analyses, products were sealed in their original packaging at 32° C, and re-tested at 5 and 10 weeks of storage. Products were purchased and analyzed in duplicate.

Moisture

Total moisture content was determined in duplicate for each elderberry product using a draft drying technique based on AOAC method 950.46 (AOAC, 2005). Aluminum drying pans were labeled according to product and replication number. The initial pan weight was determined (±0.0001 g) using an A&D Weighing analytical balance Model GH-120 (A&D Engineering, Inc., San Jose, CA). The aluminum pans were tared and approximately 3 grams of product was dropped evenly onto labeled pans. The weight of each sample was recorded to the nearest 0.0001 g. Each sample was placed in a 100° C Precision Scientific oven Model 16 (The Precision Scientific Co., Chennai, India) for approximately 16 hours. The pans were removed from the oven, cooled, and re-weighed to the nearest 0.0001 g. Percent moisture was determined using the following calculation:

% = (pan wt. + sample wt.) – (pan + dry sample wt.)/sample wt. x 100.

Water Activity

Water activity was determined in duplicate using an Aqua Lab 4TE water activity meter Model S40002169 (Decagon Devices, Inc., Pullman, WA). The instrument was calibrated prior to analyses using 0.30, 0.50 and 0.90 aw calibration solutions. Each product was thoroughly mixed and placed into a clean, dry sample cup, and water activity was determined.

Soluble Solids

Soluble solids content was determined in duplicate for syrups, tinctures, and concentrates using an ATAGO RX-5000 refractometer (ATAGO Co., LTD, Tokyo, Japan), and reported as °Brix (sucrose equivalents). Liquid elderberry samples were mixed thoroughly and dropped onto the sample port of the refractometer using a 4 mL plastic transfer pipette (Globe Scientific Inc., Paramus, NJ). The lid was closed and soluble solids were determined using the instrument.

Total Proanthocyanidins

Proanthocyanidins Purification

Freeze-dried elderberry fruit (46.8 g) was obtained from Nuts.com (Cranford, NJ), placed into a large ceramic mortar, frozen to approximately -62° C using a Revco Elite Plus Minus 80 Freezer (Thermo Fisher Scientific, Waltham, MA) and ground to form a powder using a mortar and pestle. The elderberry powder was divided evenly into six BD 50mL Falcon™ centrifuge tubes (Thermo Fisher Scientific, Waltham, MA). Twenty mL of a 70% acetone (Thermo Fisher Scientific, Waltham, MA) solution was added into each tube, shaken vigorously by hand for 1 minute, and sonicated for 10 minutes using a Branson 5510 sonicator (Branson Ultrasonics Co., Danbury, CT). The tubes were then centrifuged at 1800 x g for 10 minutes using a Damon IEC HN-S centrifuge (Damon/IEC Division, Needham Heights, MA). The elderberry supernatant was carefully decanted from each tube and combined. The extraction process was repeated three more times until a substantial loss of color was perceived from the elderberry powder. The acetone

fraction of the elderberry extract was evaporated off using a Büchi Rotavapor® rotary evaporator Model R-124 (BUCHI Corp., New Castle, DE) with a water bath temperature of 30° C. The extract was placed into a plastic bottle and cooled to ~5° C.

A 500 mL Chromaflex® glass chromatography column (PPG Industries Ohio, Inc., Cleveland, OH) was packed with Sephadex® LH-20 gel (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and preconditioned by pumping 500 mL of deionized water through it at a flow rate of 5 mL/min. The elderberry extract was loaded in the column by pumping it at the same flow rate (Figure 4). The top 2 inches of the LH-20 gel was stirred briefly to ensure even loading of the elderberry extract. Five hundred mL of deionized water were pumped through the column, which removed sugars and organic acids. This fraction was discarded. Eight hundred mL of a 25% ethyl alcohol (Thermo Fisher Scientific, Waltham, MA) solution was pumped through the column, which eluted more sugars and low-medium molecular weight flavonoids. Eight hundred mL of a 70% acetone (Thermo Fisher Scientific, Waltham, MA) solution was pumped through the column at the same flow rate and collected, which contained purified elderberry proanthocyanidins.

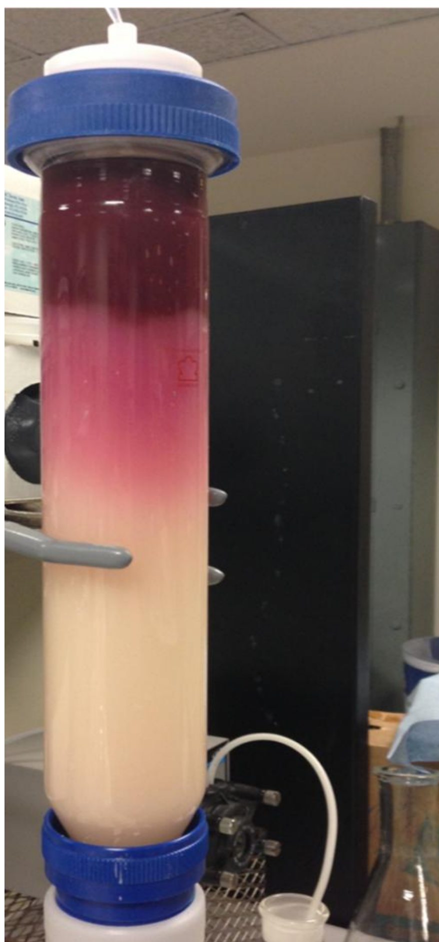


Figure 4 Isolation of elderberry proanthocyanidins using Sephadex® LH-20 gel chromatography.

The 70% acetone fraction, which contained the purified elderberry proanthocyanidins, was subjected to rotary evaporation with a water bath temperature of 30° C, to remove the acetone fraction. One hundred mL of deionized water was added to the purified proanthocyanidins, and the solution was transferred into a 60 mL HDPE bottle (Nalgene®, Rochester, NY) and frozen to -62° C using a Revco Elite Plus Minus 80 Freezer (Thermo Fisher Scientific, Waltham, MA). The purified frozen proanthocyanidins were freeze dried using a FreeZone 18 Liter Console Freeze Dry System Model 7755042 (LABCONCO, Kansas City, MO). The proanthocyanidins were

weighed (±0.0001), and the proanthocyanidin content of the original elderberry product from Nuts.com (Cranford, NJ) was calculated gravimetrically. The material was transferred into 30 mL amber vials (Med-Lab Supply Co. Inc., Miami, FL), labeled, sealed and stored at frozen (~0° C) temperature until ready for analyses.

Response Factor

Purified elderberry proanthocyanidins were used to determine a response factor for elderberry products, which is defined as the ratio between spectrophotometric absorbance at 640 nm and the quantity of purified elderberry proanthocyanidins in parts per million. Five separate elderberry proanthocyanidin dilutions were made (64, 156, 282, 427, 662 ppm) using a 70% acetone (Thermo Fisher Scientific, Waltham, MA) solution. One mL of each dilution was pipetted into individual disposable glass culture tubes. A blank was prepared by pipetting 1 mL of a 70% acetone solution into another glass culture tube. 4-(Dimethylamino)cinnamaldehyde (DMAC) (Sigma-Aldrich®, Saint Louis, MO) was dissolved into a 30% hydrochloric acid/70% methanol (Thermo Fisher Scientific, Waltham, MA) solution at a 0.1000 g into 100 mL concentration. Four mL of DMAC reagent was pipetted into each tube at 10 second intervals, and vortexed. Five minutes was allowed to pass between the addition of the DMAC reagent and spectrophotometric analysis, which gave time for the DMAC to react with the proanthocyanidins. After 5 minutes, the instrument was zeroed and the spectrophotometric absorbance at 640 nm was determined for each sample using a Hach® spectrophotometer Model DR/2500 (Hach Co., Loveland, CO). The overall

response factor was determined in duplicate by averaging the values obtained from the five diluted samples using the following calculation:

absorbance at 640 nm/ppm.

Proanthocyanidins Recovery

Proanthocyanidins recovery (%) is defined as the absorbance at 640 nm of purified elderberry proanthocyanidins which have been subjected to the proanthocyanidins assay versus the absorbance at 640 nm of purified elderberry proanthocyanidins, not having been subjected to the proanthocyanidins assay. Four dilutions (0.2477, 0.4984, 0.9849, 2.5321 g/L) of purified elderberry proanthocyanidins were prepared (±0.0001 g) using deionized water, and shaken vigorously for one minute. Each dilution was subjected to the proanthocyanidins assay outlined in the subsequent sub-chapter, entitled 'proanthocyanidins assay'.

Proanthocyanidins Assay

Total proanthocyanidins content were determined in duplicate for each sample based on a procedure by Prior and others (2010). Elderberry syrups, extracts and concentrates were diluted at an approximate 1:1 ratio with deionized water. Capsules and lozenges were either broken open or ground using a mortar and pestle, diluted to ~1:8, shaken vigorously for 5 minutes, sonicated for 10 minutes using a Branson 5510 sonicator (Branson Ultrasonics Co., Danbury, CT) and centrifuged at 1800 x g for 10 minutes using a Damon IEC HN-S centrifuge (Damon/IEC Division, Needham Heights,

MA). Dried fruit was diluted to ~1:4, homogenized using a Waring® commercial blender Model HGB55556 (Waring® Products, Odessa, FL) and centrifuged at 1800 x g for 10 minutes. Powder was diluted to ~1:40 and shaken vigorously for 5 minutes. The solutions or supernatants of each elderberry product were used for the subsequent determination of total proanthocyanidins.

The tips of Bio-Rad Poly-Prep columns Model 731-1553 (Bio-Rad Laboratories, Inc., Hercules, CA) were broken off, and the columns were inserted into a Bio-Rad Polycolumn rack Model 731-7005 (Bio-Rad Laboratories, Inc., Hercules, CA). One and two-tenths mL of Sephadex® LH-20 gel (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was dropped into each column. Five mL of deionized water were added to each column, which caused the gel to pack down to a 1 mL volume. Once the water content was drained, ~1.0 g of sample was carefully added into each column, allowed to drain gravimetrically, and the elute was discarded. Ten mL of deionized water was added to each column and allowed to drain, and the elute was discarded. Ten mL of a 25% ethyl alcohol (Thermo Fisher Scientific, Waltham, MA) solution was added to each column, allowed to drain, and the elute was discarded. Fifteen mL Falcon™ centrifuge tubes (Thermo Fisher Scientific, Waltham, MA) were inserted in between the columns and rack to act as collection vessels for the collection of a 70% acetone wash (i.e. the fraction of interest). Two and a half mL of a 70% acetone (Thermo Fisher Scientific, Waltham, MA) solution were added to each column and the eluate was collected in the 15 mL centrifuge tubes. Another 2.5 mL of the 70% acetone solution were added to each column and eluate was added into the previous collection. The columns were

discarded and the centrifuge tubes were vortexed for ~30 seconds each. One mL of each sample was transferred from the centrifuge tubes into disposable glass culture tubes, and a blank was prepared by pipetting 1 mL of a 70% acetone solution into another glass culture tube. 4-(Dimethylamino)cinnamaldehyde (DMAC) (Sigma-Aldrich®, Saint Louis, MO) was dissolved into a 30% hydrochloric acid/70% methanol (Thermo Fisher Scientific, Waltham, MA) solution at a 1.0 g/mL concentration. Four mL of DMAC reagent was pipetted into each tube at 10 second intervals, and vortexed after a five minute incubation period, which allowed DMAC to react with the proanthocyanidins. Samples were analyzed using a spectrophotometer. The instrument was zeroed and the spectrophotometric absorbance at 640 nm was measured for each sample using a Hach® spectrophotometer Model DR/2500 (Hach Co., Loveland, CO). Total proanthocyanidins were determined using the following calculation:

% proanthocyanidins = ((((abs@640 x eluate volume mL)/(response factor x volume analyzed mL x weight on column g x dilution factor x % sample solids))/% recovery)/µg per g to mg per g conversion factor) x % conversion factor.

Example calculation:

((((0.405 x 5.0)/(0.0018 x 1.0 x 0.9654 x 0.008 x 100%))/32%)/1000) x 0.1 = 44.1 % proanthocyanidins (DMAC).

Titratable Acidity and Vitamin C

Titratable acidity was determined in duplicate for fluid elderberry products by sodium hydroxide titration using a Pharm Titrando titration system Model 2.907.1020 (Metrohm Ltd., Herisau, Switzerland) (Method 942.15, AOAC 2005). Ten mL of sample was diluted into 90 mL of distilled water and titrated with 0.1 N NaOH (Thermo Fisher

Scientific, Inc., Waltham, MA) until a pH endpoint of 8.1 was reached. Titratable acidity was expressed as % citric acid using the following calculation:

(mLs of 0.1 N NaOH x 0.1 x milliequivalent factor of citric acid x 100)/10.

Vitamin C (ascorbic acid + dehydroascorbic acid) was determined in duplicate for fluid elderberry products by iodine titration using a Pharm Titrando titration system Model 2.907.1020 (Metrohm Ltd., Herisau, Switzerland) and expressed as % vitamin C (Method 967.21, AOAC 2005).

Spectrophotometric Color

The spectrophotometric color of liquid elderberry products was determined in duplicate. Samples were diluted to a 1:50 ratio with deionized water and sonicated for 10 minutes using a Branson 5510 sonicator (Branson Ultrasonics Co., Danbury, CT). Products which contained particulates were filtered using a 10 mL BD™ Luer-Lock™ syringe Model S7510-10 (BD™, Franklin Lakes, NJ) with a 30 mm x 1.2 µm GMF Membrane Titan 2 HPLC filter tip (Sun Sri, Rockwood, TN). Five mL of each sample was pipetted into disposable glass culture tubes and absorbance was determined at 520 and 430 nm using a Hach® spectrophotometer Model DR/2500 (Hach Co., Loveland, CO).

L*a*b* Color

Elderberry products were subjected to colorimetric analysis using a LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, VA) to determine L*a*b* values. Elderberry products were poured into a 2.5 inch clear glass sample cup and

placed on a pre-calibrated, 2.5 inch sample port. L*a*b* values were determined using the computer software and analysis was completed in duplicate per product with one reading per sample.

Sugars Profile

The sugars profile of elderberry products was determined based on a procedure by Richmond and others (1981). Elderberry products were diluted to an approximate 1:30 ratio using deionized water and shaken vigorously by hand for 1 minute. Products which contained particulates were filtered using a 10 mL BD™ Luer-Lock™ syringe Model S7510-10 (BD™ Franklin Lakes, NJ) with a 30 mm x 1.2 µm GMF Membrane Titan 2 HPLC filter tip (Sun Sri, Rockwood, TN). The tips of Bio-Rad Poly-Prep columns Model 731-1553 (Bio-Rad Laboratories, Inc., Hercules, CA) were broken off, and the columns were inserted into a Bio-Rad Polycolumn rack Model 731-7005 (Bio-Rad Laboratories, Inc., Hercules, CA). The columns were sequentially rinsed with methanol (Thermo Fisher Scientific, Waltham, MA) and deionized water. One (±0.1) g of Bio-Rad Bio-Rex® 100-200 mesh resin (Bio-Rad Laboratories, Inc., Hercules, CA) was added to each column after being slurried with ~5 mL of deionized water. Approximately 4 mL of deionized water was carefully added into each column and drained, which allowed the resin to settle. One half mL of each elderberry sample was carefully pipetted directly onto the resin beds, and allowed to drain. Fifteen mL Falcon™ centrifuge tubes (Thermo Fisher Scientific, Waltham, MA) were inserted in between the columns and rack to act as collection vessels for the collection of the subsequent eluate. One mL of deionized

water was carefully added into each column, twice, and the eluates were collected. Once the columns had completely drained, they were discarded and the centrifuge tubes were vortexed for ~10 seconds each. Sample eluate was transferred from the centrifuge tubes into clear HPLC autosampler vials, and sealed. The sugars profile (xyloglucans, sucrose, lactose, sucrose, mannose, fructose, glycerin, maltitol, xylitol, sorbitol) of each elderberry sample was determined using an Agilent 1100 Series G1311A QuatPump liquid chromatograph (Agilent Technologies, Inc., Santa Clara, CA) with a 300 x 780 mm Phenomenex® Rezex™ RCM-Monosaccharide Ca+2 (8%) column (Phenomenex Inc., Torrance, CA), a Bio-Rad Micro-Guard® Carbo C guard column (Bio-Rad Laboratories, Inc., Hercules, CA), and an Agilent 1200 Series refractive index detector Model G1362A (Agilent Technologies, Inc., Santa Clara, CA). The mobile phase was deionized water, of which 10 µL of each sample were injected into the system at a 0.6 mL/min flow rate. The overall run time was 30 minutes and the column temperature was 80° C. Multiple concentrations of reference standards of each type of saccharide were analyzed along with the samples. Each sample was analyzed in duplicate and each sugar was reported as %.

Anthocyanins Profile

The anthocyanins profile of all elderberry products was determined based on a procedure by Brown and Shipley (2011). Elderberry products were diluted to between a 1:2 and a 1:100 ratio using deionized water, and sonicated for 10 minutes using a Branson 5510 sonicator (Branson Ultrasonics Co., Danbury, CT). Products which

contained particulates were filtered using a 10 mL BD™ Luer-Lock™ syringe Model S7510-10 (BD™ Franklin Lakes, NJ) with a 30 mm x 1.2 µm GMF Membrane Titan 2 HPLC filter tip (Sun Sri, Rockwood, TN). Samples were transferred into amber HPLC autosampler vials, and sealed. The anthocyanins profile (cyanidin-3-sambubioside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, peonidin-3-galactoside, peonidin-3-glucoside, peonidin-3-arabinoside, cyanidin, peonidin) of each sample was determined using an Agilent 1100 Series G1312A BinPump liquid chromatograph (Agilent Technologies, Inc., Santa Clara, CA) with a 50 x 4.60 mm Phenomenex® Kintex™ 2.6µ C18 100Å column (Phenomenex Inc., Torrance, CA) and an Agilent diode array detector Model G1315A (Agilent Technologies, Inc., Santa Clara, CA). The mobile phase was comprised of two eluents (A/B). Eluent A was comprised of 99:1 deionized water:85% phosphoric acid, and eluent B contained 50:49:1 deionized water:85% phosphoric acid:acetonitrile. The eluents were injected by gradient elution (0.0-7.8 minutes, 88% A, 12% B; 7.8-11.4 minutes, 70% A, 30% B; 11.4-13.2 minutes, 60% A, 40% B; 13.2-15.0 minutes, 30% A, 70% B) at a 1.5 mL/min flow rate, along with a 20 µL sample. The overall run time was 15 minutes, the column temperature was 30° C, and anthocyanins were detected at 520 nm absorbance. Multiple concentrations of reference standards of each type of anthocyanin were analyzed along with the samples. Each anthocyanin was reported as either mg/L for fluid elderberry products or parts per million (ppm) for dried elderberry products.

Organic Acids Profile

The organic acids profile of elderberry products was determined based on a procedure by Chen and others (2013). Elderberry products were diluted to between a 1:40 and a 1:2000 ratio using deionized water, and sonicated for 10 minutes using a Branson 5510 sonicator (Branson Ultrasonics Co., Danbury, CT). Products which contained particulates were filtered using a 10 mL BD™ Luer-Lock™ syringe Model S7510-10 (BD™ Franklin Lakes, NJ) with a 30 mm x 1.2 µm GMF Membrane Titan 2 HPLC filter tip (Sun Sri, Rockwood, TN). Samples were transferred into Dionex™ PolyVials™ 0.5 mL Model 038010 (Thermo Fisher Scientific, Waltham, MA), capped using Dionex™ PolyVial™ 0.5 mL filter caps Model 038011 (Thermo Fisher Scientific, Waltham, MA) and inserted into the slots of a Dionex™ AS-DV autosampler Model 068888 (Thermo Fisher Scientific, Waltham, MA). The organic acids profile (quinic, galacturonic, malic, tartartic, fumaric, citric, isocitric) of each sample was determined using a Dionex™ ICS-2100 Reagent-Free™ ion chromatograph (Thermo Fisher Scientific, Waltham, MA) with a RFIC™ IonPac® AG11-HC 4 x 250 mm ion exchange column Model 078429 (Thermo Fisher Scientific, Waltham, MA), RFIC™ IonPac® AG11-HC 4 x 50 mm guard column Model 078430 (Thermo Fisher Scientific, Waltham, MA) and an ASRS® 300 4 mm suppressor Model 064554 (Thermo Fisher Scientific, Waltham, MA). The column temperature was 30° C, and the mobile phase was deionized water at a flow rate of 1.35 mL/min. Standards of each organic acid were analyzed along with the samples, and each sample was tested in duplicate. Multiple concentrations of reference standards of each type of organic acid were analyzed along with the samples.

The benzoic acid content of each sample was determined by high-performance liquid chromatography (HPLC). Samples were prepared the same as previously outlined within this subchapter, except sample dilutions were transferred into amber HPLC autosampler vials instead of Dionex™ PolyVials™ 0.5 mL Model 038010 (Thermo Fisher Scientific, Waltham, MA). The benzoic acid content of each sample was determined using an Agilent 1100 Series G1313A liquid chromatograph (Agilent Technologies, Inc., Santa Clara, CA) with a 4.6 x 150 mm Agilent ZORBAX® Eclipse XDB-C8 5µ column (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent diode array detector Model G1315A (Agilent Technologies, Inc., Santa Clara, CA). The mobile phase was a solution of 50% 0.025M phosphoric acid (Thermo Fisher Scientific, Waltham, MA), 35% methanol (Thermo Fisher Scientific, Waltham, MA), and 15% acetonitrile (Thermo Fisher Scientific, Waltham, MA), of which 5 µL per sample were injected into the system at a flow rate of 0.5 mL/min. The run time was 7 minutes and the detector was set at 230 nm absorbance. Multiple concentrations of a benzoic acid standard was included in each sample set and each sample was analyzed in duplicate. Each organic acid was reported as either mg/L for fluid elderberry products or parts per million (ppm) for dried elderberry products.

Statistical Analyses

Data collected from elderberry product assays and accelerated temperature (32° C) storage were evaluated within product categories using JMP 7.0.1 (SAS Institute Inc., Cary, NC) statistical software using one-way analysis of variance (ANOVA) with a

significance value of $p \leq 0.05$. Differences between means were evaluated using the Fisher's least significant difference test. A correlation analysis was also performed among the dependent variables.

Results and Discussion

Syrups

Compositional Analyses at Week 0

The analytical testing performed in this study quantified the large majority (>88.0%) of ingredients within each of the syrups, except for Sambucol® for Kids and Sambucol® syrups, which contained 30.3% and 45.1% of unknown ingredients, respectively (Tables 2+3). Maltodextrins, which were not quantified in this study, likely accounted for the large fraction of unknown ingredients within these two syrups. Other ingredients which were likely in all of the syrups, yet unquantified, may have included some organic acids (cinnamic, chlorogenic, neochlorogenic, caffic, palmitic), plant material (fiber), and possibly small fractions of sorbates. Considering that hydroxycinnamic acid derivatives (cinnamic, chlorogenic, neochlorogenic, caffic) account for the major non-flavonoid organic acids naturally present in elderberry fruit, it is theorized that the total organic acid contents of all products tested in this study were underestimated by approximately half (Thomas and others 2015). Determining the total organic acids content of elderberry products is important because there may be a relationship between the amount organic acids and the amount of fruit used for production. The organic acid profiles of all syrups were typical of elderberry, and

contained mostly citric, quinic, and malic acids (Table 6) (Whiting 1958). The exception was Dynamic Health® Black Elderberry Tonic, which contained elevated levels of galacturonic acid, likely due to the inclusion of raspberry fruit. The anthocyanin profiles of all syrups were also typical of elderberry, and contained mostly cyanidin-3-sambubioside, cyanidin-3-glucoside, and to a lesser extent, cyanidin-3-sambubioside-5-glucoside (Table 5) (Wu and others 2002, Seabra and others 2010a, Schmitzer and others 2010). Anthocyanins which include sambubioside glycosides are important chemical markers for the identification of elderberry in foods, since these markers are exclusive to elderberry (Clifford 2000, Veberic and others 2009). It was determined that glycerin comprised between 51.1% and 60.7% of the total sugars content of Nature's Answer® Black Elderberry Extract, Bio-Botanica® SE Elderberry Syrup, Bio-Botanica® AF Elderberry Syrup, and Now® Elderberry, Zinc and Echinacea Syrup; and between 13.7% and 14.3% of Nature's Way® Sugar-Free Sambucus Syrup, Nature's Way® Sambucus Syrup, and Integrative Therapeutics™ Sambucus Extract (Table 4). Additionally, Nature's Way® Sugar-Free Sambucus Syrup also contained 43.1% sorbitol and 13.9% glycerin, and its sugar contents were comprised exclusively of these two polyols. Although it was necessary for the syrups to contain added sugars for preservation and consumer acceptability purposes, their high sugars content may negatively offset the overall perception of being healthy products.

Table 2 Labeled Ingredients and Price of Elderberry Syrups

Product Code	Labeled Ingredients	Serving Size	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
EMG	N/A	N/A	N/A	N/A	3
NW SF	Standardized Elderberry BioActives® Extract, Sorbitol, Purified Water, Vegetable Source Glycerin, Natural Raspberry Flavor, Citric Acid	10 mL	$28.99/240 mL (www.vitacost.com)	$0.12/ mL	17
NW	Standardized Elderberry BioActives® Extract, Fructose, Purified Water, Vegetable Source Glycerin, Natural Raspberry Flavor, Citric Acid	10 mL	$28.99/240 mL (www.vitacost.com)	$0.12/ mL	16
Sam K	Elderberry Extract, Glucose Syrup, Purified Water, Citric Acid, Potassium Sorbate	10 mL	$24.99/230 mL (www.sambucolusa.com)	$0.11/ mL	1
NA	Black Elderberry Extract, Vegetable Glycerin, Purified Water, Citric Acid	5 mL	$14.99/120 mL (www.naturesanswer.com)	$0.12/ mL	18
Sam	Elderberry Extract, Glucose Syrup, Purified Water, Citric Acid, Potassium Sorbate	10 mL	$24.99/230 mL (www.sambucolusa.com)	$0.11/ mL	3
Bio SE	Elderberry, Glycerin	N/A	N/A	N/A	14
Bio AF	Elderberry, Glycerin	N/A	N/A	N/A	1
Dyn	Elderberry Juice Concentrate, Raspberry Puree, Invert Red Beet Syrup, Honey, Natural Raspberry Flavor	15 mL	$11.89/240 mL (www.vitacost.com)	$0.05/ mL	3
Inte	Standardized Elderberry Extract, Fructose, Purified Water, Vegetable Source Glycerin, Natural Raspberry Flavor, Citric Acid	10 mL	$15.50/120 mL (www.integrativepro.com)	$0.13/ mL	8
Now	Elderflower, Elderberry Fruit, *Echinacea purpurea*, Zinc Gluconate, Vegetable Glycerin, Water	5 mL	$16.99/120 mL (www.vitacost.com)	$0.14/ mL	4
Antho	Organic American Elderberry, Organic European Elderberry, Organic Blueberry, Organic American Elderflower, Organic Agave Nectar, Water, Organic Pure Grain Alcohol USP (3.5-5%)	5 mL	$27.95/240 mL (www.mainemedicinals.com)	$0.12/ mL	5

Table 2 continued

Honey	US Grade A Raw Honey, Organic Elderberries, Organic Apple Cider Vinegar, Propolis Echinacea, Pure Grain Alcohol, Water	5 mL	$20.99/240 mL (www.honeygardens.com)	$0.09/mL	8
Planet	European Elderberry Extract, Honey, Grain Alcohol (20-25%), Purified Water	5 mL	$33.50/240 mL (www.planetaryherbals.com)	$0.14/mL	10

Refer to Table 1 for product identification.

Table 3 Compositional Profiles of Elderberry Syrups at Week 0

Product Code	Moisture (%)	Total Proantho-cyanidins (%)	Vitamin C (%)	Total Sugars (%)	Total Antho-cyanins (%)	Total Organic Acids (%)	Total Unknown (%)
EMG	44.6*c*	0.163*def*	ND	60.1 *abcd*	0.0385*c*	0.3443*e*	-5.2
NW SF	38.7*ef*	0.360*b*	ND	57.2 *bcd*	0.0434*c*	0.1674*g*	3.5
NW	40.7*de*	0.252*c*	ND	55.4*cd*	0.0346*c*	0.4490*e*	3.2
Sam K	38.2*f*	0.142*efg*	13.5*a*	17.4*i*	0.0002*e*	0.4568*e*	30.3
NA	24.8*h*	0.092*gh*	ND	62.8*a*	0.0026*e*	1.5619*b*	10.7
Sam	33.9*g*	0.378*b*	ND	19.9*i*	0.0098 *de*	0.6709*d*	45.1
Bio SE	24.8*h*	0.203*cde*	ND	61.7 *abc*	0.0031 *de*	2.0290*a*	11.3
Bio AF	24.6*h*	0.585*a*	ND	65.4*a*	0.1048*a*	2.2131*a*	7.1
Dyn	62.4*a*	0.614*a*	13.5*a*	27.5*h*	0.0355*c*	1.1759*c*	-5.2
Inte	42.6*cd*	0.218*cd*	ND	54.7*de*	0.0741*b*	0.3732 *ef*	2.0
Now	32.4*g*	0.064*hi*	ND	61.4*ab*	0.0002*e*	0.3922*f*	5.7
Antho	54.1*b*	0.341*b*	ND	40.3*g*	0.0263 *cd*	0.7913*d*	4.4
Honey	42.3*cd*	0.131*fg*	ND	49.6*ef*	0.0062*e*	0.2938 *fg*	7.7
Planet	45.0*c*	0.021*i*	ND	46.4*fg*	0.0003*e*	0.7913*d*	7.8

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm, (vitamin C)=7.3 mg.

Sambucol® for Kids Syrup and Dynamic Health® Black Elderberry Tonic were the only two syrups tested in this study which made vitamin C claims. Initial analysis showed that they both contained vitamin C contents of 13,500 mg/100 g, had serving sizes between 5.0-14.8 mL, and exceeded their claims by approximately 20 and 65 times. The recommended daily intake for vitamin C is 60-90 mg per day per person, and daily consumption exceeding 2,000 mg is not recommended, primarily due to the potential for renal taxation, upset stomach, and diarrhea (U.S. Department of Agriculture 2008). Considering that fresh elderberry fruit and elderberry fruit juice contains approximately 29 mg/100g of vitamin C (48% daily value/100 g), and that no other syrups tested in this study contained detectable levels of vitamin C, it is theorized that Sambucol® for Kids Syrup and Dynamic Health® Black Elderberry Tonic were vitamin C fortified (U.S. Department of Agriculture 2008, Casati and others 2012). Interestingly, Sambucol® for Kids Syrup contained somewhat lower anthocyanin contents compared to Sambucol® Syrup, which may have been a result of its vitamin C contents causing the degradation of anthocyanins (González-Molina and others 2012).

Table 4 Sugar Profiles of Elderberry Syrups at Week 0

Product Code	Sucrose (%)	Sucrose (%)	Fructose (%)	Glycerin (%)	Sorbitol (%)	Total Sugars (%)
EMG	53.0*a*	4.2*f*	3.0*g*	ND	ND	60.1*abcd*
NW SF	ND	ND	ND	14.3*d*	43.1	57.2*bcd*
NW	ND	ND	41.5*a*	13.9*d*	ND	55.4*cd*
Sam K	7.8*c*	9.4*e*	0.5*i*	ND	ND	17.4*i*
NA	ND	2.4*g*	2.9*f*	57.6*b*	ND	62.8*a*
Sam	9.6*b*	10.3*d*	ND	ND	ND	19.9*i*
Bio SE	ND	4.5*f*	6.0*f*	51.1*c*	ND	61.7*abc*
Bio AF	ND	2.7*g*	2.9*g*	59.8*b*	ND	65.4*a*
Dyn	3.1*d*	11.5*c*	12.9*e*	ND	ND	27.5*h*
Inte	ND	ND	41.0*a*	13.7*d*	ND	54.7*de*
Now	ND	ND	0.7*h*	60.7*a*	ND	61.4*ab*
Antho	1.3*f*	4.8*f*	34.0*b*	0.3*g*	ND	40.3*g*
Honey	2.0*e*	20.8*a*	26.1*c*	0.9*f*	ND	49.6*ef*
Planet	ND	19.8*b*	23.1*d*	1.4*e*	ND	46.4*fg*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 5 Anthocyanin Profiles of Elderberry Syrups at Week 0

Product Code	cyan-3-sambu (mg/L)	cyan-3-sambu-5-gluco (mg/L)	cyan-3-galact (mg/L)	cyan-3-gluco (mg/L)	peonidin (mg/L)	Total Unknown (mg/L)	Total Anthocyanins (mg/L)
EMG	90.7*ab*	ND	ND	12.6*d*	16.5*a*	265.3*a*	385.1*c*
NW SF	42.1*bcd*	7.3*a*	ND	378.0*c*	ND	2.6*c*	433.6*c*
NW	31.5*bcd*	ND	ND	314.4*c*	ND	ND	345.8*c*
Sam K	0.4*cd*	0.4*d*	ND	1.3*d*	ND	ND	1.9*e*
NA	15.1*cd*	2.1*c*	ND	14.3*d*	ND	2.4*c*	25.5*e*
Sam	27.0*bcd*	4.5*bc*	ND	65.0*cd*	ND	ND	98.4*de*
Bio SE	10.6*bcd*	ND	ND	8.5*d*	ND	12.1*bc*	31.2*de*
Bio AF	130.2*a*	ND	ND	917.9*ab*	ND	ND	1048.1*a*
Dyn	62.1*abc*	ND	ND	176.7*cd*	ND	116.0*ab*	354.8*c*
Inte	59.4*abcd*	ND	ND	681.2*b*	ND	ND	740.6*b*
Now	2.0*d*	ND	ND	ND	ND	ND	2.0*e*
Antho	119.0*a*	6.3*ab*	2.1	65.6*cd*	ND	69.5*b*	262.5*cd*
Honey	9.7*cd*	ND	ND	ND	0.6*b*	51.6*b*	61.8*de*
Planet	2.8*cd*	ND	ND	0.6*d*	ND	ND	3.4*e*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses. Limit of quantitation=0.2 mg/L.

Table 6 Organic Acid Profiles of Elderberry Syrups at Week 0

Product Code	Quinic (mg/L)	Galac-turonic (mg/L)	Malic (mg/L)	Tart-aric (mg/L)	Fum-aric (mg/L)	Citric (mg/L)	Iso-citric (mg/L)	Total Organic Acids (mg/L)
EMG	201.8*f*	ND	579.1 *efg*	27.1*d*	53.4*cd*	2487.1 *gh*	92.6*de*	3443.0 *efg*
NW SF	75.3*f*	44.3*e*	87.4*h*	ND	ND	1430.7 *hi*	27.0*e*	1673.7*g*
NW	138.7*f*	40.6*e*	82.7*h*	9.3*e*	ND	4165.9*e*	52.9*e*	4489.9*e*
Sam K	291.1*f*	262.4*de*	265.1*gh*	11.7*d*	ND	3622.9 *ef*	114.4*de*	4567.6*e*
NA	3219.2*b*	371.0*cde*	1455.0 *bc*	21.6*cd*	126.3 *abc*	9834.7*b*	585.8*b*	15618.5 *b*
Sam	461.5*ef*	440.5*cde*	415.6 *fgh*	ND	13.7*cd*	5326.3 *cd*	102.9*de*	6708.9*d*
Bio SE	4879.0*a*	440.2*cde*	2928.0*a*	143.5*a*	243.0 *abc*	10887.1 *b*	769.3*ab*	20290.0 *a*
Bio AF	4270.5*a*	1202.7*b*	1888.8*b*	ND	ND	14768.7 *a*	ND	22130.7 *a*
Dyn	2591.3 *bc*	2172.2*a*	650.3 *efg*	92.7*b*	ND	5962.5*c*	289.7*c*	11758.7 *c*
Inte	555.5*ef*	ND	71.4*h*	ND	ND	3097.1 *fg*	ND	3732.3 *ef*
Now	1000.2 *de*	ND	878.4*de*	139.4*a*	138.3*ab*	1655.8 *hi*	102.5*de*	3921.5 *ef*
Antho	2561.6*c*	278.2*de*	961.9*de*	33.0*d*	60.9*c*	3867.7 *ef*	166.3*cd*	7913.1*d*
Honey	570.0*ef*	59.0*e*	670.3*ef*	57.7*c*	91.3*bc*	1330.1*i*	48.2*e*	2937.8 *fg*
Planet	1524.7*d*	31.2*e*	1267.5 *cd*	85.0*b*	96.1*bc*	4702.4 *de*	206.2*cd*	7913.2*d*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses. Honey=111.4±0.9 mg/L benzoic. Limit of quantitation=90 mg/L.

Color Analyses at Week 0

Spectrophotometric wavelengths of 520 and 430 nm were used to indicate the amount of red (anthocyanic pigment) and brown (anthocyanic degradation) pigmentations within the syrups, respectively. A 520/430 ratio was used to gain quick

insight into the quality of the syrups. Typically, ratios exceeding 1.00 denote high quality elderberry syrups, which contain greater proportions of red pigmentation compared to brown pigmentation. The 520/430 ratios exceeded 2.00 for Evergreen Manufacturing Group Elderberry Syrup, Nature's Way® Sugar-Free Sambucus Syrup, Nature's Way® Sambucus Syrup, Sambucol® for Kids Syrup, Sambucol® Syrup, Integrative Therapeutics™ Sambucus Extract, and Honey Gardens™ Elderberry Syrup, during initial testing. A 520/430 ratio of 1.23 was detected for Now® Elderberry, Zinc and Echinacea Syrup, and ratios between 0.45 and 0.92 were detected for Nature's Answer® Black Elderberry Extract, Bio-Botanica® SE Elderberry Syrup, Bio-Botanica® AF Elderberry Syrup, Dynamic Health® Black Elderberry Tonic, and AnthoImmune™ Organic Elderberry Syrup. Syrups with ratios below 1.00 generally appeared more brown, and contained strong musty odors, which would likely contribute to lowered consumer acceptability.

Colorimetric analysis using the L*a*b* color scale is a fast quantitative technique in which the L*a*b* values are used to approximate human vision. L* values represent a range of black (0) to white (100), or darkness to lightness. The a* value represents a range of green (-) to red colors (+), and b* values represent a range of blue (-) to yellow colors (+) (Nielson, 2010). In this study, significantly ($p \leq 0.05$) higher a* and b* values were detected within Evergreen Manufacturing Group Elderberry Syrup, Sambucol® for Kids Syrup, and Honey Gardens™ Elderberry Syrup, which indicates that these products are rich sources of red and yellow pigmentation (Figure 5). Interestingly, the average time between date of production and analyses of these three syrups was only 4 months, whereas the average age for the other syrups was 8 months after production. The

shorter distribution time of these syrups likely contributed to their improved color. Moderate to low correlations (p≤0.05) were observed between 520/430 ratio and L* (r=0.22), a* (r=0.50), and b* (r=0.37) values. Additionally, a strong correlation was observed between absorbance at 520 nm and absorbance at 430 nm (r=0.65) among the syrups, which demonstrates a relationship between the amounts of anthocyanic pigmentation and anthocyanic degradation within the syrups.

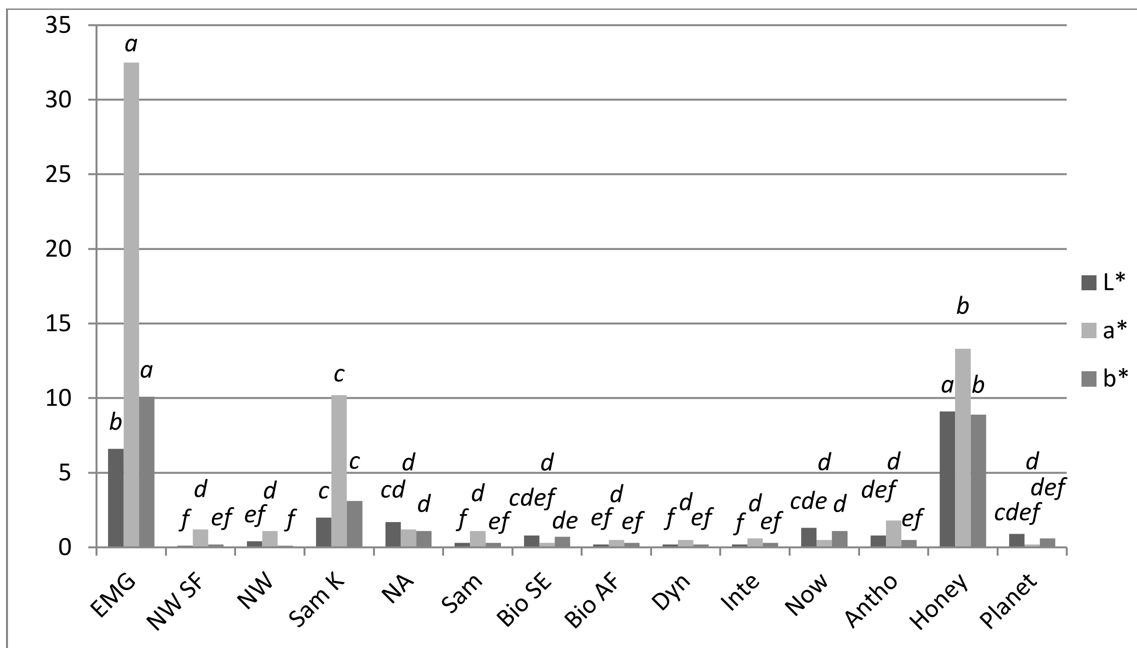


Figure 5 L*a*b* color values of elderberry syrups at week 0.
Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Effects of Accelerated Storage

An important objective of this study was to determine the effects of accelerated temperature (32° C) storage on the quality (nutrient and color stability) of elderberry syrups at 0, 5, and 10 weeks. The two syrups which contained vitamin C exhibited

significant ($p \leq 0.05$) decreases in vitamin C content throughout 10 weeks of accelerated temperature (32° C) storage. Sambucol® for Kids Syrup and Dynamic Health® Black Elderberry Tonic had vitamin C contents of 13,500, 10,700, and 9,500 mg/100 g and 13,500, 8,800, and 5,800 mg/100 g at 0, 5, and 10 weeks of storage (Figure 6). These results show that the vitamin C content degraded slower in the Sambucol® for Kids Syrup than in the Dynamic Health® Black Elderberry Tonic. This decrease may be the result of the presence of maltodextrins within Sambucol® for Kids Syrup, which may have acted as a preservative by encapsulating vitamin C and excluding oxygen. A second theory is that the presence of potassium sorbate within Sambucol® for Kids Syrup may have limited microbial growth, which otherwise may have degraded vitamin C. After 10 weeks of accelerated temperature storage, both syrups still exceeded their vitamin C claims by wide margins.

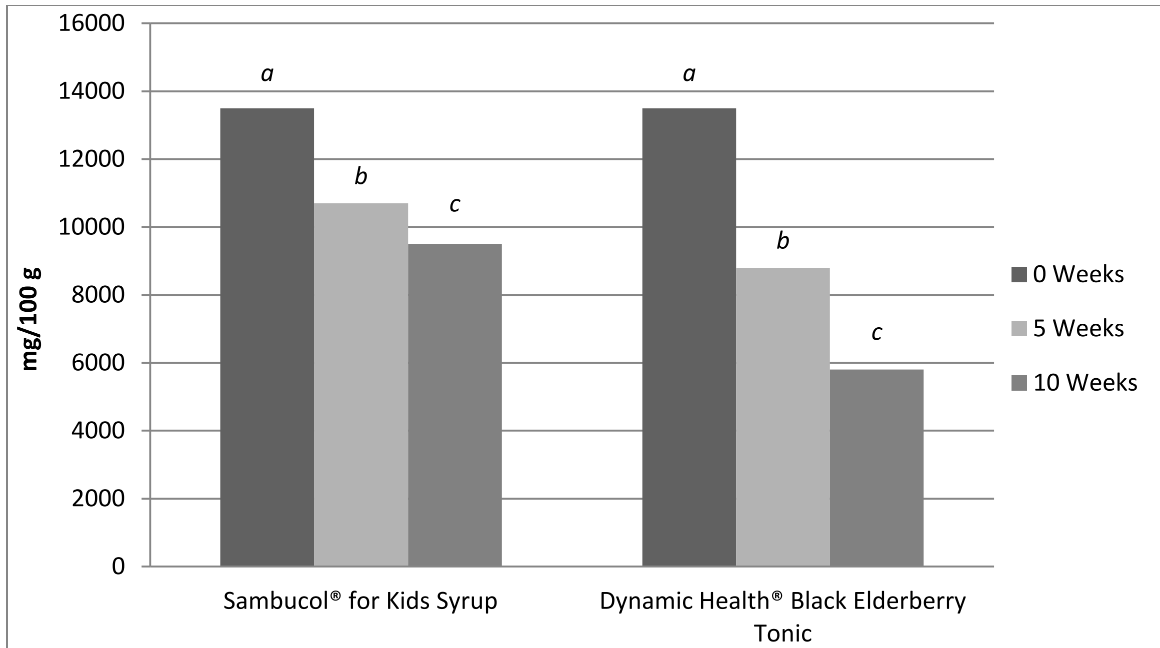


Figure 6 Vitamin C values (mg/100 g) of elderberry syrups throughout weeks of accelerated temperature (32° C) storage.

Vitamin C content by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

During the first 5 weeks of accelerated temperature (32° C) storage, significant (p≤0.05) losses of red pigmentation were observed in the Evergreen Manufacturing Group Elderberry Syrup, Nature's Way® Sugar-Free Sambucus Syrup, Sambucol® for Kids Syrup, Nature's Answer® Black Elderberry Extract, Sambucol® Syrup, Bio-Botanica® SE Elderberry Syrup, Bio-Botanica® AF Elderberry Syrup, and AnthoImmune™ Organic Elderberry Syrup (Figure 7). There was an average of a 25% reduction to the 520/430 ratio of these syrups, and Evergreen Manufacturing Group Elderberry Syrup, Sambucol® for Kids Syrup, Sambucol® Syrup, and AnthoImmune™ Organic Elderberry Syrup displayed 76%, 66%, 76%, and 49% lower 520/430 ratios throughout the first 5 weeks of storage, respectively. Interestingly, Evergreen Manufacturing Group Elderberry Syrup

and Sambucol® for Kids Syrup were among the syrups with the strongest initial red colorations according to their a* values, therefore the color of these syrups remained competitive after substantial losses to red pigmentation. Additionally, although many of the syrups experienced significantly ($p \leq 0.05$) lower 520/430 ratios throughout the first 5 weeks of accelerated temperature storage, few effects were observed between 5 weeks and 10 weeks of storage. These results indicate that the majority of the color degradation occurred within the first 5 weeks of accelerated temperature storage. It is believed that the elevated 520/430 ratios of Honey Gardens™ Elderberry Syrup observed at 5 and 10 weeks of storage are a result of experimental error. There is no scientific justification for the increase of red pigmentation throughout accelerated temperature storage, and Honey Gardens™ Elderberry Syrup contained observable particulates which may have interfered with the spectrophotometric absorbance readings. Interestingly, Honey Gardens™ Elderberry Syrup was the only elderberry syrup tested in this study which did not contain elderberry fruit as its primary ingredient, which was instead raw honey. These results are in agreement with Fernández-López and others (2013), who reported a loss of 36.2% of absorbance at 535nm throughout 6 hours of storage at 90° C, which indicated a loss of anthocyanic pigmentation.

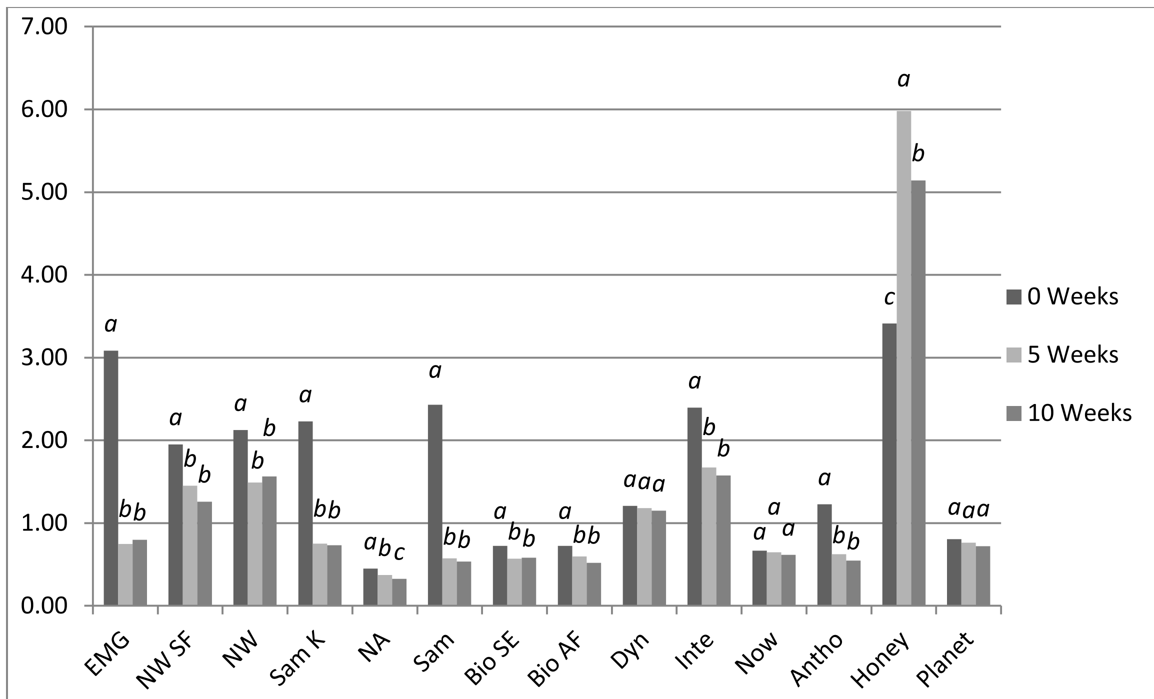


Figure 7 520/430 ratios of elderberry syrups throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. 520/430 ratios by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

In addition to the storage effects on vitamin C and color, significant ($p \leq 0.05$) decreases of anthocyanin content in elderberry syrups were detected throughout 10 weeks of accelerated temperature (32° C) storage (Figure 8). Over the course of 10 weeks, the majority of the syrups lost approximately half of their initial anthocyanin content. Bio-Botanica® AF Elderberry Syrup and Dynamic Health® Black Elderberry Tonic were the most susceptible to anthocyanin degradation among the syrups tested in this study, and contained initial anthocyanin contents of 1048.1 and 354.8 mg/L at 0 weeks of storage, 259.6 and 32.6 mg/L at 5 weeks of storage, and 72.8 and 10.5 mg/L at 10 weeks of storage, respectively. Although these two syrups lost the majority of their

anthocyanins throughout accelerated storage, these syrups still contained appreciable amounts of anthocyanins at 10 weeks of storage. These results are in agreements with González-Molina and others (2012), who reported an average loss of approximately 60% of the anthocyanin contents of an elderberry solution throughout 56 days of room temperature storage.

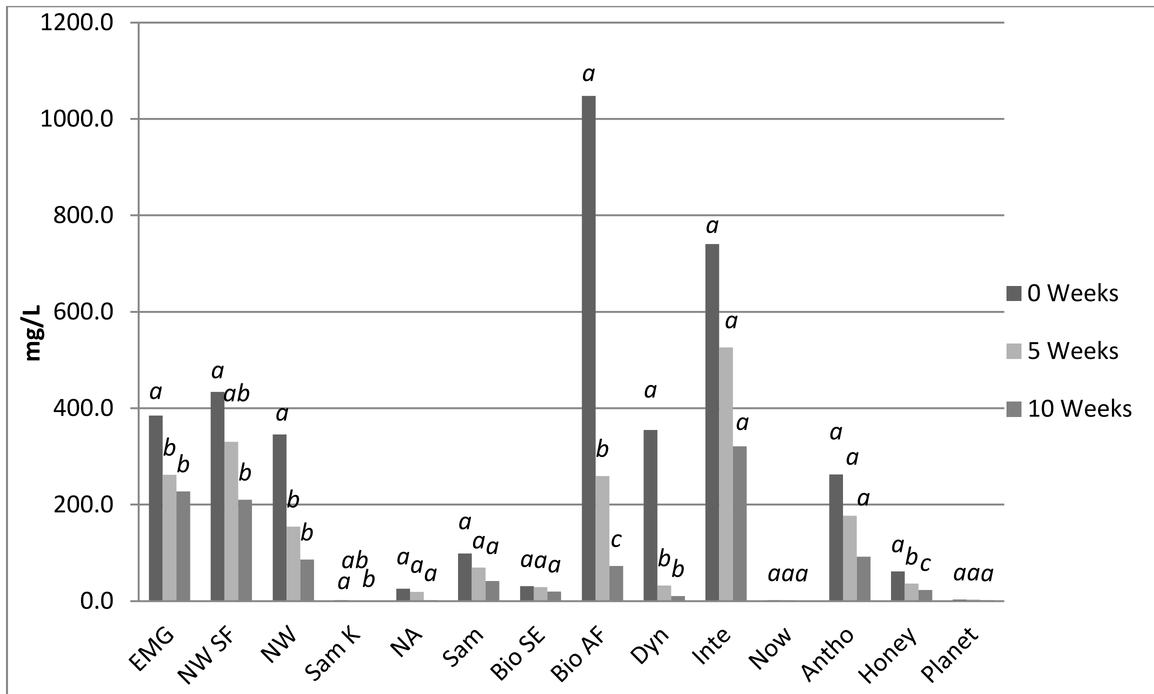


Figure 8 Total anthocyanin values (mg/L) of elderberry syrups throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total anthocyanins by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Tinctures

Compositional Analyses at Week 0

The analytical testing performed in this study quantified greater than 94.0% of the ingredients within each of the tinctures (Tables 7+8). All of the tinctures contained at least 80.4% moisture/alcohol, between 0.9% and 15.0% total sugars, and small fractions of anthocyanins (<0.015% or <140.0 mg/L). Sugars profile analyses revealed that these tinctures primarily contained naturally-occurring elderberry sugars (sucrose, fructose), with the exception of Herb Pharm® Black Elderberry Extract, which contained 12.1% glycerin (Table 9). Additionally, Gaia® Organics Black Elderberry Liquid Extract contained 8.5% sucrose, which was not detected within any of the other tinctures tested in this study. The anthocyanin profiles of the tinctures were exclusive of elderberry fruit, and contained primarily cyanidin-3-sambubioside and cyanidin-3-glucoside (Table 10). Quantum® Health Elderberry Liquid Extract and Source Naturals® Wellness Elderberry Liquid Extract™ contained appreciable amounts of anthocyanins, however the anthocyanin contents of the tinctures were relatively low compared to the syrups tested in this study. This difference was likely due to increased sugar contents of the syrups, which absorbed water and prevented the hydrolytic degradation of anthocyanins. The total organic acids content of the tinctures were comparable to the syrups tested in this study, which indicates that similar quantities of fruit were used during the production of each elderberry product. The organic acids profiles of the tinctures indicate that elderberry fruit was the exclusive fruit used within the tinctures, and contained primarily citric, quinic, and malic acids (Table 11). The organic acid results

are in agreement with Verberic and others (2009), who reported the organic acid content of fresh elderberry fruit to be 6.3 g/kg or 0.638%. All of the tinctures contained low proportions of proanthocyanidins, with the exception of Quantum® Health Elderberry Liquid Extract which contained a relatively high proanthocyanidin fraction (11,060.0 mg/L or 1.106%). In comparison, the estimated daily consumption of proanthocyanidins is between 22.5 and 57.7 mg per person, and cranberry fruit, which is considered a rich source of proanthocyanidins, contains 4,110.0 mg/kg (Gu and others 2004, Kyle and Duthie 2006, Rothwell and others 2013). Interestingly, vitamin C was not detected within any of the tinctures tested in this study, which was likely due to the susceptibility of vitamin C to oxidative and hydrolytic degradation.

Table 7 Labeled Ingredients and Price of Elderberry Tinctures

Product Code	Labeled Ingredients	Serving Size	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
Quant	Elderberry Extract, Distilled Water, Alcohol	0.6-0.9 mL	$13.49/ 60 mL (www.quantumhealth.com)	$0.22/ mL	6
Source	European Elder Berry and Flower Extract, Grain Alcohol (40%), Purified Water	5 mL	$31.98/ 240 mL (www.sourcenaturals.com)	$0.13/ mL	17
Gaia	Organic Black Elderberries, Water, Organic Grain Alcohol USP (40-50%)	1 mL	$11.99/ 30 mL (www.gaiaherbs.com)	$0.40/ mL	20
Herb	Distilled Water, Certified Organic Grain Alcohol (33-43%), Vegetable Glycerine, Black Elderberry Extractives	0.7 mL	$14.00/ 29.6 mL (www.herb-pharm.com)	$0.47/ mL	10
Planet	European Elder Berry and Flower Extract, Grain Alcohol (40%), Purified Water	5 mL	$33.50/ 240 mL (www.planetaryherbals.com)	$0.14/ mL	8

Refer to Table 1 for product identification.

Table 8 Compositional Profiles of Elderberry Tinctures at Week 0

Product Code	Moisture/ Ethanol (%)	Total Proantho-cyanidins (%)	Vitamin C (%)	Total Sugars (%)	Total Antho-cyanins (%)	Total Organic Acids (%)	Total Unknown (%)
Quant	92.2*ab*	1.106*a*	ND	3.6*bc*	0.0139*a*	0.9366*b*	2.1
Source	94.3*a*	0.067*b*	ND	2.4*c*	0.0070 *ab*	0.7382*b*	2.5
Gaia	85.6*bc*	0.058*b*	ND	7.0*b*	0.0001*b*	1.7161*a*	5.6
Herb	80.4*c*	0.058*b*	ND	15.0*a*	0.0002*b*	1.3672 *ab*	3.2
Planet	96.5*a*	0.070*b*	ND	0.9*c*	0.0003 *ab*	0.6031*b*	1.9

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm, (vitamin C)=7.3 mg.

Table 9 Sugar Profiles of Elderberry Tinctures at Week 0

Product Code	Sucrose (%)	Sucrose (%)	Fructose (%)	Glycerin (%)	Malitol (%)	Sorbitol (%)	Total Sugars (%)
Quant	ND	1.4*ab*	2.2*ab*	ND	ND	ND	3.6*bc*
Source	ND	0.9*ab*	1.5*ab*	ND	ND	ND	2.4*c*
Gaia	8.5	1.8*a*	2.8*a*	1.9*b*	ND	ND	7.0*b*
Herb	ND	1.0*ab*	1.9*ab*	12.1*a*	ND	ND	15.0*a*
Planet	ND	0.3*b*	0.6*b*	ND	ND	ND	0.9*c*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 10 Anthocyanin Profiles of Elderberry Tinctures at Week 0

Product Code	cyan-3-sambu (mg/L)	cyan-3-sambu-5-gluco (mg/L)	cyan-3-galact (mg/L)	cyan-3-gluco (mg/L)	peon-3-galact (mg/L)	Total Unknown (mg/L)	Total Anthocyanins (mg/L)
Quant	68.1*a*	6.0*a*	ND	63.1*a*	1.5*a*	0.9*a*	139.1*a*
Source	34.8*ab*	5.9*a*	ND	60.6*a*	1.5*a*	1.4*a*	69.5*ab*
Gaia	0.3*b*	ND	ND	ND	ND	0.5*a*	0.6*b*
Herb	1.5*b*	ND	ND	ND	ND	ND	1.5*b*
Planet	2.4*ab*	ND	ND	0.5*b*	ND	ND	2.8*ab*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.2 mg/L.

Table 11 Organic Acid Profiles of Elderberry Tinctures at Week 0

Product Code	Quinic (mg/L)	Galac-turonic (mg/L)	Malic (mg/L)	Tart-aric (mg/L)	Fum-aric (mg/L)	Citric (mg/L)	Iso-citric (mg/L)	Total Organic Acids (mg/L)
Quant	3683.6 *a*	285.6*a*	1394.4 *a*	80.7*b*	62.8*a*	3754.6*b*	145.3*b*	9366.3*b*
Source	1212.6 *b*	9.3*c*	1494.3 *a*	ND	ND	4501.1*b*	158.3*b*	7381.7*b*
Gaia	2371.8 *b*	105.8*b*	2887.8 *a*	79.9*ab*	15.1*b*	11282.2 *a*	418.6*a*	17161.1*a*
Herb	2133.6 *b*	170.9*b*	2041.3 *a*	120.9*a*	ND	8879.9 *ab*	320.5 *ab*	13672.2*ab*
Planet	1043.2 *b*	ND	1493.3 *a*	ND	ND	3383.2*b*	111.3*b*	6031.0*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=90 mg/L.

Color Analyses at Week 0

The initial 520/430 ratios of all tinctures were between 0.66 and 1.02 (Figure 9). As previously mentioned, if 520/430 ratios are below 1.00 this indicates poor product color due to the polymerization of red-colored anthocyanic pigmentation into brown-

colored degradation products. All of the tinctures tested in this study appeared brown in color and contained strong musty odors, which would likely result in decreased consumer acceptability. These results are substantiated by the fact that the tinctures contained appreciable amounts of organic acids and low concentrations of anthocyanins, which may indicate that appreciable amounts of fruit were used, but losses of red colored anthocyanic pigments were detected. Although alcohol is an excellent extractive of anthocyanins and other phenolics, it is theorized that glycerin is a better preservative based on the evaluation of elderberry syrups. The high levels of ethanol within the tinctures likely resulted in the alcoholic denaturation of the elderberry polyphenolic and anthocyanin contents (Lapornik and others 2005, Tay and others 2014). The average a* value of the tinctures tested in this study was 0.7, whereas the average a* value of the syrups was 4.6, further demonstrating that the tinctures contained less red pigmentation compared to the syrups tested in this study. In comparison, Casati and others (2012) reported that the average a* value of elderberry juice was approximately 20, and that approximately half of the juices' a* value was lost throughout 185 days of storage at 40° C.

Effects of Accelerated Storage

The 520/430 ratios decreased over accelerated temperature storage (32° C), which indicates similar degradation of tincture color compared to elderberry syrups over storage time. At 0, 5, and 10 weeks of storage, the average 520/430 ratios among all tinctures were 0.85, 0.58, and 0.56, respectively, which equates to a reduction of

31.6% throughout the first 5 weeks of storage, and an additional 3.2% between 5 and 10 weeks of storage (Figure 9). Apparently, the majority of anthocyanic degradation occurred throughout the first 5 weeks of accelerated storage. Interestingly, Quantum® Health Elderberry Liquid Extract and Source Naturals® Wellness Elderberry Liquid Extract™ were the only two tinctures tested in this study which contained appreciable amounts of anthocyanins, and these two tinctures showed the sharpest declines in 520/430 ratios as their anthocyanin contents declined (Figure 10). These results demonstrated a relationship between the amount of anthocyanins within the tinctures and the intensity of red pigmentation. A strong correlation ($r=0.62$) ($p \leq 0.05$) was observed between the 520/430 ratio and total anthocyanins among the tinctures.

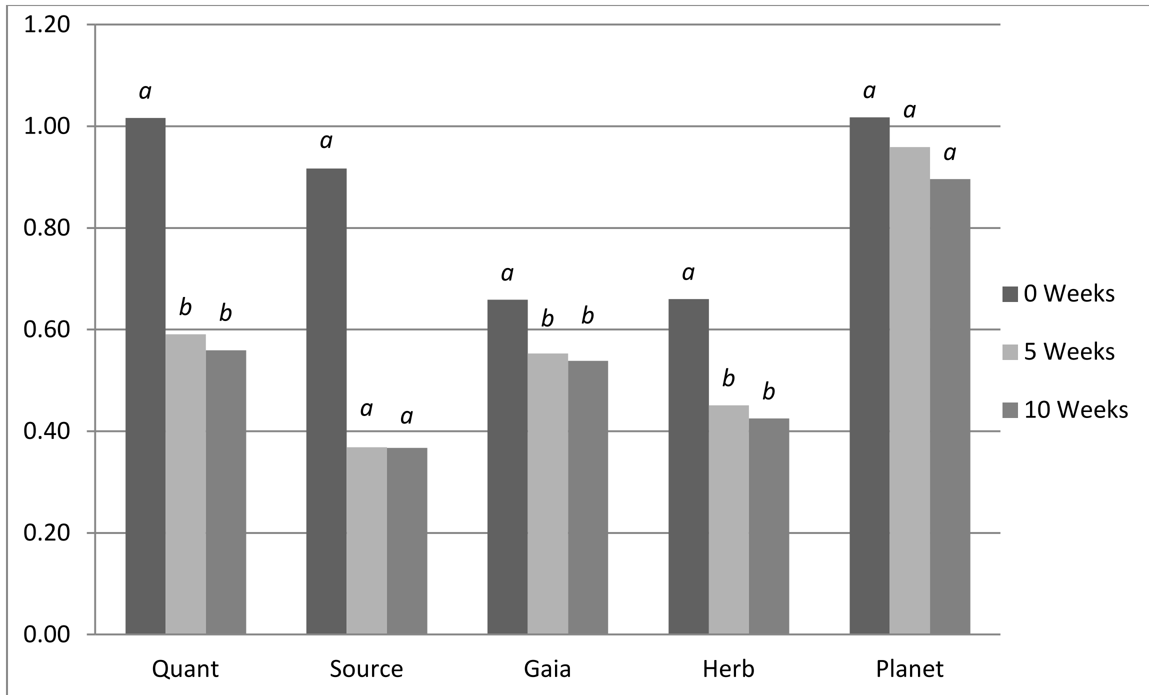


Figure 9 520/430 ratios of elderberry tinctures throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. 520/430 ratios by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

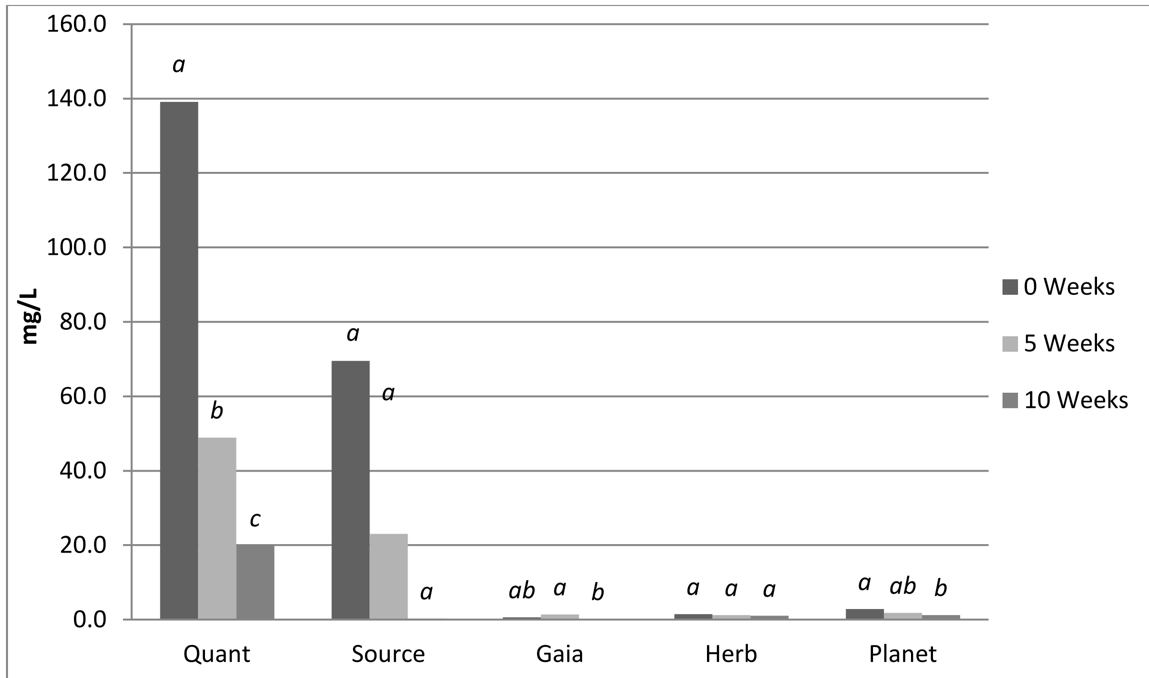


Figure 10 Total anthocyanin values (mg/L) of elderberry tinctures throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total anthocyanins by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Concentrates

Compositional Analyses at Week 0

The analytical testing performed in the study quantified greater than 90.7% of the ingredients within each of the concentrates (Tables 12+13). Kerr Elderberry Concentrate contained very rich fractions of proanthocyanidins (7.4% or 74,220.0 mg/L), anthocyanins (0.8% or 8,442.0 mg/L), vitamin C (14.5% or 145,000.0 mg/L), and organic acids (12.6% or 125,810.0 mg/L) during initial testing, which indicates that a substantial amount of fruit was used for its production. Sambu® Wild Grown Elderberry Concentrate and Natural Sources® Natural Elderberry Concentrate contained

appreciable amounts of sugars and organic acids, however also contained very low proportions of anthocyanins and proanthocyanidins. Although it is likely that considerable amounts of fruit were used for their production based on organic acids data, a lack of anthocyanins and proanthocyanidins indicates poor product stability. The nutrient profiles of these two concentrates make them more comparable to the syrups tested in this study. All of the concentrates tested in this study contained sugars, anthocyanins, and organic acids profiles which were indicative of elderberry fruit (Tables 14-16).

Table 12 Labeled Ingredients and Price of Elderberry Concentrates

Product Code	Labeled Ingredients	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
Kerr	Elderberry Juice Concentrate	N/A	N/A	4
Sambu	Elderberry Juice Concentrate, Elderflower Extract, Honey	$36.49/504 mL (www.vitacost.com)	$0.07/mL	13
Nat	Elderberry Juice Concentrate	$21.10/480 mL (www.vitacost.com)	$0.04/mL	5

Refer to Table 1 for product identification.

Table 13 Compositional Profiles of Elderberry Concentrates at Week 0

Product Code	Moisture (%)	Total Proantho-cyanidins (%)	Vitamin C (%)	Total Sugars (%)	Total Antho-cyanins (%)	Total Organic Acids (%)	Total Unknown (%)
Kerr	42.6*a*	7.422*a*	14.5	27.2*c*	0.8442*a*	12.5810 *a*	-5.1
Sambu	33.0*c*	0.012*b*	ND	54.8*a*	0.0005*b*	2.8402*b*	9.3
Nat	41.6b	0.093*b*	ND	45.8*b*	0.0010*b*	3.7072*b*	8.8

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm, (vitamin C)=7.3 mg.

Table 14 Sugar Profiles of Elderberry Concentrates at Week 0

Product Code	Sucrose (%)	Sucrose (%)	Fructose (%)	Glycerin (%)	Malitol (%)	Sorbitol (%)	Total Sugars (%)
Kerr	ND	13.5*b*	13.8*b*	ND	ND	ND	27.2*c*
Sambu	4.6*a*	22.3*a*	28.0*a*	ND	ND	ND	54.8*a*
Nat	0.9*b*	14.9*b*	28.8*a*	ND	ND	1.6	45.8*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 15 Anthocyanin Profiles of Elderberry Concentrates at Week 0

Product Code	cyan-3-sambu (mg/L)	cyan-3-sambu-5-gluco (mg/L)	cyan-3-galact (mg/L)	cyan-3-gluco (mg/L)	cyan-3-arabino (mg/L)	Total Unknown (mg/L)	Total Anthocyanins (mg/L)
Kerr	1127.1*a*	ND	ND	7315.1*a*	ND	ND	8442.1*a*
Sambu	1.2*b*	ND	ND	0.4*b*	ND	3.9*a*	5.4*b*
Nat	2.4*b*	ND	0.3	4.9*b*	1.4	1.9*b*	10.1*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.2 mg/L.

Table 16 Organic Acid Profiles of Elderberry Concentrates at Week 0

Product Code	Quinic (mg/L)	Galac-turonic (mg/L)	Malic (mg/L)	Tart-aric (mg/L)	Fum-aric (mg/L)	Citric (mg/L)	Iso-citric (mg/L)	Total Organic Acids (mg/L)
Kerr	13139.8*a*	17427.6*a*	15526.0*a*	1031.1*a*	414.3*a*	74854.7*a*	3416.9*a*	125810.4*a*
Sambu	3560.0*b*	2223.8*b*	2857.8*b*	ND	192.3*a*	18915.6*b*	652.4*b*	28401.9*b*
Nat	1798.3*b*	4997.9*b*	10268.6*a*	540.4*a*	165.5*a*	18375.2*b*	265.3*b*	37072.3*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses. Nat=666.3±315.7 mg/L benzoic. Limit of quantitation=90 mg/L.

Color Analyses at Week 0

The color of elderberry concentrates is one of the most important characteristics to food and beverage manufacturers because concentrates are commonly utilized as natural sources of red pigmentation in lieu of synthetically produced lakes and dyes. Kerr Elderberry Concentrate, Sambu® Wild Grown Elderberry Concentrate, and Natural Sources® Natural Elderberry Concentrate displayed 520/430 ratios of 1.82, 0.89, and 1.37 during initial analyses, respectively (Figure 11). Although the 520/430 ratios were comparable among the concentrates, it is important to note that the absorbances at both 520 nm and 430 nm were observed to be significantly ($p \leq 0.05$) higher for the Kerr Elderberry Concentrate compared to Sambu® Wild Grown Elderberry Concentrate and Natural Sources® Natural Elderberry Concentrate. These results indicate that Kerr Elderberry Concentrate contained strong color density and was a rich source of anthocyanic pigmentation compared to the other products tested in this study.

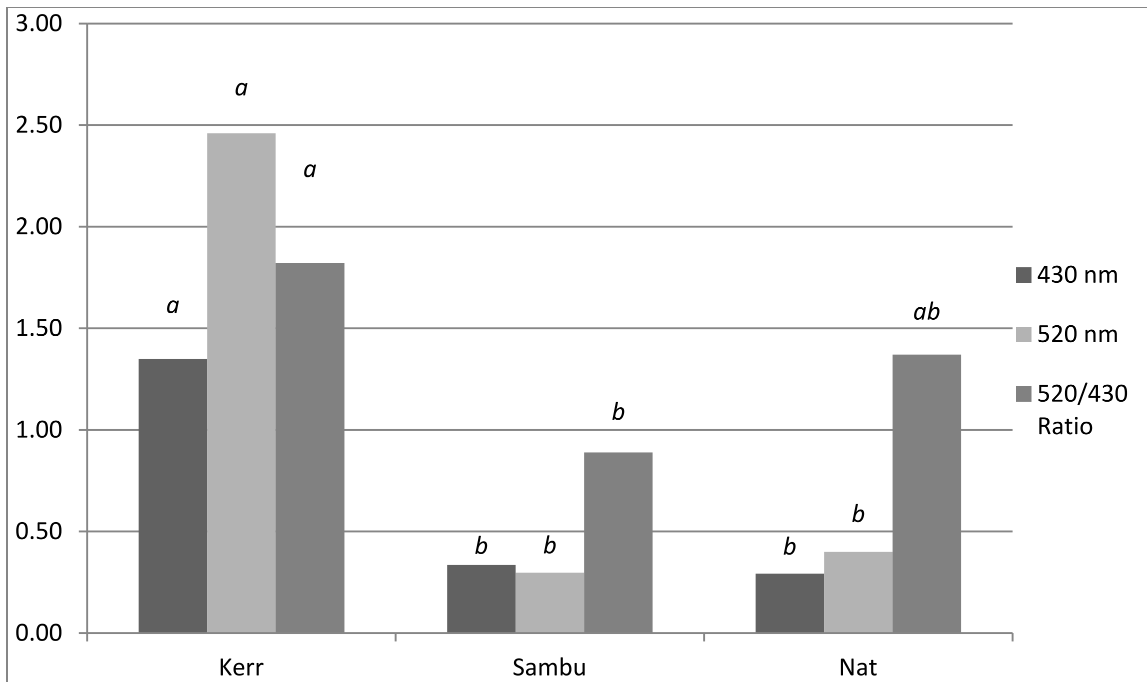


Figure 11 Spectrophotometric color values of elderberry concentrates at week 0. Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among concentrates based on one-way ANOVA. n=2 replicates, 2 analyses.

Effects of Accelerated Storage

Similar trends were observed in regards to decreasing levels of color and anthocyanins among the concentrates over storage time compared to the syrups and tinctures tested in this study. At 0, 5, and 10 weeks of storage, average 520/430 ratios were 1.82, 1.56, 1.40 (Kerr Elderberry Concentrate), 0.89, 0.64, 0.57 (Sambu® Wild Grown Elderberry Concentrate), and 1.37, 1.11, 1.04 (Natural Sources® Natural Elderberry Concentrate), respectively (Figure 12). These results indicate that some of the red colored anthocyanic pigments within the concentrates were subjected to degradation effects, and were polymerized into brown colored degradation products throughout storage.

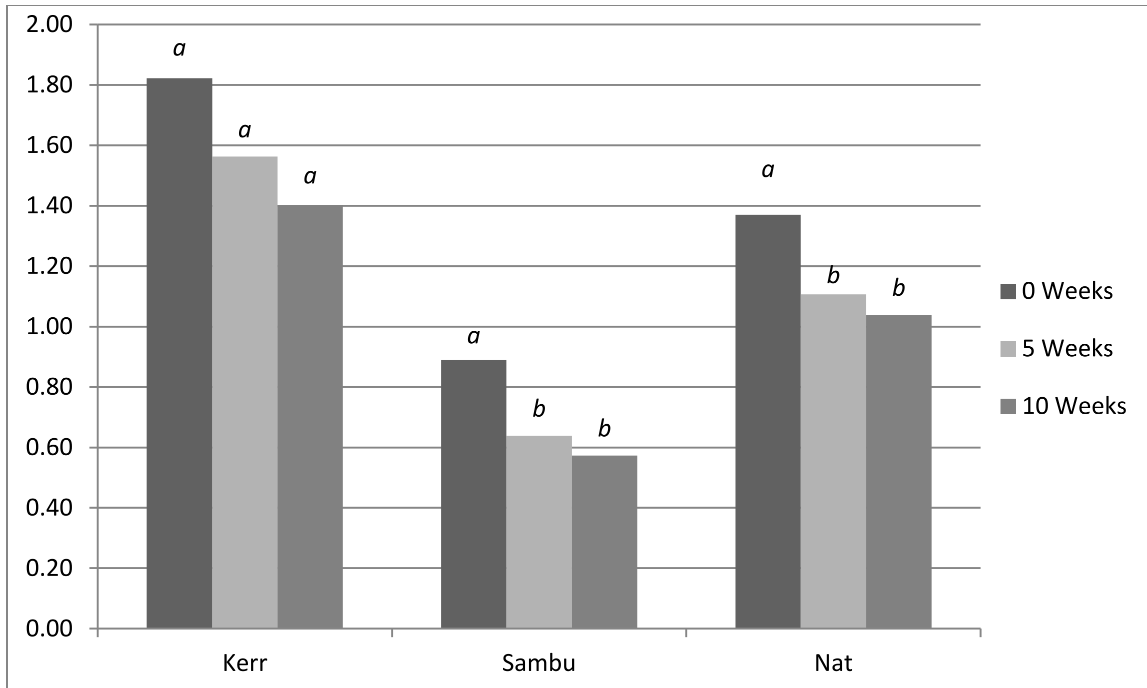


Figure 12 520/430 ratios of elderberry concentrates throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. 520/430 ratios by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

In addition to the loss of red color intensity throughout storage, significant ($p \leq 0.05$) losses to anthocyanin contents of the elderberry concentrates were observed. At 0, 5, and 10 weeks of storage, total anthocyanins were 8,442.1, 223.6, 62.3 (Kerr Elderberry Concentrate), 5.4, 6.6, 2.8 (Sambu® Wild Grown Elderberry Concentrate), and 10.1, 3.1, 0.8 (Natural Sources® Natural Elderberry Concentrate) mg/L, respectively. These results are in agreement with Arslan (2015), who reported a loss of 51% of cyanidin-3-glucoside within sour cherry concentrate after 150 days of room temperature (24° C) storage. Additionally, substantial losses to the proanthocyanidins and vitamin C contents of were detected within Kerr Elderberry Concentrate during the

first 5 weeks of accelerated storage, which was expected due to the large fractions of these compounds and their relative instability at high temperatures (Figure 13).

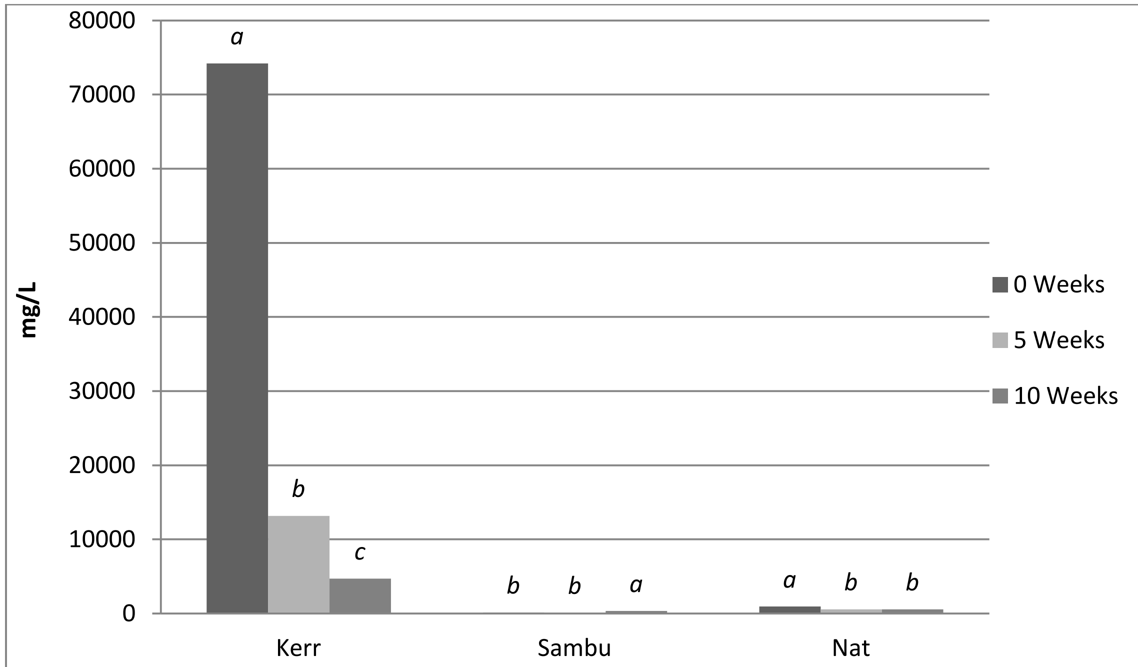


Figure 13 Total proanthocyanidin values (mg/L) of elderberry concentrates throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total proanthocyanidins by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Capsules

Compositional Analyses at Week 0

The analytical testing performed in this study identified and quantified between 32.9% and 74.4% of the total compounds within the elderberry capsules (Tables 17+18). Xyloglucans (hemicelluloses) were detected within all of the capsules, which indicated that the capsules were comprised of appreciable amounts of plant or fruit cell wall material, and that the capsules likely contained elderberry pomace or other plant

material (Table 19). Although large amounts of organic acids were detected within all of the capsules, substantially more anthocyanins were detected within Eclectic Institute™ Elderberry Capsules than within Nature's Way® Elderberry Capsules or Swanson® Premium Brand Elderberry Capsules (Tables 20+21). Large fractions of proanthocyanidins were detected within both Swanson® Premium Brand Elderberry Capsules and Eclectic Institute™ Elderberry Capsules.

Both Nature's Way® Elderberry Capsules and Swanson® Premium Brand Elderberry Capsules contained anthocyanins and organic acids profiles typical of elderberry fruit, constituting cyanidin-3-glucoside, cyanidin-3-sambubioside, and cyanidin-3-sambubioside-5-glucoside anthocyanins, as well as citric, quinic, and malic organic acids. Interestingly however, Eclectic Institute™ Elderberry Capsules had anthocyanins and organic acids profiles ,which were not indicative of elderberry fruit. Eclectic Institute™ Elderberry Capsules contained cyanidin-3-galactoside, and peonidin anthocyanins, as well as fumaric, low quinic, and substantially more isocitric acids than is typically detected for elderberry fruit. Additionally, neither cyanidin-3-sambubioside or cyanidin-3-sambubioside-5-glucoside were detected within Eclectic Institute™ Elderberry Capsules, which are important chemical markers for the presence of elderberry fruit (Clifford 2000, Veberic and others 2009). Based on these results, it is theorized that no elderberry fruit was used for the production of Eclectic Institute™ Elderberry Capsules, and that blackberry fruit was used as the primary ingredient, which still resulted in a high-value, anthocyanin-rich product.

Table 17 Labeled Ingredients and Price of Elderberry Capsules

Product Code	Labeled Ingredients	Serving Size	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
NW	Elderberry Fruit, Elderflower, Gelatin, Silica	2 Capsules (1150 mg)	$12.99/ 100 Capsules (www.vitacost.com)	$0.13/ Capsule	5
Swan	Elderberry Fruit, Elder Flowers, Gelatin, Microcrystalline Cellulose, Magnesium Stearate, Silica	1 Capsule (575 mg)	$6.49/ 120 Capsules (www.swansonvitamins.com)	$0.05/ Capsule	5
Eclectic	Wild Harvested Freeze-Dried Elderberry Fruit, Freeze-Dried Blackberry Fruit, Larix Arabinogalactan, Organic Ginger Root, Clove Bud, Hypromellose, Purified Water	2 Capsules (950 mg)	$14.90/ 50 Capsules (www.eclecticherb.com)	$0.30/ Capsule	4

Refer to Table 1 for product identification.

Table 18 Compositional Profiles of Elderberry Capsules at Week 0

Product Code	Moisture (%)	Total Proanthocyanidins (%)	Total Sugars (%)	Total Anthocyanins (%)	Total Organic Acids (%)	Total Shell (%)	Total Unknown (%)
NW	3.7*b*	0.013*b*	8.4*c*	0.0096*b*	3.9195*b*	16.8	67.1
Swan	2.6*c*	2.866*ab*	52.8*a*	0.0029*b*	2.5762*c*	13.6	25.6
Eclectic	4.4*a*	3.543*a*	29.1*b*	0.2501*a*	6.8637*a*	17.8	38.1

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. n=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm.

Table 19 Sugar Profiles of Elderberry Capsules at Week 0

Product Code	Xylo-glucans (%)	Sucrose (%)	Sucrose (%)	Fructose (%)	Glycerin (%)	Sorbitol (%)	Total Sugars (%)
NW	5.6*b*	0.5*b*	1.0*b*	3.0*b*	ND	ND	10.1*c*
Swan	55.1*a*	1.7*a*	1.1*b*	1.0*c*	ND	2.4	61.1*a*
Eclectic	8.3*a*	ND	14.1*a*	13.1*a*	ND	ND	35.4*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. Values do not include shell material. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 20 Anthocyanin Profiles of Elderberry Capsules at Week 0

Product Code	cyan-3-sambu (ppm)	cyan-3-sambu-5-gluco (ppm)	cyan-3-galact (ppm)	cyan-3-gluco (ppm)	cyan-3-arabino (ppm)	cyanidin (ppm)	Total Anthocyanins (ppm)
NW	36.7*a*	3.4	ND	76.6*b*	ND	ND	115.0*b*
Swan	3.4*b*	ND	1.8*b*	1.4*c*	4.0*b*	5.2*b*	33.5*b*
Eclectic	ND	ND	316.4*a*	2199.0*a*	92.0*a*	78.9*a*	3043.9*a*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. Values do not include shell material. *n*=2 replicates, 2 analyses. Eclectic=45.9*a* ppm peon-3-gluco, Swan=8.8*b* ppm peon-3-gluco, Eclectic=67.6 ppm peon-3-arabino, Swan=9.2*b* ppm total unknown, Eclectic=313.2*a* ppm total unknown. Limit of quantitation=0.2 ppm.

Table 21 Organic Acid Profiles of Elderberry Capsules at Week 0

Product Code	Quinic (ppm)	Galac-turonic (ppm)	Malic (ppm)	Tart-aric (ppm)	Fum-aric (ppm)	Citric (ppm)	Iso-citric (ppm)	Total Organic Acids (ppm)
NW	9949.6*a*	45.1*a*	6644.1*b*	500.5*b*	2106.4*a*	26376.5*b*	1305.6*b*	47087.3*b*
Swan	9194.0*a*	1021.3*a*	14402.3*a*	1405.8*a*	ND	3487.2*c*	209.1*b*	29824.2*c*
Eclectic	2465.5*b*	788.0*a*	13855.1*a*	1047.1*a*	1070.1*a*	34282.2*a*	29844.0*a*	83520.1*a*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. Values do not include shell material. *n*=2 replicates, 2 analyses. NW=169.8*a* ppm benzoic, Swan=104.6*a* ppm benzoic, Eclectic=168.3*a* ppm benzoic. Limit of quantitation=90 ppm.

Effects of Accelerated Storage

Similar to the other products tested in this study, significant ($p \leq 0.05$) losses of anthocyanin contents were detected in the elderberry capsules throughout accelerated temperature (32° C) storage (Figure 14). Although almost ⅓ of total anthocyanins were lost throughout 10 weeks of accelerated storage within Eclectic Institute™ Elderberry Capsules, a substantial amount of anthocyanins remained at the end of this study. No other notable storage effects were observed within the capsules, which indicates that elderberry capsules appeared to maintain color and nutrient stability throughout storage. The positive stability characteristics of the capsules are likely the result of the lack of water and encapsulation, which excludes oxygen and likely maintains anthocyanin stability.

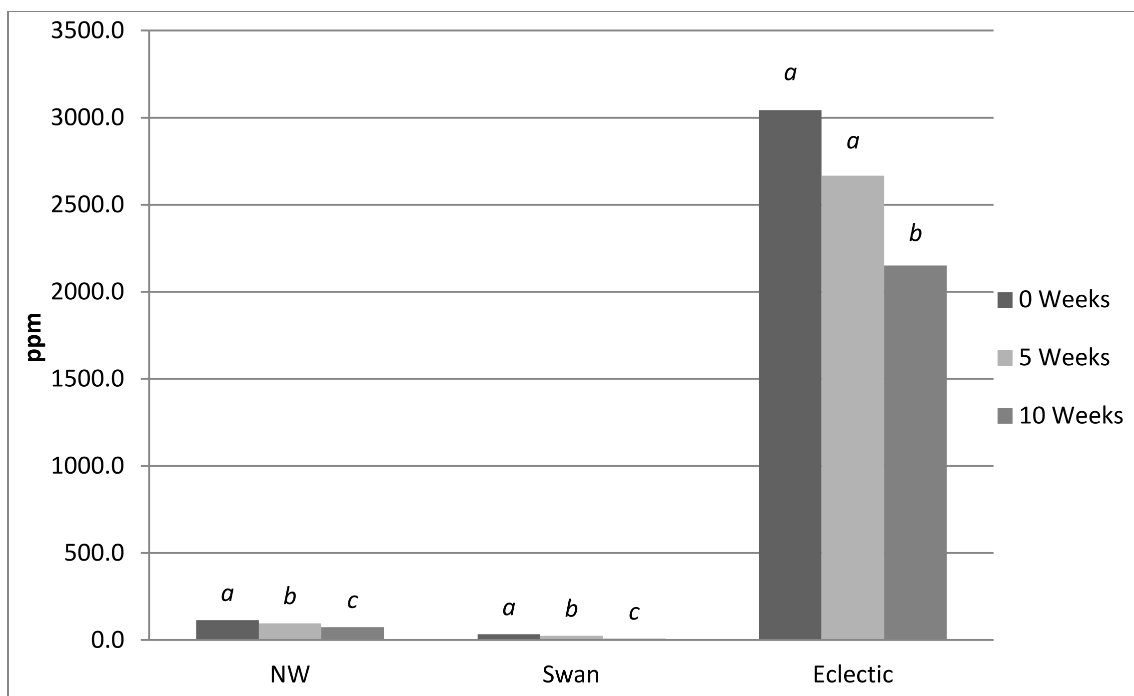


Figure 14 Total anthocyanin values (ppm) of elderberry capsules throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total anthocyanins by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Lozenges

Compositional Analyses at Week 0

The analytical testing performed in this study determined between 16.7% and 83.3% of the compounds within the elderberry lozenges (Tables 22+23). Sorbitol was detected as the primary sugar within Now® Elderberry and Zinc Lozenges, Sambucol® Chewable Tablets, and Rubini® ProFlavon Elderberry Complex Lozenges, and contributed to their moderate and high sugar contents (Table 24). Planetary™ Herbals Full Spectrum™ Elderberry Tablets contained the highest amount of xyloglucans among the lozenges analyzed, which indicated that it contained the highest amount of plant or

fruit cell wall material. Significantly ($p \leq 0.05$) higher levels of organic acids were detected within Sambucol® Chewable Tablets, which may have indicated that more fruit was used during production (Table 26). Although Sambucol® Chewable Tablets contained the highest organic acid contents, it contained the lowest amount of xyloglucans, which indicates that Sambucol® Chewable Tablets were processed to remove hemicellulose and other plant material, and possibly contained elderberry juice or concentrate. All of the lozenges tested in this study contained appreciable amounts of anthocyanins, and displayed anthocyanin profiles which were indicative of elderberry fruit (Table 25). Now® Elderberry and Zinc Lozenges, Sambucol® Chewable Tablets, and Rubini® ProFlavon Elderberry Complex Lozenges displayed high levels of fumaric and citric acids, and low levels of quinic acid, which indicated that another fruit was added along with elderberry in their production. These lozenges may contain additions of grape, raspberry, or cherry fruit; and/or fumaric and citric acids were added as flavor enhancers or antimicrobials. Now® Elderberry and Zinc Lozenges, Sambucol® Chewable Tablets, and Planetary™ Herbals Full Spectrum™ Elderberry Tablets were chewable tablets, and likely contained excipients, such as tableting agents (dibasic calcium phosphate, maltodextrin, tapioca starch), hydrocolloids (cellulose, acacia gum, hypromellose), and/or antiadherents (magnesium stearate, silica, stearic acid, colloidal silicon dioxide). Rubini® ProFlavon Elderberry Complex Lozenges appeared darker in color, were much chewier and gummier than the other lozenges tested in this study, and likely contained hydrocolloids as its other major fraction of ingredients, which were not detected by these analyses. All of the lozenges contained substantial

proanthocyanidin fractions compared to the other products tested in this study, which may indicate that lozenges maintain the stability characteristics of elderberry color and phytochemicals.

Table 22 Labeled Ingredients and Price of Elderberry Lozenges

Product Code	Labeled Ingredients	Serving Size	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
Now	Vitamin C (Ascorbic Acid and Sodium Ascorbate), Elderberry Fruit Concentrate, *Echinacea purpurea*, Bee Propolis, Slippery Elm Bark, Zinc Gluconate, Fructose, Cellulose, Vegetable Source Stearic Acid, Vegetable Source Magnesium Stearate, Silica, Natural Vanilla Flavor, Natural Raspberry Flavor	1 Lozenge	$7.99/30 Lozenges(www.vitacost.com)	$0.27/ Lozenge	5
Sam	Dried Elderberry Extract, Ascorbic Acid, Sorbitol, Xylitol, Natural Flavors, Silica, Magnesium Stearate, Hypromelose, Maltodextrin, Tapioca Starch	1 Tablet	$12.99/30 Tablets (www.sambucolusa.com)	$0.43/ Tablet	5
Rub	Sorbitol and Malitol Syrup, Gum Arabic, Elderflower Extract (8.5%), Honey (2.8%), Elderberry Extract (1.4%), Citric Acid, Vitamin C, Beeswax, Stevia	1 Lozenge (465 mg)	$11.33/24 Lozenges (www.rubiny.ie)	$0.47/ Lozenge	2
Planet	Elderberry, Elderberry Flower Extract, Elderberry Fruit Extract, Dibasic Calcium Phosphate, Stearic Acid, Acacia Gum, Modified Cellulose Gum, Silica	2 Tablets (1050 mg)	$9.98/42 Tablets (www.planetaryherbals.com)	$0.24/ Tablet	6

Refer to Table 1 for product identification.

Table 23 Compositional Profiles of Elderberry Lozenges at Week 0

Product Code	Moisture (%)	Total Proantho-cyanidins (%)	Total Sugars (%)	Total Antho-cyanins (%)	Total Organic Acids (%)	Total Unknown (%)
Now	0.9*d*	0.229*c*	80.4*a*	0.0236*c*	0.7836*c*	17.7
Sam	2.2*c*	1.151*a*	70.0*b*	0.0551*b*	8.3912*a*	18.2
Rub	9.7*a*	0.558*b*	33.4*c*	0.0681*a*	1.5499*b*	54.7
Planet	4.2*b*	0.228*c*	20.5*d*	0.0057*d*	1.7528*b*	73.3

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm.

Table 24 Sugar Profiles of Elderberry Lozenges at Week 0

Product Code	Xylo-glucans (%)	Sucrose (%)	Sucrose (%)	Fructose (%)	Malitol (%)	Sorbitol (%)	Total Sugars (%)
Now	2.4*c*	ND	0.6*c*	16.9*a*	ND	60.8*a*	80.4*a*
Sam	1.5*d*	ND	4.4*a*	4.4*b*	ND	59.8*a*	70.0*b*
Rub	3.0*b*	ND	1.3*b*	2.0*d*	6.0	27.1*b*	33.4*c*
Planet	18.2*a*	ND	ND	2.3*c*	ND	ND	20.5*d*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 25 Anthocyanin Profiles of Elderberry Lozenges at Week 0

Product Code	cyan-3-sambu (ppm)	cyan-3-sambu-5-gluco (ppm)	cyan-3-galact (ppm)	cyan-3-gluco (ppm)	peon-3-galact (ppm)	Total Unknown (ppm)	Total Anthocyanins (ppm)
Now	29.1*c*	1.5*b*	0.8	203.5*c*	3.1*a*	1.3*b*	236.3*c*
Sam	125.7*a*	ND	ND	425.6*b*	ND	ND	551.2*b*
Rub	63.6*b*	6.5*a*	ND	604.5*a*	6.5*a*	ND	681.0*a*
Planet	22.9*c*	ND	ND	27.7*d*	ND	11.7*a*	56.5*d*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.2 ppm.

Table 26 Organic Acid Profiles of Elderberry Lozenges at Week 0

Product Code	Quinic (ppm)	Galac-turonic (ppm)	Malic (ppm)	Tart-aric (ppm)	Fumaric (ppm)	Citric (ppm)	Iso-citric (ppm)	Total Organic Acids (ppm)
Now	1265.2*b*	11.3*b*	753.4*c*	135.5*b*	4541.9*b*	463.0*d*	549.3*b*	7836.3*c*
Sam	3250.6*a*	1869.8*a*	5386.5*a*	926.6*a*	52582.9*a*	17819.8*a*	1921.3*a*	83911.9*a*
Rub	204.4*c*	45.8*b*	512.6*c*	129.7*b*	3402.4*b*	10815.8*b*	246.9*c*	15498.9*b*
Planet	3199.6*a*	309.8*b*	2354.8*b*	237.9*b*	434.6*c*	10173.1*c*	671.0*b*	17528.3*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Now=117.0*a* ppm benzoic, Sam=154.6*a* ppm benzoic, Rub=141.4*a* ppm benzoic, Planet=147.5*a* ppm benzoic. Limit of quantitation=90 ppm.

Effects of Accelerated Storage

Similar to the other products tested in this study, significant (p≤0.05) losses to the anthocyanin contents of the elderberry lozenges were detected throughout accelerated temperature (32° C) storage, with the exception of Planetary™ Herbals Full Spectrum™ Elderberry Tablets. In this product, relatively low anthocyanins were initially detected (Figure 15). Similar to the capsule anthocyanins results, approximately ⅓ of total anthocyanins were lost after the first 5 weeks of accelerated storage. No other notable storage effects were observed among the lozenges, which indicated that elderberry lozenges are a product that can maintain color and nutrient stability throughout storage.

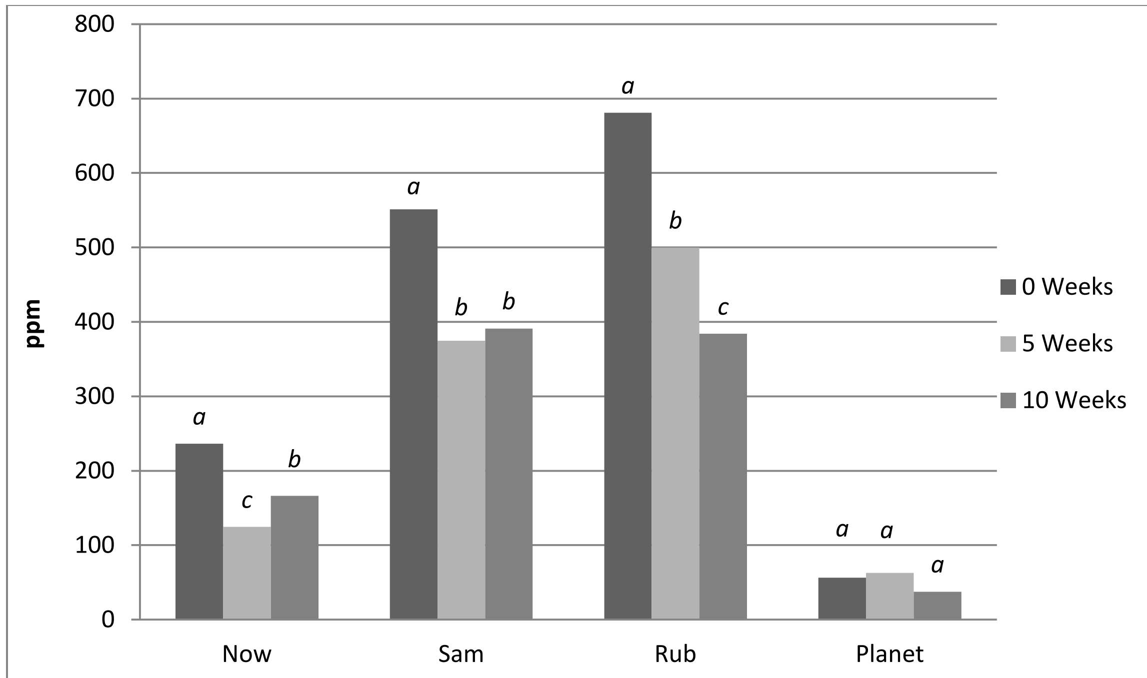


Figure 15 Total anthocyanin values (ppm) of elderberry lozenges throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total anthocyanins by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Dried Fruit

Compositional Analyses at Week 0

Of the three brands of dried elderberry fruit tested in this study, only 25.3% to 38.8% of ingredients were identified during initial compositional analyses (Tables 27+28). The large unknown fractions were likely dietary fiber (pectin, cellulose), vitamins (A, B6), minerals (iron, phosphorous, potassium, calcium), flavonols (quercetin, kaempferol), as well as small fractions of unidentified organic acids (cinnamic, chlorogenic, neochlorogenic, caffeic, palmitic). Moisture contents varied between 8.4 and 12.6%, and water activity levels were between 0.47 and 0.54. These values are

typical for dried fruit products. In comparison, sweetened dried cranberries have moisture contents between 15 and 20%, and values exceeding 20% are considered 'too wet' for positive consumer acceptability. The sugar profiles of all three brands of dried elderberry fruit consisted of fructose, sucrose, and xyloglucans, which are sugars naturally contained within elderberry fruit (Table 29). As with many other elderberry products in this study, the anthocyanin and organic acid profiles of the dried fruit were comprised of cyanidin-3-glucoside and cyanidin-3-sambubioside anthocyanins, as well as citric, quinic, and malic acids, which are representative of elderberry fruit (Tables 30+31). Interestingly, Florida Herb House Minced Dried Elderberries contained significantly ($p \leq 0.05$) more organic acids, and slightly higher proanthocyanidins and anthocyanins compared to the other two dried fruit products tested in this study. Considering that both Florida Herb House Minced Dried Elderberries and Florida Herb House Dried Elderberries displayed the same lot number upon delivery, it is theorized that the higher nutrient values observed within the minced product were a result of better extraction efficiencies during the preparative phases of analyses.

Table 27 Labeled Ingredients and Price of Dried Elderberry Fruit

Product Code	Labeled Ingredients	Retail Price (USD as of April 2016)	Weighted Price (USD)	Average Months in Distribution Before Analysis
Front	Elderberry Fruit	$18.00/16 oz (www.frontiercoop.com)	$1.13/oz	3
Flor M	Elderberry Fruit	N/A	N/A	15
Flor W	Elderberry Fruit	$22.99/16 oz (www.floridaherbhouse.com)	$1.44/oz	15

Refer to Table 1 for product identification.

Table 28 Compositional Profiles of Dried Elderberry Fruit at Week 0

Product Code	Moisture (%)	Total Proantho-cyanidins (%)	Total Sugars (%)	Total Antho-cyanins (%)	Total Organic Acids (%)	Total Unknown (%)
Front	12.6*a*	0.282*a*	21.7*a*	0.0158*b*	4.1598*c*	61.2
Flor M	8.4*b*	0.976*a*	15.2*b*	0.0705*a*	7.1445*a*	68.2
Flor W	8.9*b*	0.685*a*	10.4*c*	0.0241*b*	5.2655*b*	74.7

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm.

Table 29 Sugar Profiles of Dried Elderberry Fruit at Week 0

Product Code	Xyloglucans (%)	Sucrose (%)	Sucrose (%)	Fructose (%)	Glycerin (%)	Total Sugars (%)
Front	5.3*a*	0.3*a*	6.0*a*	10.0*a*	0.2*a*	21.7*a*
Flor M	5.7*a*	ND	3.3*b*	6.3*b*	ND	15.2*b*
Flor W	3.4*b*	0.1*a*	2.6*c*	4.4*c*	0.1*a*	10.4*c*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 30 Anthocyanin Profiles of Dried Elderberry Fruit at Week 0

Product Code	cyan-3-sambu (ppm)	cyan-3-sambu-5-gluco (ppm)	cyan-3-galact (ppm)	cyan-3-gluco (ppm)	cyan-3-arabino (ppm)	Total Unknown (ppm)	Total Anthocyanins (ppm)
Front	157.9*a*	ND	ND	ND	ND	ND	157.9*b*
Flor M	74.7*b*	ND	ND	568.4*a*	ND	61.7*a*	704.8*a*
Flor W	44.8*b*	2.4	1.4	178.7*b*	2.0	7.1*b*	241.0*b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.2 ppm.

Table 31 Organic Acid Profiles of Dried Elderberry Fruit at Week 0

Product Code	Quinic (ppm)	Galac-turonic (ppm)	Malic (ppm)	Tart-aric (ppm)	Fumaric (ppm)	Citric (ppm)	Iso-citric (ppm)	Total Organic Acids (ppm)
Front	7828.5 c	ND	5337.8*b*	293.9*a*	681.6*a*	26062.4 *c*	1343.2 *c*	41598.3 *c*
Flor M	9039.1 *a*	ND	10325.6 *a*	264.2*a*	399.4*b*	49612.1 *a*	1804.7 *a*	71445.0 *a*
Flor W	8280.6 *b*	185.3	5563.2*b*	ND	363.7*b*	36619.6 *b*	1642.3 *b*	52654.5 *b*

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. ND=non-detected. n=2 replicates, 2 analyses. Front=102.2*a* ppm benzoic, Flor W=97.3*a* ppm benzoic. Limit of quantitation=90 ppm.

Effects of Accelerated Storage

Similar to the other products tested in this study, significant ($p \leq 0.05$) losses to the proanthocyanidin and anthocyanin contents of the dried elderberry fruit were detected throughout accelerated temperature (32° C) storage (Figures 16+17). After 10 weeks of storage time, less proanthocyanidins (66.4% decrease) and anthocyanins (35.7% decrease) were detected among all of the brands of dried elderberry fruit tested in this study. The only other product in this study to show significant ($p \leq 0.05$) losses of proanthocyanidins throughout accelerated storage was Kerr Elderberry Concentrate, in which a substantial proanthocyanidin content was detected during initial testing. These results indicate that although dried elderberry fruit contains appreciable amounts of healthful nutrients, there are other elderberry products with better stability characteristics throughout accelerated storage. Due to the relatively inexpensive cost of dried elderberry fruit, it is suggested to producers to

investigate other value-added products for the utilization of elderberry fruit for the creation of elderberry products which consumers can enjoy.

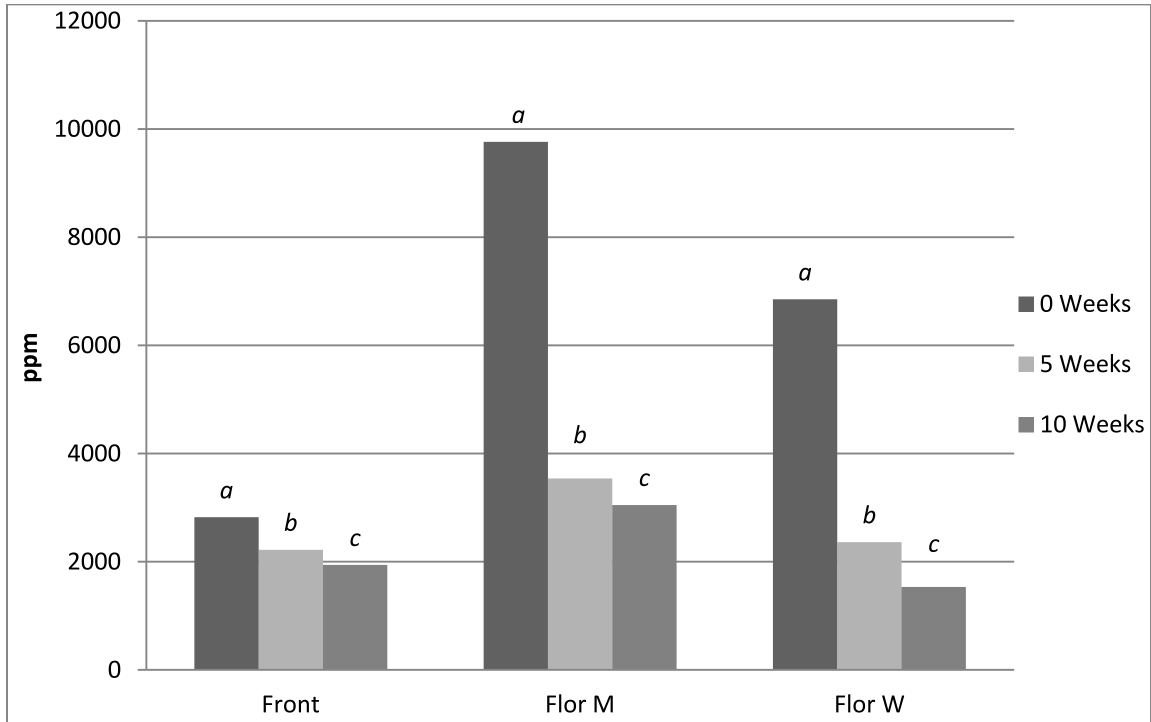


Figure 16 Total proanthocyanidin values (ppm) of dried elderberry fruit throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total proanthocyanidins by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

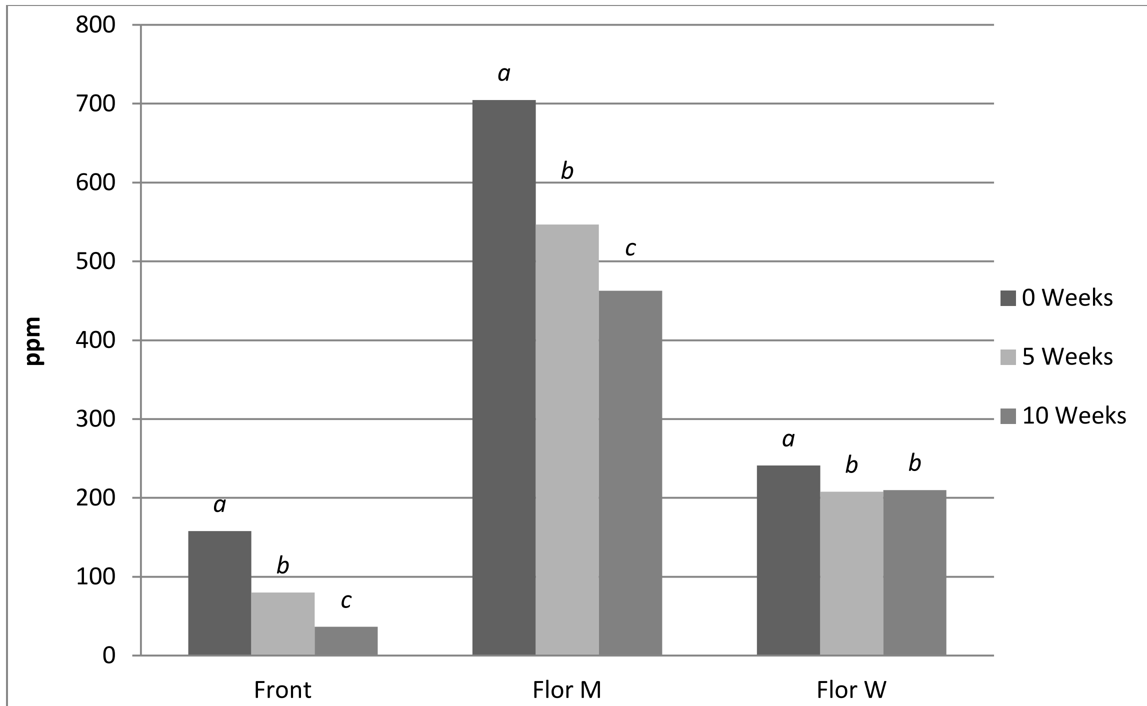


Figure 17 Total anthocyanin alues (ppm) of dried elderberry fruit throughout weeks of accelerated temperature (32° C) storage.

Refer to Table 1 for product identification. Total anthocyanins by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Powder

Compositional Analyses at Week 0

Although there are other elderberry powders available within the wholesale market, NP Nutra® Elderberry P.E. 10:1 was the only powdered elderberry product to be tested in this study. Very high concentrations of proanthocyanidins (40.781% or 407,810.0 ppm) were detected within the powder during initial analyses, which indicates that NP Nutra® Elderberry P.E. 10:1 powder was processed with the objective of concentrating elderberry fruit flavonoids (Table 32). Ten times the amount of anthocyanins were detected within the powder compared to the amounts detected

within the dried elderberry fruits tested in this study (Table 34). Very low moisture and sugar contents were detected within the powder, and the organic acids content was approximately one-tenth of the value observed within the dried elderberry fruit (Tables 33+25). The powder was observed to be dark purple in color, which indicates its suitability as a natural food colorant. These results demonstrate that NP Nutra® Elderberry P.E. 10:1 powder has great potential for utilization within other value-added food products.

Table 32 Compositional Profile of Elderberry Powder at Week 0

Product Code	**Moisture (%)**	**Total Proantho-cyanidins (%)**	**Total Sugars (%)**	**Total Antho-cyanins (%)**	**Total Organic Acids (%)**	**Total Unknown (%)**
NP Nutra	0.1	40.781	3.3	0.1619	0.5095	55.1

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=2 replicates, 2 analyses per variable. Limit of quantitation (proanthocyanidins)=0.51 ppm.

Table 33 Sugar Profile of Elderberry Powder at Week 0

Product Code	**Xylo-glucans (%)**	**Sucrose (%)**	**Sucrose (%)**	**Fructose (%)**	**Glycerin (%)**	**Malitol (%)**	**Total Sugars (%)**
NP Nutra	ND	2.7	0.7	ND	ND	ND	3.3

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. Limit of quantitation=0.05%.

Table 34 Anthocyanin Profile of Elderberry Powder at Week 0

Product Code	cyan-3-sambu (ppm)	cyan-3-sambu-5-gluco (ppm)	cyan-3-galact (ppm)	cyan-3-gluco (ppm)	cyan-3-arabino (ppm)	peon-3-gluco (ppm)	Total Anthocyanins (ppm)
NP Nutra	33.0	ND	34.0	923.7	12.8	438.0	1619.1

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. NP Nutra=184.0 ppm total unknown. Limit of quantitation=0.2 ppm.

Table 35 Organic Acid Profile of Elderberry Powder at Week 0

Product Code	Quinic (ppm)	Galac-turonic (ppm)	Malic (ppm)	Tartaric (ppm)	Fumaric (ppm)	Citric (ppm)	Iso-citric (ppm)	Total Organic Acids (ppm)
NP Nutra	254.2	ND	1826.8	1648.1	ND	1003.6	175.6	5094.9

Refer to Table 1 for product identification. Treatments not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. ND=non-detected. *n*=2 replicates, 2 analyses. NP Nutra=377.4±5.7 ppm benzoic. Limit of quantitation=90 ppm.

Effects of Accelerated Storage

Significant (p≤0.05) losses (29.9%) to the proanthocyanidins content of NP Nutra® Elderberry P.E. 10:1 powder were observed throughout the first 5 weeks of accelerated temperature (32° C) storage, although no further losses were observed between 5 and 10 weeks of storage (Figure 18). After 5 weeks of storage, the powder still contained a very high proanthocyanidin fraction (28.622%). No other significant effects were detected within the powder throughout accelerated storage. These results indicate that NP Nutra® Elderberry P.E. 10:1 powder has excellent stability characteristics compared to the other products tested in this study, and is a product

with promising potential to enhance color and phytochemical properties of other value-added food products.

Figure 18 Total proanthocyanidin values (ppm) of NP Nutra® Elderberry P.E. 10:1 powder throughout weeks of accelerated temperature (32° C) storage. Total proanthocyanidins by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=2 replicates, 2 analyses.

Conclusions

Of the syrups tested in this study, Nature's Way® Sugar-Free Sambucus Syrup, Nature's Way® Sambucus Syrup, and Integrative Therapeutics™ Sambucus Extract were the syrups which utilized elderberry fruit most efficiently, due to their relatively low organic acid contents (i.e. amount of fruit used for production), high phytochemical contents, and excellent color stability throughout accelerated temperature (32° C) storage. Additionally, these three syrups were in the distribution chain for averages of

17 (NW SF), 16 (NW), and 8 (Inte) months, with the average time in distribution of 8 months among all syrups. This demonstrates their ability to retain their competitive properties throughout extended storage. Generally, there is a well established market for elderberry syrups, and they represent a good elderberry product due to their relatively high phytochemical contents and good stability.

Of the tinctures tested in this study, Quantum® Health Elderberry Liquid Extract contained the most appreciable amounts of anthocyanins and proanthocyanidins. Overall the elderberry tinctures were a poor product for the utilization of elderberry fruit due to their high moisture/alcohol contents, low phytochemical contents, poor color and nutrient stability, relatively high costs ($3.90/oz-$14.10/oz), and organic acid contents which were comparable to the syrups tested in this study. Although alcohol is an excellent extractive of elderberry color and phytochemicals, it may not be the best medium for preservation, due to its low surface tension, which can expose anthocyanin molecules to hydrolytic or oxidative degradation (Lapornik and other 2005, Tay and others 2014). Additionally, the high ethanol content of the tinctures likely resulted in the denaturation of elderberry polyphenolics (Lapornik and others 2005, Tay and others 2014). Future product development studies should investigate the use of additives or processing techniques to improve the nutrient and color stability of elderberry tinctures.

Of the concentrates tested in this study, Kerr Elderberry Concentrate was the only concentrate which contained substantial amounts of vitamin C, anthocyanins, proanthocyanidins, and red pigmentation. Although large concentrations of organic acids were detected in Sambu® Wild Grown Elderberry Concentrate and Natural

Sources® Natural Elderberry Concentrate, they contained low proportions of anthocyanins and proanthocyanidins, and high amounts of sugars, which makes these two concentrates more comparable to the syrups tested in this study. These results identify Kerr Elderberry Concentrate as an excellent source of elderberry pigmentation and phytochemicals.

Of the capsules tested in this study, Eclectic Institute™ Elderberry Capsules contained the highest levels of anthocyanins, organic acids, and proanthocyanidins. Although interestingly, neither cyanidin-3-sambubioside or cyanidin-3-sambubioside-5-glucoside anthocyanins were detected within Eclectic Institute™ Elderberry Capsules, indicating that it was made with another (non-elderberry) fruit and likely mislabeled. Generally, some anthocyanin degradation was detected among all of the capsules throughout accelerated storage, however the capsules displayed better color and nutrient stability compared to the fluid elderberry products tested in this study. The low moisture content and encapsulation of the anthocyanins may have excluded oxygen more effectively, which most likely reduced oxidative degradation.

All of the lozenges tested in this study contained appreciable amounts of phytochemicals, and displayed positive nutrient and color stability characteristics throughout accelerated storage. These results identify lozenges as an effective product form for the delivery of elderberry nutrients. Relatively high levels of proanthocyanidins, anthocyanins, and organic acids were detected within Rubini® ProFlavon Elderberry Complex Lozenges, which distinguishes Rubini® ProFlavon Elderberry Complex Lozenges as a valuable elderberry product. Sambucol® Chewable Tablets also contained high

phytochemical contents, however high levels of galacturonic and fumaric acids were detected within this product, which is atypical of elderberry fruit.

Although appreciable amounts of phytochemicals were initially detected within the dried elderberry fruit tested in this study, significant ($p \leq 0.05$) losses of proanthocyanidins were observed throughout accelerated storage. Although there is a market for dried elderberry fruit, it may be desirable for processors to utilize elderberry fruit into other value-added elderberry products, since the fruit has a relatively low cost and moderate stability characteristics.

NP Nutra® Elderberry P.E. 10:1 was the only powdered elderberry product tested in this study, and displayed very high concentrations of proanthocyanidins and anthocyanins, as well as low moisture, sugars, and organic acid levels. These results indicate that NP Nutra® Elderberry P.E. 10:1 powder was formulated with the intention of maximizing phytochemical content. Additionally, NP Nutra® Elderberry P.E. 10:1 powder had excellent nutrient and color stability throughout accelerated storage, which further distinguishes it as a high quality, value-added elderberry product.

Overall, this study evaluated a wide array of value-added elderberry fruit products, which were produced with varying intentions (sugar-free, high polyphenolics, contains vitamin C, use of by-product, etc.) and consumer markets (wholesale, health food, general markets). Although there is substantial research which implicates elderberry fruit as a healthful food, there is some skepticism by the researchers regarding the healthfulness of some of the products tested in this study. High proportions of moisture were detected within the elderberry tinctures. Elderberry

syrups contained high amounts of added sugars, and capsules and lozenges likely contained large fractions of excipients and tableting agents. These ingredients displace or offset the healthfulness of elderberry phytochemicals. Although many of the nutrients which were evaluated within the elderberry products remained unchanged throughout accelerated temperature (32° C) storage, losses of proanthocyanidins, anthocyanins, vitamin C, and color were observed among many of the products. Overall, better color and nutrient stability was observed within the dried elderberry products (capsules, lozenges, dried fruit, powder) compared to the fluid products (syrups, tinctures, concentrates) throughout storage time. Elderberry fruit was utilized more effectively within the elderberry syrups compared to the tinctures. The high sugar content within the syrups likely contributed to the preservation of elderberry phytochemicals and color by lowering water activity and preventing hydrolytic reactions, and likely contributed towards improved consumer acceptability. Future studies should investigate the effects of processing techniques and/or additives on the phytochemical and color stability of elderberry products; as well as identify the amount of flavonols (quercetin, kaempferol, rutin), flavonol glycosides, and additional organic acids (cinnamic, chlorogenic, neochlorogenic, caffic) within elderberry products. The results of this study provide insight into the chemistry and processing of elderberry fruit products, which has value to consumers who desire to make informed elderberry purchases, and to value-added elderberry fruit processors who want to improve upon their products and increase their competitiveness within elderberry fruit markets.

CHAPTER 3. COPIGMENTATION OF ELDERBERRY (*SAMBUCUS NIGRA*) TINCTURES TO ENHANCE NUTRIENT AND COLOR STABILITY THROUGHOUT STORAGE

Objectives

The objectives of this study were to determine how additions of rosemary extract, tannic acid, black carrot color, purple sweet potato color, and enzymatically modified isoquercitrin affected the color, anthocyanin content, phenolic content, and antioxidant activity of elderberry tinctures at 0, 2, 4, and 6 weeks of storage at 21° C, with one week of accelerated temperature (32° C) storage which occurred between 5 and 6 weeks.

Materials and Methods

Experimental Design

Elderberry tinctures were selected for product development within this study due to the unfavorable results of the commercialized tinctures analyzed in chapter 2. Previous studies have identified the effect of copigmentation as an effective treatment for the enhancement of color, pigment stability, and antioxidant activity of anthocyanin-rich systems, and copigment additives were selected based on favorable results from previous research (Boulton 2001, Talcott and others 2003, Del Pozo-Insfran and others, Bąkowska-Barczak 2005, Rein 2005, Kammerer and others 2007). Although, previous research has shown that wines containing 10%-21% ethanol were susceptible to copigmentation reactions, no studies have demonstrated the effectiveness of

copigments within anthocyanin-rich products containing high ethanol content, such as tinctures (>25% ethanol) or liqueurs (15%-55% ethanol) (Boulton 2001).

Five copigmentation additives were tested three different concentrations against a control: rosemary extract (100, 200, 300 mg/100mL), tannic acid (2.5, 5.0, 7.5 mg/100mL), black carrot color (100, 200, 300 mg/100mL), purple sweet potato color (100, 200, 300 mg/100mL), and enzymatically modified isoquercitrin (25, 50, 75 mg/100mL) (Table 36). Levels of each additive were chosen to represent a range of recommended usage to upper limit levels, based on supplier specifications. The dependent variables tested in this study included: pH, titratable acidity, L*a*b* color, monomeric anthocyanins, color density, polymeric color, % polymeric color, total phenolics, and antioxidant activity (% inhibition of DPPH radical per 20 mg of tincture, IC_{50} of DPPH radical, total antioxidants). Each tincture was produced in triplicate, tested initially, and retested at 2, 4, and weeks storage at 21° C, with one week of accelerated temperature (32° C) storage which occurred between weeks 5 and 6.

Preparation of Elderberry Tinctures

Thirty-six pounds of elderberry fruit were harvested from Heath Hill Farm (Sumner, ME), frozen, delivered to the University of Maine Dr. Matthew Highlands Pilot Plant, and cleaned by hand to remove stems, leaves, and foreign material (Figure 19). The frozen elderberry fruit was then cooked on a double boiler for approximately 40 minutes, so that the temperature of the fruit exceeded 71° C for approximately 10 minutes to inactivate cyanogenic glycoside toxins (Conn 1979). The cooked fruit was

processed using an ACME™ Supreme Juicerator® Model 6001 (ACME™ Juicer Mfg. Co., Sierra Madre, CA), which yielded 61.6% (22.2 lbs) juice and 38.4% (13.8 lbs) pomace. The juice was immediately frozen and held at -26° C until further use.

The moisture content of the pomace was determined to be 71.9% using a draft-drying technique based on AOAC method # 950.46 (AOAC 2005). The pomace (4,800 g) was separated into six 800 gram batches and placed into six 64 ounce wide mouth mason jars (Ball Corp., Broomfield, CO). Each jar was filled with 880 grams (~1100 mL) of 95% ethanol (Thermo Fisher Scientific, Inc., Waltham, MA), which resulted in a 1:1.1 marc (pomace) to menstruum (ethanol) ratio. The mixture was gently stirred using a stir rod to ensure complete homogenization, and the jars were sealed with their respective lids. The jars were placed into a covered box to eliminate light, and held at 21° C for a period of 6 weeks. Once per day throughout storage the jars were gently inverted ten times each, which aided in the extraction process.

After 6 weeks, the frozen elderberry juice was defrosted for 2 days under refrigeration temperature (3.3° C). Once the juice was defrosted, both the juice and the extract were separately filtered through a number 35, 500 micron, stainless steel USA standard testing sieve (W.S. Tyler® Industrial Group, Mentor, OH). Approximately 9.8 L of the elderberry juice (°brix=11.5) was mixed with approximately 5.3 L of the elderberry extract (°brix=22.2) and 0.9 L of 95% ethanol (Thermo Fisher Scientific, Inc., Waltham, MA), which yielded a final elderberry tincture (°brix=18.3) containing 30.1% alcohol, which is a typical alcohol content for tinctures. The elderberry tincture was then vacuum

filtered through a 150 mm diameter porcelain Büchner funnel, lined with cheesecloth to remove solids.

The elderberry tincture (12 L) was separated into sixteen, 750 mL treatment groups. Copigment additives were weighed (±0.0001) according to each treatment, and mixed into each batch of tincture using a magnetic stir bar for approximately 5 minutes (Table 36). Each 750 mL treatment group was then subdivided into three 250 mL wide-mouth, amber glass bottles (Environmental Express®, Charleston, SC) for a total of 48 samples (16 treatments, in triplicate). Samples were stored in a covered cardboard box throughout storage and analyses.

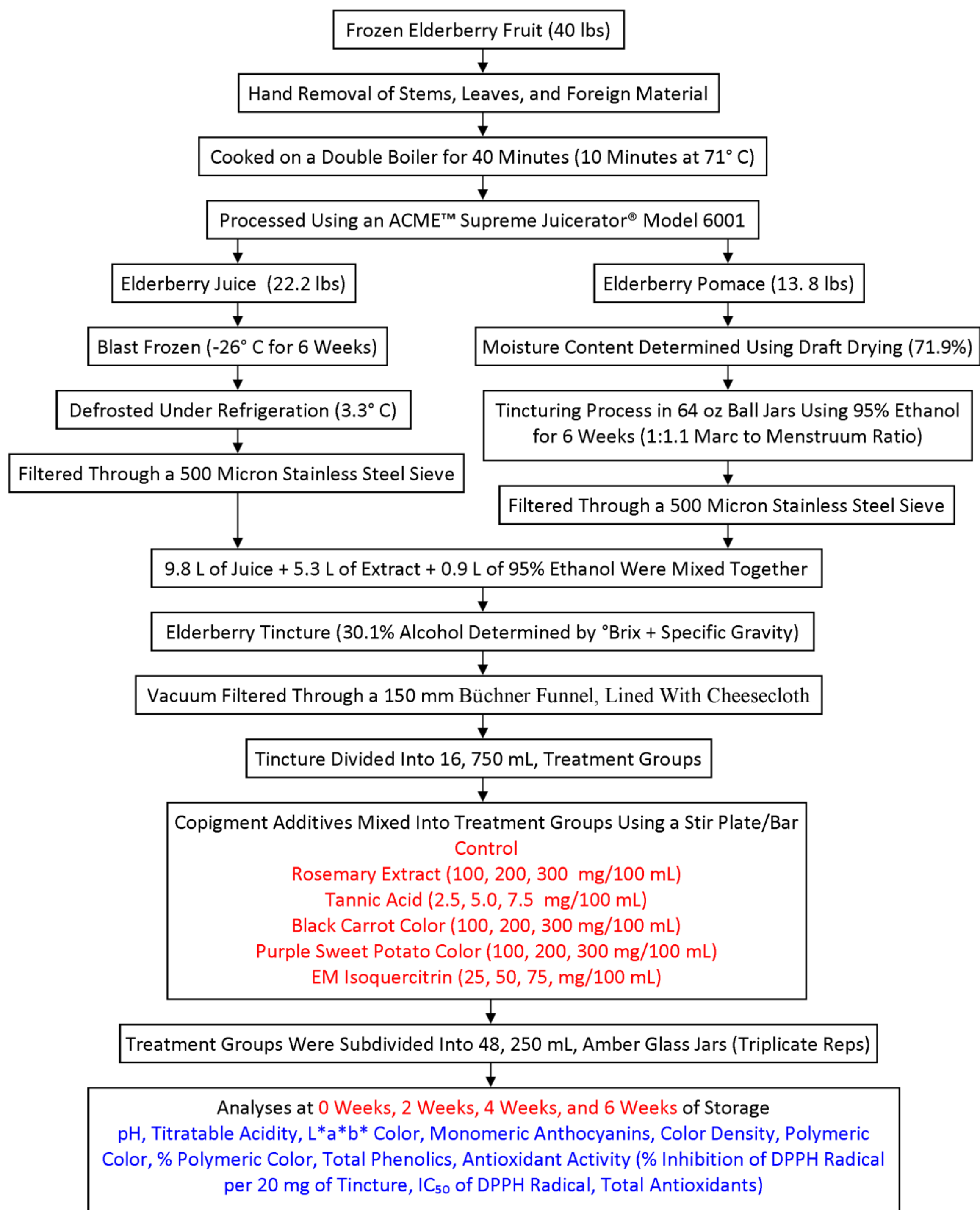


Figure 19 Flow chart of the preparation of elderberry tinctures and treatment groups.

Table 36 Concentration of Each Additive Used for the Copigmentation of Elderberry Tinctures

Treatment Code	Copigment Additive	Copigment Concentration (mg/100mL)	Copigment Source
Control	N/A	N/A	N/A
RME100	Rosemary Extract	100.0	Water Soluble Herbalox® Rosemary Extract (Kalsec®, Inc., Kalamazoo, MI)
RME200	Rosemary Extract	200.0	
RME300	Rosemary Extract	300.0	
TA2.5	Tannic Acid	2.5	(Graham Chemical™ Corp., Barrington, IL)
TA5.0	Tannic Acid	5.0	
TA7.5	Tannic Acid	7.5	
BCC100	Black Carrot Color	100.0	(Food Ingredient Solutions, LLC., Teterboro, NJ)
BCC200	Black Carrot Color	200.0	
BCC300	Black Carrot Color	300.0	
PSPC100	Purple Sweet Potato Color	100.0	San Red YM (San-Ei Gen F.F.I. (U.S.A.), Inc., New York, NY)
PSPC200	Purple Sweet Potato Color	200.0	
PSPC300	Purple Sweet Potato Color	300.0	
EMIQ25	EM Isoquercitrin	25.0	Sanmelin® Powder R-20 (San-Ei Gen F.F.I. (U.S.A.), Inc., New York, NY)
EMIQ50	EM Isoquercitrin	50.0	
EMIQ75	EM Isoquercitrin	75.0	

n=3.

pH and Titratable Acidity

pH was determined using an Orion™ PerpHecT™ LogR meter Model (Thermo Fisher Scientific, Inc., Orion™, Waltham, MA) with an Accumet® probe Cat. No. 13-620-289 (Thermo Fisher Scientific, Inc., Accumet®, Waltham, MA). The pH meter was calibrate using 4.0 and 7.0 calibration solutions. Titratable acidity was determined by diluting 10 mL of sample with 90 mL of distilled water, and titrating with 0.1 N NaOH (Thermo Fisher Scientific, Inc., Waltham, MA) until a pH endpoint of 8.1 was reached. Titratable acidity was determined in duplicate based on a procedure by Cliff and others (2007), and expressed as % citric acid using the following calculation:

(mLs of 0.1 N NaOH x 0.1 x milliequivalent factor of citric acid x 100)/10.

L*a*b* Color

Elderberry treatments were subjected to colorimetric analysis using a LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, VA) to determine L*a*b* values. Elderberry treatments were poured into a 2.5 inch clear glass sample cup with a black ring and disk, and placed on a pre-calibrated 2.5 inch sample port. L*a*b* values were determined using the computer software.

Monomeric Anthocyanins

Total monomeric anthocyanins were determined in duplicate based on AOAC method # 2005.02 (AOAC 2005), and a procedure by Lee and others (2005). One L of 0.025 M potassium chloride buffer solution was prepared by mixing 1.86 g of KCl (Thermo Fisher Scientific, Inc., Waltham, MA) with 981 mL of distilled water, and adjusted to pH 1.0 using 12 N HCl (Thermo Fisher Scientific, Inc., Waltham, MA). One L of 0.4 M sodium acetate buffer solution was prepared by mixing 54.43 g of sodium acetate trihydrate (Thermo Fisher Scientific, Inc., Waltham, MA) with 960 mL of distilled water, and adjusted to pH 4.5 using 12 N HCl. Elderberry tinctures were volumetrically diluted with each of the buffer solutions in 50 mL polyethylene centrifuge tubes (VWR International, LLC., Radnor, PA) to a 1:101.5 ratio (dilution factor = 0.009852217) and vortexed. The instrument was zeroed, and the absorbances of the dilutions were determined at 520 nm and 700 nm using a Beckman Coulter™ DU® 530 Life Science

UV/Vis spectrophotometer with Fisherbrand™ disposable methacrylate cuvettes, Cat. No. 14-386-21 (Thermo Fisher Scientific, Inc., Waltham, MA). Total monomeric anthocyanins was determined as cyanidin-3-glucoside equivalents as mg/L, using the following calculation:

(((Abs520nm pH 1.0 - Abs700nm pH 1.0) - (Abs520nm pH 4.5 - Abs700nm pH 4.5))/molar absorbance of cyanidin-3-glucoside) x 1000 x molecular wt. of cyanidin-3-glucoside x dilution factor.

Color Density and Polymeric Color

Color density and polymeric color were determined in duplicate based on a procedure by Giusti and Wrolstad (2001). Fifteen mL of bisulfite solution was prepared by dissolving 5 g of potassium metabisulfite (Thermo Fisher Scientific, Inc., Waltham, MA) into 15 mL of distilled water. Elderberry tinctures were diluted in 50 mL centrifuge tubes (VWR International, LLC., Radnor, PA) to a 1:67 ratio with distilled water. Diluted samples (2.8 mL) were volumetrically transferred into each of two 4.5 mL Fisherbrand™ disposable polystyrene cuvettes (Thermo Fisher Scientific, Inc., Waltham, MA). For each sample, 0.2 mL of bisulfite solution was added to one of the cuvettes and 0.2 mL of distilled water was added to the other. The sample solutions were allowed to equilibrate for 15 minutes, and their absorbances were determined at 420 nm, 520 nm, and 700 nm using a Beckman Coulter™ DU® 530 Life Science UV/Vis spectrophotometer with Fisherbrand™ disposable methacrylate cuvettes, Cat. No. 14-386-21 (Thermo Fisher Scientific, Inc., Waltham, MA). Color density, polymeric color, and % polymeric color were determined using the following calculations:

Color density = ((Abs420nm water sample - Abs700nm water sample) + (Abs520nm water sample - Abs700nm water sample)) x dilution factor.

Polymeric color = ((Abs420nm bisulfite sample - Abs700nm bisulfite sample) + (Abs520nm bisulfite sample - Abs700nm bisulfite sample)) x dilution factor.
% Polymeric color = (polymeric color/color density) x 100.

Total Phenolics

Total phenolics were determined in duplicate based on a procedure by Velioglu and others (1998). Elderberry tinctures were diluted in 50 mL centrifuge tubes (VWR International, LLC., Radnor, PA) to a 1:100 ratio with acidified methanol (0.1% v/v formic acid (Thermo Fisher Scientific, Inc., Waltham, MA) in methanol (Thermo Fisher Scientific, Inc., Waltham, MA)). Diluted samples (200 µL) were mixed with 1.5 mL of Folin-Ciocalteu (Sigma-Aldrich Co., LLC., St. Louis, MO) reagent (diluted 1:10 with distilled water) in glass, screw-top, test tubes (Corning, Inc., Corning, NY), and allowed to stand at room temperature for 5 minutes. Sodium bicarbonate (Thermo Fisher Scientific, Inc., Waltham, MA) solution (6 g/100 mL distilled water) was then added to each test tube (1.5 mL), and the solutions were allowed to stand for 90 minutes at room temperature. After 90 minutes, the absorbance of each sample was read at 725 nm using a Beckman Coulter™ DU® 530 Life Science UV/Vis spectrophotometer with Fisherbrand™ disposable methacrylate cuvettes Cat. No. 14-386-21 (Thermo Fisher Scientific, Inc., Waltham, MA). A standard curve based on gallic acid (Thermo Fisher Scientific, Inc., Waltham, MA) standards of 0, 50, 100, 150, and 200 µg/mL was used to determine the regression equation. Total phenolics were determined as gallic acid equivalents as mg/L using the following calculation:

(((abs/slope) - (y-intercept/slope)) x total volume of sample)/sample weight.
e.g. (((0.199/0.0056) - (-0.053/0.0056)) x 10.1 mL)/0.100 g = 4545.0 µg GAE/mL.

Antioxidants

Total antioxidants were determined based on procedures by Brand-Williams and others (1995), D'Souza (2006), and Plank and others (2012). A 0.1 mM DPPH solution was prepared by mixing 40 mg of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich®, Saint Louis, MO) radical into 500 mL of methanol (Thermo Fisher Scientific, Inc., Waltham, MA) for approximately 20 minutes, in a covered flask to exclude light. Distilled water (500 mL) was then added to the solution, and mixed for an additional 20 minutes. The absorbance of the DPPH solution was determined at 517 nm against a distilled water blank using a Beckman Coulter™ DU® 530 Life Science UV/Vis spectrophotometer with Fisherbrand™ disposable methacrylate cuvettes, Cat. No. 14-386-21 (Thermo Fisher Scientific, Inc., Waltham, MA), to determine target absorbance (Abs target = Abs of DPPH solution/2).

For each tincture sample, three subsamples were weighed into 60 mL amber, wide mouth, packer bottles (Qorpak®, Bridgeville, PA) to the nearest 0.1 mg. The weight of the subsamples were determined to be between 3.0 mg and 25.0 mg, with the goal of bracketing the target absorbance during final spectrophotometric analysis at 517 nm. Fifty mL of the 0.1 mM DPPH solution were added to each sample. The samples were swirled gently, covered, and placed on a BenchRocker™ 3d variable speed lab rocker Model B3D2300 (Benchmark Scientific, Inc., Edison, NJ), set to high for 4 hours at 21° C. After 4 hours of incubation, the absorbance of the samples were determined at 517 nm

against a distilled water blank. A standard curve based on trolox (Thermo Fisher Scientific, Inc., Waltham, MA) standards of 0.2, 0.4, 0.6, and 0.8 mg/mL were used to determine the regression equation. Total antioxidants was determined as trolox equivalents in μg/100 g, % inhibition in 20 mg of sample, and IC_{50} (amount of sample in mg required to cause a 50% inhibition of DPPH) using the following calculations:

μg TE/100 g = ((((-y-intercept of standard curve/2)/(2 x slope of standard curve x (((y-intercept of standard curve/2) - (y-intercept of data))/slope of data))/molecular weight of trolox) x 1000) x 100).

e.g. ((((-0.893/2)/(2 x -0.8625 x (((0.893/2) - (0.473555727))/-0.01467))/250) x 1000) x 100) = 56.139 μg TE/100 g.

% Inhibition of DPPH per 20 mg of Tincture = (100 - ((absorbance of sample/absorbance of DPPH blank) x 100)).

IC_{50} = ((50 - y-intercept of data)/slope of data).

Statistical Analyses

Statistical differences among data to determine if copigment treatments and storage time had any effects were evaluated using JMP 7.0.1 (SAS Institute Inc., Cary, NC) statistical software using one-way analysis of variance (ANOVA) with a significance value of $p \leq 0.05$. Differences between means were evaluated using the Fisher's least significant difference test. A correlation analysis was also performed among dependent variables.

Results and Discussion

pH and Titratable Acidity

pH is defined as the negative logarithm of the hydrogen ion concentration of an aqueous solution, and is used to measure its degree of acidity or alkalinity. In beverage manufacturing, pH is primarily used to control the growth of microorganisms and prevent spoilage by denaturation, however it has an equally important role in anthocyanin-rich beverages. The pH of anthocyanin-rich beverages determines which anthocyanin chromophore is the most dominant one within the solution, which can include either the red flavylium cation, blue or red quinonoidal base, colorless carbinol pseudobase, or colorless chalcone (Brouillard 1982, Hubbermann 2006). Anthocyanins are most stable around pH 3.0, in which the red flavylium cation dominates and gives the beverage a red color (Brouillard 1982, Rein 2005, Hubbermann 2006).

In this study, the average pH of elderberry tinctures was 4.8, with no significant differences ($p \leq 0.05$) among copigment treatments based on one-way ANOVA (Table 37). Although significant differences were noticed among weeks of storage, these results were likely due to experimental error during the calibration of the pH meter. Typically, elderberry juice has a pH of 3.5-5.0 which categorizes it as a low acid product, in which acidulants, such as malic or citric acid, are often added to lower the pH to increase shelf-stability and reduce microbial contamination (Byers and others 2012, Casati and others 2012, Garofulić and others 2012). Although spoilage was not a concern with the elderberry tinctures in this study, due to their ethanol content of 30.1%, it was

important to evaluate if any of the copigment treatments affected the pH levels or if there were any associations between pH and color stability.

Table 37 pH Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*ab*
RME100	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
RME200	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
RME300	4.8±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
TA2.5	4.8±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
TA5.0	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
TA7.5	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
BCC100	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
BCC200	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
BCC300	4.7±0.0*c*	4.9±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
PSPC100	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
PSPC200	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
PSPC300	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
EMIQ25	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
EMIQ50	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*
EMIQ75	4.7±0.0*c*	4.8±0.0*a*	4.8±0.0*b*	4.8±0.0*b*

Refer to Table 36 for treatment identification. pH values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD).

Similar to pH, titratable acidity is also used to measure the acidity of a solution, and is defined as the quantity of protons present in a solution during titration with a strong base to a neutral endpoint. Titratable acidity is the measure of the amount of predominate acid in a solution, whereas pH is a measure of the strength of acid. Determining the titratable acidity of beverages is of great importance to beverage

manufactures because titratable acidity is a predictor of perceived tartness when related to a beverages sugar content.

In this study, the average titratable acidity (% citric acid) of elderberry tinctures was 0.57, with no significant differences ($p \leq 0.05$) among copigment treatments based on one-way ANOVA (Table 38). Although significant differences were noticed over storage time, as well as difference among pH levels, these results were likely due to experimental error during the calibration of the pH meter. Typically, elderberry juice contains 0.57-0.67 titratable acidity (% citric acid) and total soluble solids of 11-12° brix (Byers and others 2012). Although the determination of soluble solids was not an objective of this study, elderberry tinctures were initially determined to be 18.3° brix.

In a study by Garofulić and others (2012), six different elderberry wines were produced with varying levels of acidity (9.1-19.0 g/L), soluble solids (10.3-17.3° brix), and sugar contents (0.4-8.9 %), which were then subjected to sensory analysis. It was determined that wines which were produced with the highest amounts of sugar and water resulted in the best sugar/acid ratios, and had the best sensory characteristics during consumer evaluation (Garofulić and others 2012).

Table 38 Titratable Acidity (% Citric Acid) Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	0.58±0.00*a*	0.56±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
RME100	0.58±0.00*a*	0.57±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
RME200	0.58±0.00*a*	0.57±0.00*b*	0.57±0.00*b*	0.57±0.00*c*
RME300	0.58±0.00*a*	0.56±0.00*b*	0.56±0.00*b*	0.57±0.00*b*
TA2.5	0.57±0.00*a*	0.57±0.00*ab*	0.57±0.00*b*	0.57±0.00*b*
TA5.0	0.57±0.00*a*	0.56±0.00*b*	0.57±0.00*ab*	0.57±0.00*b*
TA7.5	0.58±0.00*a*	0.56±0.00*b*	0.57±0.00*b*	0.56±0.00*b*
BCC100	0.57±0.00*a*	0.56±0.00*b*	0.56±0.00*ab*	0.57±0.00*ab*
BCC200	0.58±0.00*a*	0.56±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
BCC300	0.57±0.00*a*	0.57±0.00*a*	0.57±0.00*a*	0.57±0.00*a*
PSPC100	0.58±0.00*a*	0.57±0.00*a*	0.57±0.00*b*	0.57±0.00*b*
PSPC200	0.58±0.00*a*	0.57±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
PSPC300	0.58±0.00*a*	0.57±0.00*b*	0.56±0.00*b*	0.57±0.00*b*
EMIQ25	0.58±0.00*a*	0.57±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
EMIQ50	0.58±0.00*a*	0.57±0.00*b*	0.57±0.00*b*	0.57±0.00*b*
EMIQ75	0.57±0.00*a*	0.56±0.00*b*	0.56±0.00*b*	0.57±0.00*b*

Refer to Table 36 for treatment identification. Titratable acidity values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD).

L*a*b* Color

In this study, the average L*, a*, and b* color values of elderberry tinctures was 1.96, 3.86, and 0.96, regardless of copigment treatment or storage time, respectively. Although some significant differences (p≤0.05) were noticed among copigment treatments (Table 39), the only noteworthy effects to L*a*b* color among copigment treatments occurred with the additions of either black carrot color at 300 mg/100mL (BCC300) or purple sweet potato color at 300 mg/100mL (PSPC300). Regardless of storage time, BCC300 and PSPC300 consistently had the lowest L*, a*, and b* color

values, indicating that these two treatments were darker, less red, and bluer than the other copigment treatments tested in this study. Although significant effects were noticed within these two treatments, it is unlikely that these results are indicative of copigment reactions. It is more likely that these effects to L*a*b* color were simply a result of the initial deep purple color of the black carrot and purple sweet potato additives, and their additions above their recommended usage levels, which are typically 10-100 mg/100mL for beverages.

Table 39 Mean L*a*b* Color Values of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	L*	a*	b*
Control	2.07±0.39*ab*	4.04±0.77*a*	1.07±0.29*a*
RME100	1.91±0.42*abcd*	3.82±1.02*a*	0.96±0.22*abc*
RME200	2.12±0.32*ab*	4.08±0.83*a*	1.05±0.23*a*
RME300	1.94±0.28*abc*	3.80±0.89*a*	0.99±0.22*ab*
TA2.5	2.04±0.29*ab*	4.04±0.86*a*	1.04±0.21*a*
TA5.0	1.93±0.32*abcd*	3.73±0.79*a*	0.92±0.19*abc*
TA7.5	2.11±0.27*ab*	4.12±0.86*a*	1.03±0.21*a*
BCC100	2.15±0.23*a*	4.15±0.65*a*	1.01±0.19*a*
BCC200	1.95±0.27*abc*	3.81±0.67*a*	0.91±0.17*abc*
BCC300	1.67±0.19*d*	3.51±0.67*a*	0.82±0.17*bc*
PSPC100	1.95±0.37*abc*	3.70±1.00*a*	0.90±0.29*abc*
PSPC200	2.04±0.21*ab*	3.97±0.71*a*	0.95±0.20*abc*
PSPC300	1.72±0.33*cd*	3.60±0.80*a*	0.79±0.18*c*
EMIQ25	1.89±0.39*bcd*	3.69±0.96*a*	0.95±0.24*abc*
EMIQ50	1.96±0.31*abc*	3.84±0.96*a*	1.01±0.27*a*
EMIQ75	1.95±0.37*abc*	3.84±0.97*a*	0.98±0.26*ab*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different (p≤0.05) among copigment treatments based on one-way ANOVA. *n*=16 analyses (±SD).

Regardless of copigment treatment, average L* values of elderberry tinctures were 2.00, 1.89, 1.94, and 2.02 at 0, 2, 4, and 6 weeks of storage (Figure 20). Few significant differences ($p \leq 0.05$) were noticed among weeks of storage based on one-way ANOVA (Table 40), which indicates that the darkness/lightness of elderberry tinctures was not affected throughout 5 weeks of storage at room temperature (21° C) and throughout one week of accelerated temperature storage (32° C).

Table 40 L* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	1.81±0.18*a*	2.15±0.20*a*	2.10±0.78*a*	2.22±0.05*a*
RME100	2.09±0.12*a*	2.07±0.21*a*	1.77±0.76*a*	1.72±0.40*a*
RME200	2.09±0.06*a*	2.00±0.44*a*	2.40±0.45*a*	2.00±0.05*a*
RME300	2.15±0.12*a*	1.99±0.08*a*	1.76±0.50*a*	1.85±0.20*a*
TA2.5	2.10±0.14*a*	1.98±0.38*a*	2.12±0.45*a*	1.94±0.33*a*
TA5.0	1.96±0.22*a*	1.95±0.42*a*	1.91±0.37*a*	1.88±0.38*a*
TA7.5	2.24±0.17*a*	2.02±0.19*a*	2.23±0.49*a*	1.95±0.09*a*
BCC100	2.06±0.06*ab*	1.97±0.33*b*	2.40±0.16*a*	2.15±0.09*ab*
BCC200	1.95±0.08*ab*	1.65±0.36*b*	2.04±0.13*ab*	2.16±0.17*a*
BCC300	1.70±0.07*ab*	1.52±0.05*b*	1.59±0.27*ab*	1.87±0.13*a*
PSPC100	2.07±0.14*a*	1.64±0.51*a*	1.98±0.32*a*	2.11±0.40*a*
PSPC200	1.93±0.08*a*	2.05±0.29*a*	1.94±0.23*a*	2.25±0.05*a*
PSPC300	1.67±0.11*a*	1.82±0.08*a*	1.44±0.59*a*	1.93±0.13*a*
EMIQ25	2.03±0.05*a*	2.02±0.69*a*	1.51±0.12*a*	1.98±0.29*a*
EMIQ50	2.07±0.10*ab*	1.71±0.29*b*	1.82±0.24*ab*	2.25±0.34*a*
EMIQ75	2.00±0.14*a*	1.75±0.49*a*	2.06±0.48*a*	1.99±0.42*a*

Refer to Table 36 for treatment identification. L* color values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD).

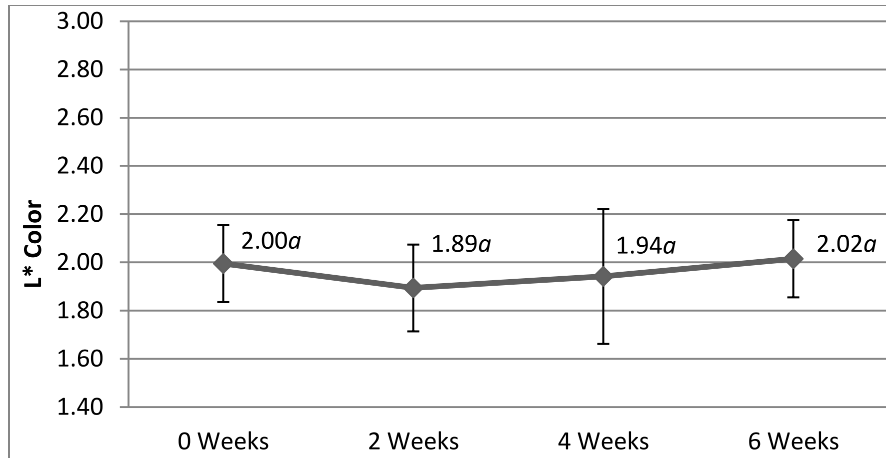


Figure 20 Mean L* color values among copigment treatments over storage time. L* color values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=48 analyses (±SD).

Average a* values of elderberry tinctures were 4.89, 3.60, 3.70, and 3.25 at 0, 2, 4, and 6 weeks of storage (Figure 21). Significant differences (p≤0.05) were noticed among all copigment treatments between 0 and 2 weeks, as well as at 4 and 6 weeks of storage based on one-way ANOVA (Table 41). These results indicate that there were detectable degradations of red color within elderberry tinctures during the initial 2 weeks of room temperature storage (21° C), as well as during the one week accelerated temperature storage (32° C), which occurred between 5 and 6 weeks of storage. It is likely that anthocyanin pigmentation metabolized into colorless and brown phenolic degradation compounds, resulting in elderberry tinctures with less red color during storage.

Table 41 a* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	4.58±0.44*a*	4.02±0.34*a*	3.75±1.51*a*	3.82±0.12*a*
RME100	5.01±0.33*a*	3.99±0.41*ab*	3.34±1.18*b*	2.93±0.60*b*
RME200	4.89±0.06*a*	3.75±0.78*bc*	4.50±0.74*ab*	3.20±0.03*c*
RME300	5.10±0.09*a*	3.80±0.20*b*	3.27±0.68*bc*	3.03±0.24*c*
TA2.5	5.05±0.24*a*	3.74±0.69*b*	4.09±0.80*ab*	3.28±0.59*b*
TA5.0	4.61±0.36*a*	3.71±0.85*ab*	3.57±0.57*ab*	3.04±0.56*b*
TA7.5	5.19±0.25*a*	3.89±0.37*bc*	4.20±0.84*b*	3.19±0.14*c*
BCC100	4.89±0.07*a*	3.79±0.57*b*	4.50±0.25*a*	3.44±0.10*b*
BCC200	4.68±0.23*a*	3.25±0.40*c*	4.03±0.31*b*	3.27±0.07*c*
BCC300	4.44±0.13*a*	3.23±0.59*b*	3.19±0.48*b*	3.17±0.38*b*
PSPC100	5.10±0.23*a*	2.73±0.28*b*	3.66±0.55*b*	3.31±0.74*b*
PSPC200	4.97±0.15*a*	3.67±0.78*b*	3.77±0.32*b*	3.48±0.15*b*
PSPC300	4.54±0.16*a*	3.71±0.27*ab*	2.89±1.06*b*	3.25±0.28*b*
EMIQ25	5.07±0.11*a*	3.76±0.62*b*	2.85±0.25*c*	3.09±0.45*bc*
EMIQ50	5.10±0.18*a*	3.24±1.16*b*	3.64±0.38*b*	3.38±0.50*b*
EMIQ75	5.03±0.12*a*	3.26±0.92*b*	3.92±0.79*ab*	3.15±0.53*b*

Refer to Table 36 for treatment identification. a* color values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD).

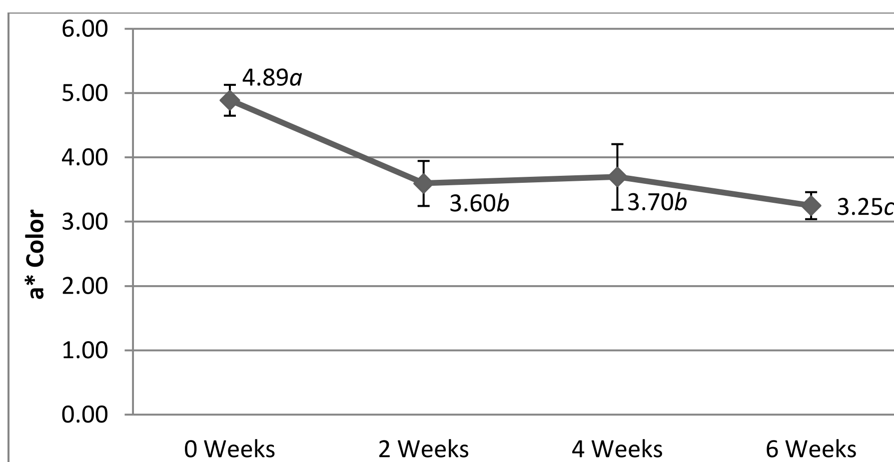


Figure 21 Mean a* color values among copigment treatments over storage time. a* color values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=48 analyses (±SD).

Average b* values of elderberry tinctures were 1.15, 0.75, 0.94, and 1.01 at 0, 2, 4, and 6 weeks of storage (Figure 22). Overall, b* color values were significantly ($p \leq 0.05$) higher at 0 weeks compared to 2, 4, and 6 weeks storage based on one-way ANOVA (Table 42). These results indicate that the elderberry tinctures initially lost some of their yellow color and turned slightly bluer during the first 2 weeks of storage. A strong correlation ($r=0.78$) existed between a* and b* color values throughout weeks of storage. However, an inverse relationship was noted between a* and b* color values between 4 and 6 weeks of storage. As previously stated, it is likely that degradation of anthocyanin pigmentation occurred and colorless and brown phenolic degradation compounds formed (Gómez-Míguez and others 2006). Based on these results, it is theorized that the initial breakdown of anthocyanin pigmentation first resulted in the formation of colorless compounds, and later developed into brown compounds as storage time increased. After 2 weeks of storage, b* values began to increase, which signified the formation of these brown degradation compounds, which is substantiated by the inverse relationship between a* and b* color values which occurred between 4 and 6 weeks of storage. As the elderberry tinctures lost their red color over time due to the degradation of anthocyanins, more yellow color was detected possibly as brown degradation compounds were formed, but only after the first 2 weeks of storage, in which colorless degradation compounds formed.

Table 42 b* Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	1.18±0.11*ab*	0.84±0.10*b*	0.95±0.47*ab*	1.32±0.14*a*
RME100	1.19±0.11*a*	0.89±0.11*ab*	0.82±0.31*b*	0.94±0.16*ab*
RME200	1.17±0.07*a*	0.79±0.22*b*	1.21±0.24*a*	1.02±0.04*ab*
RME300	1.31±0.07*a*	0.84±0.09*b*	0.82±0.17*b*	0.99±0.02*b*
TA2.5	1.15±0.05*a*	0.82±0.16*a*	1.13±0.28*a*	1.07±0.21*a*
TA5.0	1.07±0.08*a*	0.77±0.24*a*	0.91±0.19*a*	0.92±0.17*a*
TA7.5	1.24±0.07*a*	0.81±0.10*c*	1.13±0.26*ab*	0.96±0.01*bc*
BCC100	1.10±0.05*ab*	0.76±0.14*c*	1.21±0.10*a*	0.97±0.03*b*
BCC200	0.98±0.08*a*	0.66±0.11*b*	1.04±0.10*a*	0.95±0.03*a*
BCC300	1.02±0.03*a*	0.67±0.16*b*	0.75±0.14*b*	0.84±0.14*ab*
PSPC100	1.18±0.10*a*	0.54±0.10*b*	0.89±0.17*a*	0.99±0.28*a*
PSPC200	1.15±0.04*a*	0.73±0.22*c*	0.87±0.05*bc*	1.05±0.10*ab*
PSPC300	0.96±0.03*a*	0.67±0.10*bc*	0.64±0.21*c*	0.90±0.10*ab*
EMIQ25	1.25±0.03*a*	0.84±0.16*bc*	0.68±0.07*c*	1.02±0.15*b*
EMIQ50	1.22±0.07*a*	0.70±0.24*b*	0.96±0.13*ab*	1.16±0.19*a*
EMIQ75	1.19±0.04*a*	0.66±0.33*b*	1.06±0.22*a*	1.02±0.18*a*

Refer to Table 36 for treatment identification. b* color by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD).

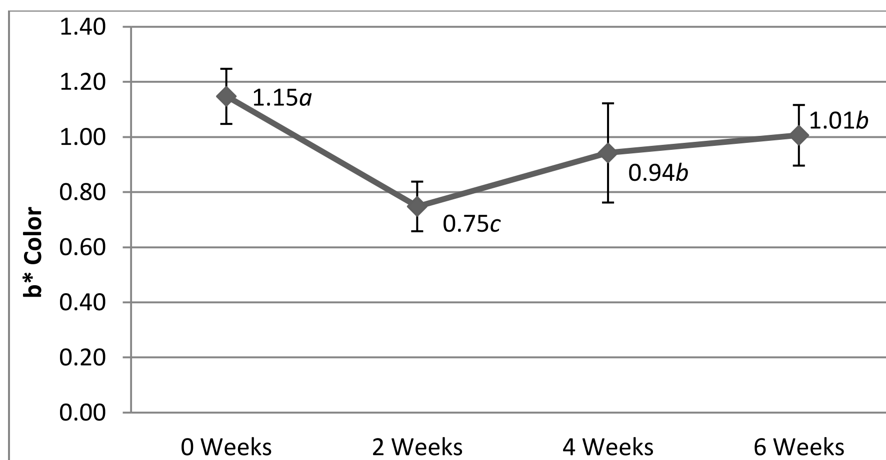


Figure 22 Mean b* color values among copigment treatments over storage time. b* color values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=48 analyses (±SD).

Monomeric Anthocyanins

Anthocyanins in the monomeric form are directly responsible for the red or blue color of anthocyanin-rich foods or beverages. Total monomeric anthocyanins were determined within elderberry tinctures using the pH differential method (2005.02, AOAC 2005). At pH 1.0, monomeric anthocyanins exist in the red colored flavylium cation form, and at pH 4.5 exist in the colorless hemiketal form (Brouillard 1982, Clifford 2000, AOAC 2005, Hubbermann 2006). The amount of monomeric anthocyanins within a beverage is calculated based on the difference of the pigments' absorbance at 520 nm between 1.0 and 4.5 pH. Degraded, polymeric anthocyanins are not expressed using this method because they are resistant to color change, despite shifts in pH, and absorb at both 1.0 and 4.5 pH used in this method (AOAC 2005).

In this study, the average monomeric anthocyanin content of elderberry tinctures was 5,561.6 mg/L, regardless of copigment treatment or weeks of storage. These results are in agreement with Garofulić and others (2012), who reported that the average anthocyanin contents in raw elderberry fruit and elderberry wine to be 8,527.0 and 3597.8 mg/L. In this study, some significant differences ($p \leq 0.05$) were detected among copigment treatments based on one-way ANOVA (Table 43), which were similar to L*a*b* color results. Noteworthy effects to monomeric anthocyanins among copigment treatments occurred with the additions of either black carrot color at 300 mg/100mL (BCC300) or purple sweet potato color at 300 mg/100mL (PSPC300). Regardless of weeks of storage, BCC300 and PSPC300 consistently had the highest monomeric anthocyanin contents compared to the other copigment treatments tested

in this study. These results simply indicate that black carrot or purple sweet potato color additives contributed to the overall anthocyanin content of elderberry tinctures. Unfortunately, this data does not support any inferences into the efficacy of the copigment additives as successful copigments within elderberry tinctures. It is likely that the high ethanol content (30.1%) of the tinctures prevented, or dissociated, intermolecular complexes between the copigment additives and the elderberry fruit anthocyanins, which otherwise would have resulted in successful copigmentation and enhanced pigment stability of the elderberry tinctures (Mazza and Brouillard 1990, Brouillard and others 1991, Gutiérrez 2003). These results are in agreement with Brouillard and others (1991), who evaluated the effects of ten different cosolvents on the extent of the copigmentation reaction, and determined that all of the cosolvents reduced the effect of copigmentation compared to water. The hydrogen-bonded network of water molecules, and the ability of water to hydrogen bond with flavonoids, strongly contributes to the stability of copigment complexes (Brouillard and others 1991). These factors validate water as the most important cofactor for the copigment phenomenon, and that non-polar solvents, such as ethanol, disrupt hydrogen bonds which are required for effective intermolecular complexation.

Table 43 Mean Monomeric Anthocyanin Values (mg/L) of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	Monomeric Anthocyanins (mg/L)
Control	5433.2±625.6*b*
RME100	5631.4±595.2*ab*
RME200	5400.5±435.5*b*
RME300	5613.3±446.5*ab*
TA2.5	5614.0±547.3*ab*
TA5.0	5590.8±455.8*ab*
TA7.5	5628.8±736.5*ab*
BCC100	5456.8±739.7*b*
BCC200	5618.3±683.5*ab*
BCC300	5701.2±657.2*ab*
PSPC100	5607.1±641.7*ab*
PSPC200	5598.9±784.9*ab*
PSPC300	5969.5±674.4*a*
EMIQ25	5438.7±493.5*b*
EMIQ50	5416.2±626.0*b*
EMIQ75	5267.2±682.7*b*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=16 analyses (±SD).

Although few differences were detected among anthocyanin contents of elderberry tinctures, there were significant ($p \leq 0.05$) effects over storage time based on one-way ANOVA (Table 44). Average monomeric anthocyanins of elderberry tinctures were 6269.2, 5825.0, 5296.4, and 4856.0 mg/L at 0, 2, 4, and 6 weeks of storage, among all copigment treatments (Figure 23). Overall, there was a linear (R^2=0.9987) degradation of the anthocyanin content of the elderberry tinctures throughout storage time, with 23% of the anthocyanin content degrading between 0 and 6 weeks of storage. There was a moderate correlation (r=0.49) between monomeric anthocyanins

and a* color among all treatments, which indicates a positive relationship between pigment concentration and the intensity of red color. Interestingly, there were no apparent effects to the anthocyanin content of elderberry tinctures during the one week of accelerated temperature (32° C) storage, which occurred between weeks 5 and 6.

Table 44 Mean Monomeric Anthocyanin Values (mg/L) of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	6337.9±392.7*a*	5499.6±92.4*b*	5044.2±177.9*c*	4850.8±124.3*c*
RME100	6457.4±249.6*a*	5791.8±39.5*b*	5305.4±26.2*c*	4971.0±92.1*d*
RME200	5537.0±529.6*a*	5823.6±69.0*a*	5389.8±40.1*a*	4851.6±88.1*b*
RME300	6025.9±87.2*a*	5820.5±129.1*ab*	5686.7±54.6*b*	4920.0±143.4*c*
TA2.5	6295.7±278.9*a*	5884.1±147.4*b*	5266.4±79.2*c*	5010.0±81.6*c*
TA5.0	6103.9±79.1*a*	5847.5±113.7*b*	5431.9±119.5*c*	4979.7±132.5*d*
TA7.5	6458.9±289.9*a*	6039.4±200.5*b*	5378.6±70.7*c*	4638.2±44.2*d*
BCC100	6029.8±1162.0*a*	5836.4±116.8*a*	5256.0±97.4*ab*	4705.1±140.1*b*
BCC200	6491.6±202.8*a*	5939.1±107.4*b*	5213.8±29.0*c*	4830.1±199.9*d*
BCC300	6434.3±183.3*a*	6089.5±249.8*a*	5403.3±185.1*b*	4877.8±250.8*c*
PSPC100	6333.2±76.4*a*	5992.4±189.6*b*	5329.2±89.4*c*	4773.6±208.4*d*
PSPC200	6486.0±210.3*a*	6098.3±207.5*b*	5221.0±54.0*c*	4590.4±60.6*d*
PSPC300	6892.0±495.3*a*	5857.1±110.3*b*	5943.9±132.1*b*	5185.1±82.9*c*
EMIQ25	6033.8±72.5*a*	5619.8±219.4*b*	5303.0±163.3*b*	4798.2±189.2*c*
EMIQ50	6290.2±382.3*a*	5498.8±143.2*b*	4787.1±240.6*c*	5088.8±142.0*bc*
EMIQ75	6099.1±321.7*a*	5561.7±212.0*a*	4782.3±472.6*b*	4625.5±229.8*b*

Refer to Table 36 for treatment identification. Monomeric anthocyanin values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 2 analyses.

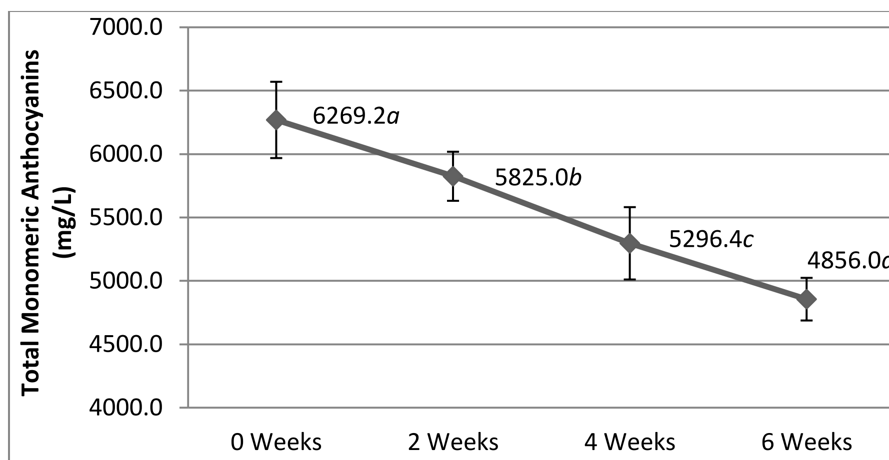


Figure 23 Mean total monomeric anthocyanins values (mg/L) among copigment treatments over storage time.

Monomeric anthocyanin values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=96 analyses (±SD).

Color Density and Polymeric Color

Determining color density is important for understanding depth of color and can provide information into the degradation characteristics of elderberry tinctures. Color density is calculated as the sum of absorbances at $\lambda_{vis\text{-}max}$ (520 nm) and at 420 nm, after subtracting for haze (700 nm). In the case of elderberry tinctures, color density can be defined as the sum of monomeric red colored anthocyanin pigments, brown colored polymeric anthocyanin pigments, and brown colored melanoidin pigments. Increases of the color density of anthocyanin-rich beverages generally signifies the conversion of anthocyanin pigmentation into degraded, brown colored anthocyanin-tannin pigments.

In this study, the average color density of elderberry tinctures was 12.8, regardless of copigment treatment or weeks of storage. Similar to previous color results, the only noteworthy effects among copigment treatments occurred with the additions of either black carrot color at 300 mg/100mL (BCC300) or purple sweet potato color at

300 mg/100mL (PSPC300), although some significant differences ($p \leq 0.05$) were noticed based on one-way ANOVA (Table 45). Regardless of weeks of storage, BCC300 and PSPC300 consistently had the highest color density compared to the other copigment treatments tested in this study. As previously stated, this result is likely due to the fact that the black carrot and purple sweet potato additives were dark purple when initially added into the elderberry tinctures and resulted in a darker colored tincture overall.

Table 45 Mean Color Density Values of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	Color Density
Control	11.8±1.5*e*
RME100	13.2±1.5*bc*
RME200	13.0±0.9*bc*
RME300	12.4±1.2*cde*
TA2.5	12.7±1.9*bcde*
TA5.0	13.0±1.3*bc*
TA7.5	12.5±1.1*cde*
BCC100	13.4±1.2*bc*
BCC200	13.1±1.4*bc*
BCC300	13.8±1.0*ab*
PSPC100	12.5±1.7*cde*
PSPC200	12.3±1.1*cde*
PSPC300	14.7±1.2*a*
EMIQ25	12.9±1.4*bcd*
EMIQ50	11.8±2.2*de*
EMIQ75	11.8±0.6*e*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=24 analyses (±SD).

Although few differences to the color density of elderberry tinctures were detected among copigment treatments, there were significant ($p \leq 0.05$) effects among weeks of storage based on one-way ANOVA (Table 46). The average color density of elderberry tinctures was 12.2, 12.7, 12.5, and 13.9 at 0, 2, 4, and 6 weeks of storage, among all copigment treatments (Figure 24). A moderate inverse correlation ($r=-0.31$) existed between color density and a* color value, which indicates a relationship between the depth of color and intensity of redness within the elderberry tinctures. Based on these results, it can be inferred that there were losses to anthocyanin pigmentation and increases of brown colored degradation products throughout storage time (Gómez-Míguez and others 2006). Interestingly, as there were noticeable decreases to the a* values of elderberry tinctures during the one week of accelerated temperature (32° C) storage, which occurred between weeks 5 and 6, there were also significant increases to color density during this time period.

Table 46 Color Density Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	11.5±1.2*ab*	12.9±0.5*a*	12.4±0.9*ab*	10.2±1.8*b*
RME100	11.8±0.5*c*	12.5±0.8*bc*	13.9±1.4ab	14.6±1.4*a*
RME200	12.2±0.3*a*	12.9±1.1*a*	13.7±0.5*a*	13.4±1.1*a*
RME300	12.1±0.4*a*	12.1±0.2*a*	12.8±1.3*a*	12.6±2.4*a*
TA2.5	12.0±0.3*a*	12.8±0.9*a*	11.5±0.9*a*	14.5±3.2*a*
TA5.0	11.8±0.1*c*	12.4±1.2*bc*	14.3±1.2*a*	13.6±0.6*ab*
TA7.5	11.9±0.4*b*	12.2±0.7*b*	11.9±1.1*b*	13.9±0.5*a*
BCC100	12.6±0.6*bc*	12.4±0.7*c*	14.2±1.5*ab*	14.7±0.2*a*
BCC200	12.7±0.4*b*	12.3±0.8*b*	12.2±0.3*b*	15.4±0.6*a*
BCC300	13.7±0.2*b*	13.6±0.8*b*	12.7±0.7*b*	15.0±0.6*a*
PSPC100	12.0±0.7*b*	13.5±0.6*ab*	10.2±0.8*c*	14.1±1.0*a*
PSPC200	12.3±0.2*a*	11.8±0.3*a*	12.9±0.6*a*	12.3±2.3*a*
PSPC300	13.7±0.3*b*	14.3±1.1*b*	14.6±0.5*ab*	16.0±1.4*a*
EMIQ25	11.8±0.4*b*	13.1±0.7*b*	11.9±1.3*b*	14.8±0.7*a*
EMIQ50	12.0±0.6*b*	12.4±0.4*b*	8.6±0.2*c*	14.2±0.7*a*
EMIQ75	11.6±0.8*b*	11.5±0.2*b*	11.4±0.4*b*	12.6±0.3*a*

Refer to Table 36 for treatment identification. Color density values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 2 analyses.

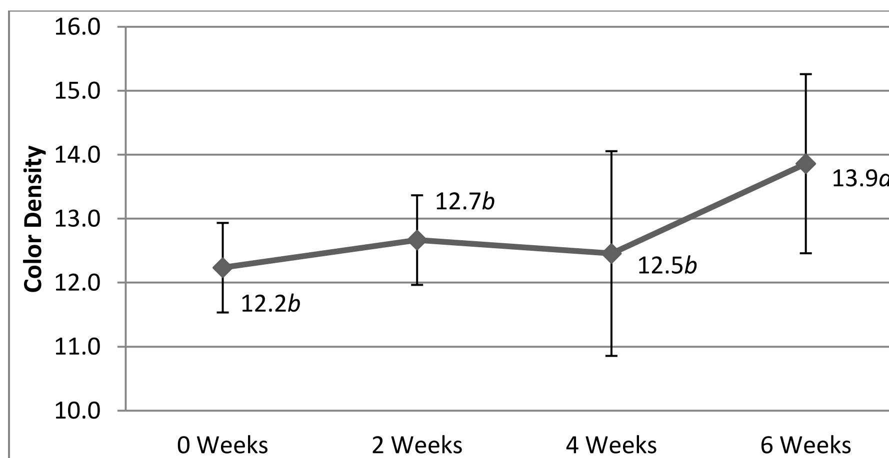


Figure 24 Mean color density values among copigment treatments over storage time. Color density values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=96 analyses (±SD).

Polymeric color is a measurement of brown colored, non-monomeric anthocyanin pigments, also referred to as tannins. Both polymeric color and color density determinations provide a similar function, which is to understand the degradation and shelf-life characteristics of anthocyanin-rich beverages. The difference being that polymeric color measures the depth of only brown pigmentation, whereas color density measures the depth of red and brown pigmentation combined. Polymeric color can be understood as the color density of a beverage excluding the monomeric anthocyanins. The assay takes advantage of the fact that anthocyanins in their monomeric form are easily bleached by bisulfite, whereas polymeric anthocyanin-tannin and melanoidin pigments are resistant and retain their color during bisulfite treatment (Giusti and Wrolstad 2001).

In this study, the average polymeric color of elderberry tinctures was 6.8, regardless of copigment treatment or weeks of storage. Unlike previous color results, no discernible effects to polymeric color were noticed among copigment treatments (Table 47). These results indicate that copigment treatments did not prevent browning within elderberry tinctures and supports a lack of copigmentation, which is likely due to the high ethanol content (30.1%) of the tinctures which prevented intermolecular complexation between anthocyanins and copigments. Interestingly, although significant ($p \leq 0.05$) effects to a* color, monomeric anthocyanins, and color density were detected within BCC300 and PSPC300 treatments, no distinguishable effects were observed to polymeric color within these treatments. These result infer that although additions of black carrot or purple sweet potato color contributed significantly to the increase of red

pigmentation within elderberry tinctures, and that these added pigments did not degrade as readily as those naturally present within elderberry fruit. The lack of additional browning within BCC300 or PSPC300 treatments is likely due to the acylated structure of the anthocyanins contained in the black carrot and purple sweet potato copigments (Giusti and Wrolstad 2003, Bąkowska-Barczak 2005). Acylated anthocyanins contain covalently bonded organic or phenolic acids, which have an increased resistance to polymerization into brown colored degradation pigments (Giusti and Wrolstad 2003, Bąkowska-Barczak 2005). Conversely, elderberry fruit contains non-acylated anthocyanins, which occur primarily as anthocyanin glycosides that readily polymerize due to weak networks of hydrogen bonds (Giusti and Wrolstad 2003).

Table 47 Mean Polymeric Color Values of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	Polymeric Color
Control	6.6±1.0*abc*
RME100	7.4±1.4*a*
RME200	7.1±1.0*ab*
RME300	6.9±0.9*abc*
TA2.5	7.0±1.4*abc*
TA5.0	7.0±1.4*abc*
TA7.5	6.6±1.2*abc*
BCC100	7.3±1.5*a*
BCC200	6.8±1.5*abc*
BCC300	6.7±1.3*abc*
PSPC100	6.5±1.3*abc*
PSPC200	6.2±0.9*bc*
PSPC300	7.1±1.5*ab*
EMIQ25	6.7±1.5*abc*
EMIQ50	6.3±1.7*abc*
EMIQ75	6.0±0.9*c*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. *n*=24 analyses (±SD).

Although few differences to the polymeric color of elderberry tinctures were detected among copigment treatments, there were significant ($p \leq 0.05$) effects among storage time based on one-way ANOVA (Table 48). The average polymeric color of elderberry tinctures was 5.7, 6.5, 6.5, and 8.3 at 0, 2, 4, and 6 weeks of storage, among all copigment treatments (Figure 25). These results indicate that between 0 and 6 weeks of storage, there were noticeable increases to the amount of brown pigmentation within elderberry tinctures, regardless of copigment treatment. Additionally, there were sharp increases to polymeric color during the one week of accelerated temperature storage (32° C), which occurred between 5 and 6 weeks of storage. Strong inverse

correlations were noted between polymeric color and a* color (r=-0.46), as well as polymeric color and monomeric anthocyanins (r=-0.51). These relationships substantiate the belief that the red colored, anthocyanin pigments within the elderberry tinctures experienced observable degradations into colorless and brown colored anthocyanin-tannin pigments throughout storage time. It is also theorized that between 0 and 4 weeks of storage, the majority of polymerized anthocyanins occurred as colorless pigments, which subsequently degraded into brown colored pigments after 4 weeks. This theory is based on the evidence that although monomeric anthocyanins degraded linearly (R^2=0.9987) throughout storage, there were no significant changes to the values of a* color, color density, or polymeric color between 2 and 4 weeks of storage among elderberry tinctures. Additionally, a strong correlation (r=0.78) existed between polymeric color and color density among elderberry tinctures, which was expected due to the fact that both variables are measures of pigment degradation and depth of color.

Table 48 Polymeric Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	5.7±0.7*a*	7.4±0.5*a*	6.7±1.1*a*	6.5±1.3*a*
RME100	5.8±0.3*c*	6.8±0.7*bc*	7.8±0.3*ab*	9.1±1.2*a*
RME200	5.8±0.3*c*	7.3±0.4*b*	6.9±0.1*b*	8.4±0.7*a*
RME300	6.0±0.1*b*	6.7±0.2*ab*	7.2±1.0*ab*	7.8±1.0*a*
TA2.5	5.7±0.3*b*	6.6±0.8*b*	6.6±0.6*b*	9.0±0.9*a*
TA5.0	5.8±0.2*b*	6.1±0.7*b*	8.3±1.0*a*	7.7±1.6*ab*
TA7.5	5.8±0.0*b*	6.3±0.7*b*	6.0±1.2*b*	8.4±0.5*a*
BCC100	5.6±0.2*c*	6.8±1.0*bc*	8.0±1.2*ab*	8.9±0.5*a*
BCC200	5.7±0.5*b*	6.3±0.2*b*	5.8±0.5*b*	9.1±0.6*a*
BCC300	5.7±0.3*b*	6.1±0.9*b*	6.5±0.8*b*	8.7±0.3*a*
PSPC100	5.4±0.1*c*	6.8±0.6*b*	5.3±0.5*c*	8.3±0.6*a*
PSPC200	5.3±0.3*b*	6.0±0.3*ab*	6.7±0.6*a*	7.0±1.2*a*
PSPC300	5.7±0.4*c*	6.7±0.3*bc*	6.8±0.2*b*	9.3±1.0*a*
EMIQ25	5.5±0.1*b*	6.2±0.9*b*	6.0±1.0*b*	8.9±0.6*a*
EMIQ50	5.4±0.4*c*	7.0±0.5*b*	4.2±0.5*d*	8.6±0.5*a*
EMIQ75	5.5±0.6*b*	5.5±0.5*b*	5.7±0.6*b*	7.2±0.4*a*

Refer to Table 36 for treatment identification. Polymeric color values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 2 analyses.

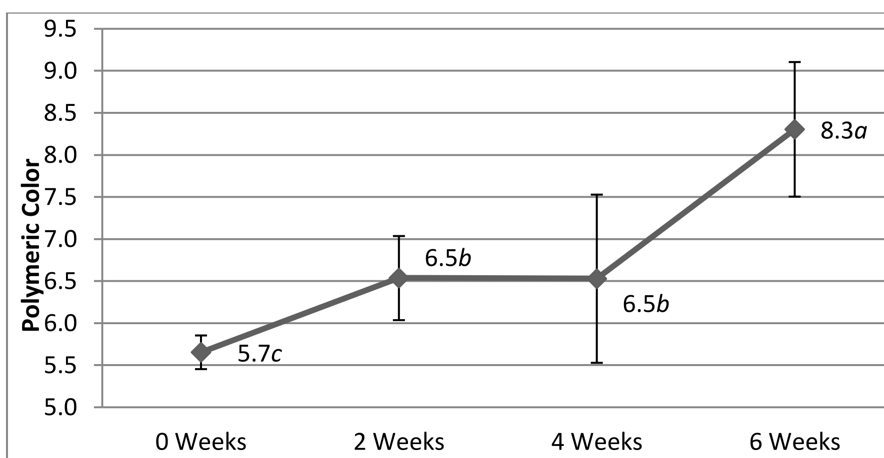


Figure 25 Mean polymeric color values among copigment treatments over storage time. Polymeric color values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. *n*=96 analyses (±SD).

Percent polymeric color is defined as the percentage of pigment contribution by tannins, or non-monomeric anthocyanins. It is simply calculated as polymeric color divided by color density, and provides additional insight into the browning characteristics of anthocyanin-rich beverages.

In this study, the average % polymeric color was 52.6%, regardless of copigment treatment or weeks of storage. Interestingly, elderberry tinctures which contained either black carrot or purple sweet potato color at the 200 mg/100mL or 300 mg/100mL levels (BCC200, BCC300, PSPC200, PSPC300) consistently exhibited the lowest % polymeric color throughout weeks of storage and overall, which were significantly ($p \leq 0.05$) lower than the control treatment based on one-way ANOVA (Table 49). Based on previous color density and polymeric color results, it was expected that BCC300 and PSPC300 treatments would exhibit the lowest % polymeric color values, however it was unexpected that the % polymeric color of BCC200 and PSPC200 treatments would be significantly lower than the control. Although there were no discernible effects to color density or polymeric color within the BCC200 and PSPC200 treatments, the analysis of % polymeric color indicated that there were observable effects to the depth of pigmentation within elderberry tinctures when either black carrot color or purple sweet potato color was added at a 200 mg/100mL level. As previously mentioned, the recommended usage level for either of these copigment additives is between 10-100 mg/mL within beverages. This study demonstrated that these copigment additives must be utilized in excess of their recommended upper usage levels of 100 mg/100mL in order to affect the color of elderberry tinctures. Adding more than what is

recommended may not be a cost-effective technique for increasing the depth of color within elderberry tinctures. Although it is unlikely that any of the copigment additives tested in this study truly caused effective copigmentation within elderberry tinctures, this result should not discourage their potential use within other anthocyanin-rich food and beverage systems. As previously mentioned, the lack of copigmentation within elderberry tinctures is most likely a result of the high ethanol content of the tinctures (30.1%), which likely prevented intermolecular interactions among elderberry anthocyanins and copigment additives (Mazza and Brouillard 1990, Brouillard and others 1991, Gutiérrez 2003).

Table 49 Mean Polymeric Color Values (%) of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	% Polymeric Color
Control	56.2±6.4*a*
RME100	55.5±5.4*abc*
RME200	54.4±6.4*abc*
RME300	55.9±5.6*ab*
TA2.5	55.0±7.8*abc*
TA5.0	53.2±7.4*abcd*
TA7.5	52.8±6.4*abcd*
BCC100	54.1±7.0*abc*
BCC200	50.9±6.0*bcd*
BCC300	48.8±7.2*d*
PSPC100	51.5±5.8*abcd*
PSPC200	50.6±5.5*cd*
PSPC300	48.3±6.6*d*
EMIQ25	51.0±6.8*abcd*
EMIQ50	52.8±6.9*abcd*
EMIQ75	50.7±5.7*cd*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=24 analyses (±SD).

Although few differences to the % polymeric color of elderberry tinctures were detected among copigment treatments, there were significant ($p \leq 0.05$) effects among storage time based on one-way ANOVA (Table 50). The average % polymeric color of elderberry tinctures was 46.3%, 51.7%, 52.3%, and 60.1% at 0, 2, 4, and 6 weeks of storage, among all copigment treatments (Figure 26). Regardless of copigment treatment, elderberry tinctures gained an average of 13.8% polymeric color, or pigment contribution by tannins, between 0 and 6 weeks of storage. Similar to polymeric color, these results indicate that elderberry tinctures experienced significant browning

between 0 weeks and 2 weeks of storage, and again between 4 weeks and 6 weeks of storage which included one week of accelerated temperature (32° C) storage.

Table 50 Percent Polymeric Color Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	49.8±1.1*c*	57.9±4.6*ab*	53.5±4.7*bc*	63.6±4.7*a*
RME100	49.1±0.4*c*	54.2±2.7*b*	56.5±3.9*b*	62.2±2.1*a*
RME200	47.6±2.3*c*	56.6±3.0*b*	50.6±2.1*c*	62.9±1.9*a*
RME300	49.9±1.0*b*	55.2±1.9*b*	56.0±4.0*ab*	62.4±5.6*a*
TA2.5	47.7±1.6*b*	51.9±5.2*b*	56.8±2.0*ab*	63.7±9.4*a*
TA5.0	49.6±1.9*a*	49.1±6.7*a*	57.6±3.1*a*	56.4±12.3*a*
TA7.5	48.8±2.0*b*	51.9±6.0*ab*	50.0±6.0*b*	60.6±4.6*a*
BCC100	44.2±1.1*b*	55.1±5.3*a*	56.6±2.9*a*	60.6±2.8*a*
BCC200	45.3±2.5*c*	51.4±2.2*b*	47.6±3.4*bc*	59.4±1.9*a*
BCC300	41.6±1.6*c*	44.5±3.9*c*	51.1±4.3*b*	57.9±3.0*a*
PSPC100	44.6±2.1*c*	50.3±2.3*bc*	52.3±4.6*b*	58.6±2.5*a*
PSPC200	42.9±1.4*c*	50.4±1.8*b*	51.9±2.3*b*	57.0±1.4*a*
PSPC300	41.4±2.4*c*	47.3±2.1*b*	46.4±1.9*b*	58.2±1.2*a*
EMIQ25	46.4±2.0*b*	46.8±4.4*b*	50.4±3.7*b*	60.6±4.9*a*
EMIQ50	44.9±1.1*b*	56.7±2.7*a*	49.1±4.6*b*	60.4±2.7*a*
EMIQ75	47.7±2.4*b*	47.7±5.0*b*	49.9±6.6*ab*	57.5±2.4*a*

Refer to Table 36 for treatment identification. Percent polymeric color values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 2 analyses.

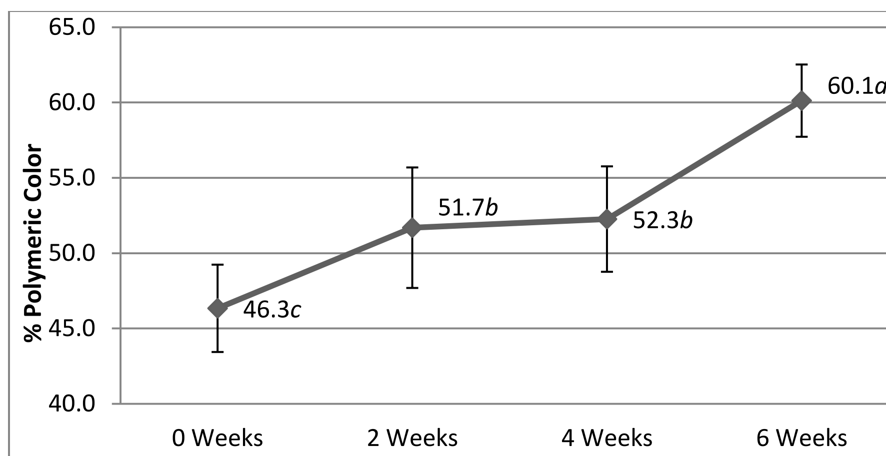


Figure 26 Mean polymeric color values (%) among copigment treatments over storage time.

Percent polymeric color values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=96 analyses (±SD).

Total Phenolics

Elderberry fruit is considered to be one of the richest sources of phenolic compounds among edible fruits and vegetables, containing approximate phenolic contents between 1,270-1,950 mg/100g fresh weight (Wu and others 2004, Rimpapa and others 2007). Casati and others (2012) reported that the phenolic contents of elderberry juice were 10,060 mg GAE/L, and 3.1-3.3 times higher than blueberry juice. The phenolic compounds in elderberry fruit have basically been identified as anthocyanins, tannins, proanthocyanidins, organic acids, and hydroxycinnamic acid derivatives (Lee and Finn 2007). These compounds are powerful antioxidants and have been implicated for their ability to reduce the risk of cardiovascular disease and cancer, improve visual acuity and cognition, as well as provide anti-inflammatory and immunostimulatory benefits (Özgen and others 2010). Additionally, phenolics contribute strongly to the bitter tastes of food and beverage products and contribute as

a preservative due to their natural antioxidant capacity (Proestos and others 2013). It was among the objectives of this study to determine how copigment additives and storage time affected the total phenolic content of elderberry tinctures.

In this study, total phenolics was determined using the Folin-Ciocalteu method based on a procedure by Velioglu and others (1998). The average total phenolics of elderberry tinctures was 3,804.1 mg GAE/L, regardless of copigment treatment or weeks of storage. These results are similar to those by Schmitzer and others (2010), who reported a phenolic content of elderberry wine to be 2004.1 mg GAE/L. Unfortunately, no significant ($p \leq 0.05$) effects were detected among copigment treatments based on one-way ANOVA (Table 51). At both 2 and 4 weeks of storage, the control treatment contained the least amount of phenolics among all of the copigment treatments, and contained the second lowest amount of phenolics overall (Table 51). Although not statistically significant, these results are likely due to the fact that all of the copigment additives initially contained phenolic compounds, which increased the phenolic contents of the tinctures compared to the control. Unfortunately, these results do not indicate effective copigmentation of elderberry anthocyanins within elderberry tinctures.

Table 51 Mean Total Phenolic Values (mg GAE/L) of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	Total Phenolics (mg GAE/L)
Control	3699.0±729.3*a*
RME100	3791.0±707.5*a*
RME200	3849.1±650.3*a*
RME300	3989.2±778.3*a*
TA2.5	3974.8±638.7*a*
TA5.0	3794.7±758.5*a*
TA7.5	3801.1±737.4*a*
BCC100	3824.3±585.1*a*
BCC200	3724.6±627.2*a*
BCC300	3713.3±579.7*a*
PSPC100	3848.7±563.7*a*
PSPC200	3917.3±792.6*a*
PSPC300	3879.8±704.3*a*
EMIQ25	3621.3±825.2*a*
EMIQ50	3723.1±708.1*a*
EMIQ75	3827.4±722.0*a*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=24 analyses (±SD).

Although no significant ($p \leq 0.05$) differences to the total phenolic content of elderberry tinctures were detected among copigment treatments, there were significant effects among storage time based on one-way ANOVA (Table 52). The average total phenolics of elderberry tinctures was 4454.1, 4129.5, 3869.3, and 2763.6 mg GAE/L at 0, 2, 4, and 6 weeks of storage, respectively, among all copigment treatments (Figure 27). Regardless of copigment treatment, elderberry tinctures lost an average of 38.0% total phenolics between 0 and 6 weeks of storage. These results are in agreement with Casati and others (2012), who reported an average of a 40% reduction of phenolic contents of elderberry juice throughout the first 30 days of storage at 40° C.

Correlations were detected between total phenolics and a* color (r=0.24), b* color (r=-0.37), total monomeric anthocyanins (r=0.75), color density (r=-0.30), polymeric color (r=-0.58), and % polymeric color (r=-0.60). Due to fact that anthocyanins are direct contributors to total phenolic content, it was expected that there would be relationships between total phenolics and color values within elderberry tinctures (Samee and others 2006). Additionally, the presence of some phenolic compounds, such as tannins, promote the formation of brown colored anthocyanin-tannin pigments. Interestingly, the control treatment contained the second lowest total phenolics overall and the lowest polymeric color at weeks 6 of storage, indicating that the presence of phenolics promotes browning within elderberry tinctures.

Table 52 Total Phenolic Values (mg GAE/L) of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	4496.9±146.6*a*	3829.7±778.8*ab*	3694.3±155.4*b*	2774.9±109.2*c*
RME100	4536.0±165.3*a*	4048.9±162.1*b*	3833.5±348.9*b*	2745.8±64.7*c*
RME200	4307.5±489.4*a*	4166.4±176.3*a*	4060.1±238.3*a*	2862.3±68.2*b*
RME300	4896.7±215.5*a*	4150.5±91.9*b*	4027.7±376.4*b*	2881.7±136.4*c*
TA2.5	4587.1±105.5*a*	4344.3±280.9*a*	3930.6±102.9*b*	3037.1±233.1*c*
TA5.0	4409.7±47.7*a*	4293.5±256.2*a*	3865.8±199.6*b*	2609.8±53.5*c*
TA7.5	4424.8±160.4*a*	4087.0±45.0*b*	4069.8±253.5*b*	2622.8±40.4*c*
BCC100	4415.7±49.7*a*	4166.4±83.2*b*	3768.7±39.2*c*	2946.5±105.0*d*
BCC200	4331.6±108.3*a*	4033.0±38.5*b*	3778.4±171.1*c*	2755.5±113.8*d*
BCC300	4061.0±136.6*a*	3940.9±78.0*a*	4047.1±351.0*a*	2804.0±184.6*b*
PSPC100	4466.8±60.0*a*	4121.9±99.5*b*	3765.5±185.9*c*	3040.4±154.3*d*
PSPC200	4899.7±393.7*a*	4109.2±138.8*b*	3807.6±231.7*b*	2852.6±75.4*c*
PSPC300	4427.8±15.6*a*	4207.7±200.8*ab*	4137.8±34.1*b*	2745.8±213.7*c*
EMIQ25	4397.7±159.4*a*	4125.1±131.5*b*	3250.8±87.6*c*	2257.0±68.7*d*
EMIQ50	4214.3±107.6*a*	4153.7±129.4*a*	3940.3±252.6*a*	2583.9±91.8*b*
EMIQ75	4391.7±86.0*a*	4293.5±147.9*a*	3930.6±230.1*b*	2694.0±196.4*c*

Refer to Table 36 for treatment identification. Total phenolic values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 2 analyses.

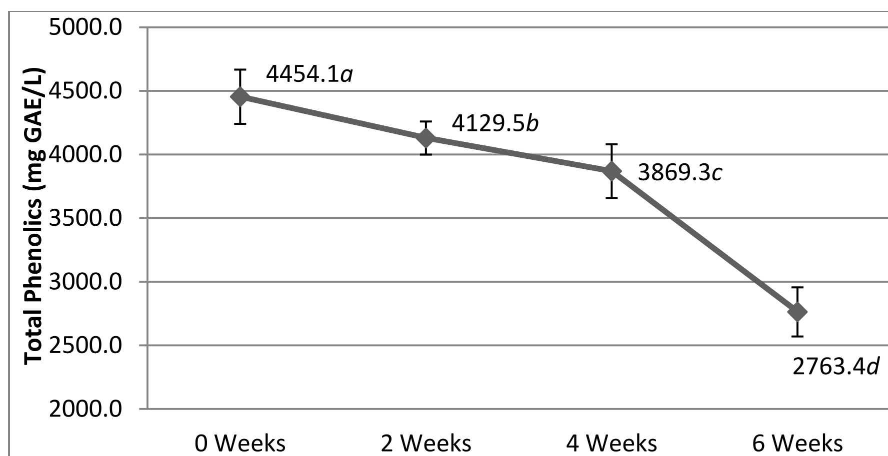


Figure 27 Mean total phenolic values (mg GAE/L) among copigment treatments by weeks of storage.

Total phenolic values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=96 analyses (±SD).

Antioxidants

The antioxidant activities of edible fruits and vegetables are directly related to their phenolic contents (Wu and others 2004, Rimpapa and others 2007). Considering that elderberry fruit is one of the richest sources of phenolic compounds, it is also among the most abundant in antioxidants (Wu and others 2004, Özgen and others 2010). Many of the healthful properties of elderberry fruit are a direct result of their high antioxidant activity. Generally, small, purple-black berries are very high in antioxidants, and have been implicated in holistic medicine for their curative properties (Bagchi and others 2004, Özgen and others 2010).

In this study, antioxidant capacity was determined by measuring the reaction of elderberry tinctures to 50 mL of a 0.1 mM DPPH solution, based on a procedure by Brand-Williams and others (1995), and was expressed as % inhibition of DPPH radical per 20 mg of tincture, IC_{50} of DPPH radical, and total antioxidants (µg TE/100 g). The

average % inhibition per 20 mg of tincture was 78.7%, regardless of copigment treatment or storage time. Notably, the three treatments which contained purple sweet potato color caused the greatest % inhibition of DPPH among all of the copigment treatments, and the control treatment displayed one of the lowest vales for % inhibition per 20 mg of tincture (Table 53). These results indicate that the addition of copigment additives may have resulted in limited improvements to the antioxidant capacity of elderberry tinctures, which is likely a result of the additives initially containing phenolic compounds. It is unlikely that the addition of copigment additives resulted in effective copigmentation, or that any improvements to the antioxidant capacity of elderberry tinctures could be attributed to copigmentation. Additionally, it should be noted that rosemary extract, black carrot color, and purple sweet potato color were added into elderberry tinctures at much higher levels than the other copigment additives tested in this study (Table 36), which provoked greater % inhibition of DPPH.

Table 53 Mean Inhibition of DPPH Radical per 20 mg of Elderberry Tinctures (%) by Copigment Treatment

Copigment Treatment	% Inhibition per 20 mg
Control	77.7±1.7*cd*
RME100	78.0±1.7*bcd*
RME200	79.2±1.4*abc*
RME300	79.9±1.8*a*
TA2.5	77.8±1.7*bcd*
TA5.0	77.3±2.9*d*
TA7.5	77.8±2.2*bcd*
BCC100	78.1±1.4*bcd*
BCC200	79.1±3.0*abcd*
BCC300	79.6±2.1*ab*
PSPC100	80.2±2.2*a*
PSPC200	80.2±0.9*a*
PSPC300	80.0±1.9*a*
EMIQ25	77.6±1.6*cd*
EMIQ50	77.7±2.4*cd*
EMIQ75	79.2±1.9*abcd*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=18 analyses (±SD).

In this study, no antioxidant data was reported at week 0 due to experimental error. Although few significant differences ($p \leq 0.05$) to % inhibition of DPPH were observed among weeks of storage within individual copigment treatments (Table 54). It was observed that there were decreases to % inhibition of DPPH during storage, and that there was a statistically significant decline of % inhibition of DPPH overall, based on one-way ANOVA (Figure 28). The average % inhibition of DPPH per 20 mg of tincture was 79.5%, 78.7%, and 77.9% at 2, 4, and 6 weeks of storage, among all copigment treatments (Figure 28). Correlations were observed between % inhibition of DPPH and a* color (r=0.36), monomeric anthocyanins (r=0.65), color density (r=-0.70), polymeric

color (r=-0.73), % polymeric color (r=-0.66), and total phenolics (r=0.79), indicating strong relationships among red color, phenolic content, and antioxidant capacity of elderberry tinctures. It is surmised that the loss of anthocyanins and phenolics within elderberry tinctures resulted in the decline of red color and antioxidant capacity throughout storage time.

Table 54 Percent Inhibition of DPPH Radical per 20 mg of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	.	79.2±1.1*a*	76.9±1.3*a*	77.0±2.0*a*
RME100	.	79.1±0.9*a*	77.9±1.4*a*	77.1±2.4*a*
RME200	.	80.3±0.6*a*	78.5±0.4*a*	78.9±2.3*a*
RME300	.	80.1±1.3*a*	80.6±2.1*a*	79.1±2.3*a*
TA2.5	.	78.2±0.5*a*	77.4±0.7*a*	77.7±3.2*a*
TA5.0	.	78.4±0.8*a*	79.6±2.0*a*	74.0±1.4*b*
TA7.5	.	77.6±3.1*a*	79.0±1.5*a*	76.7±1.8*a*
BCC100	.	79.1±0.3*a*	78.4±0.7*ab*	76.7±1.7*b*
BCC200	.	81.3±2.9*a*	78.7±0.5*a*	77.3±3.8*a*
BCC300	.	79.8±1.9*a*	79.5±0.9*a*	79.5±3.6*a*
PSPC100	.	80.6±1.2*a*	78.8±1.2*a*	81.3±3.3*a*
PSPC200	.	79.7±0.7*a*	80.1±0.1*a*	80.6±1.6*a*
PSPC300	.	81.0±0.7*a*	79.8±0.7*a*	79.3±3.2*a*
EMIQ25	.	78.9±1.1*a*	78.0±0.7*ab*	76.0±1.4*b*
EMIQ50	.	78.9±0.4*a*	77.8±0.4*a*	76.4±4.2*a*
EMIQ75	.	79.3±0.9*a*	78.8±0.9*a*	79.4±3.6*a*

Refer to Table 36 for treatment identification. Percent inhibition of DPPH radical values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 3 analyses.

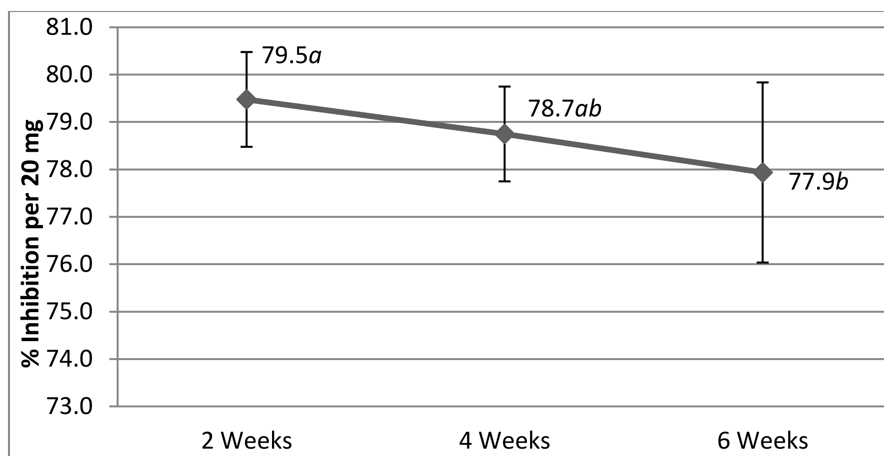


Figure 28 Mean inhibition of DPPH radical per 20 mg of elderberry tinctures (%) among copigment treatments over storage time.

Percent inhibition of DPPH radical values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=144 analyses (±SD).

A second way of measuring the antioxidant capacity of elderberry tinctures is by reporting the IC_{50} of DPPH radical. IC_{50} is calculated as the amount of tincture in milligrams required to cause 50% inhibition of a 50 mL, 0.1 mM DPPH solution during 4 hours of incubation at room temperature. In this study, the average IC_{50} (mg) of elderberry tinctures was 3.0 mg, regardless of copigment treatment or weeks of storage. Although only few significant differences ($p \leq 0.05$) were observed in regards to the IC_{50} of elderberry tinctures, based on one-way ANOVA, it was noted that there were step-wise decreases to the IC_{50} values as amounts of copigment additives increased within elderberry tinctures; and the control treatment exhibited one of the highest IC_{50} values (Table 55). Similar to previous antioxidant results, these results indicate that the copigment additives used in this study contributed to the antioxidant capacity of

elderberry tinctures, likely as a result of the increased inherent phenolic contents within the copigment additives.

Table 55 Mean IC_{50} (mg) of DPPH Radical Values of Elderberry Tinctures by Copigment Treatment

Copigment Treatment	IC_{50} (mg)
Control	3.7±0.9*ab*
RME100	3.3±1.2*abc*
RME200	2.9±0.8*bcd*
RME300	3.0±1.2*abcd*
TA2.5	3.8±1.1*ab*
TA5.0	4.0±1.2*a*
TA7.5	3.1±0.9*abc*
BCC100	3.1±1.2*abc*
BCC200	2.5±1.3*cd*
BCC300	2.3±0.9*cd*
PSPC100	3.0±1.1*bcd*
PSPC200	3.1±1.3*abcd*
PSPC300	2.6±0.9*cd*
EMIQ25	3.0±1.3*abcd*
EMIQ50	2.9±1.8*bcd*
EMIQ75	2.0±0.5*d*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different ($p \leq 0.05$) among copigment treatments based on one-way ANOVA. n=18 analyses (±SD).

Similar to previous antioxidant results, significant ($p \leq 0.05$) effects to the IC_{50} (mg) values of elderberry tinctures were detected throughout storage time, based on one-way ANOVA (Table 56). The IC_{50} of elderberry tinctures was 2.5 mg, 2.8 mg, and 3.8 mg at 2, 4, and 6 weeks of storage among all copigment treatments, respectively (Figure 29). These results demonstrate that greater amounts of tincture were required to provoke a 50% inhibition of DPPH as time progressed, and that there were losses to the

antioxidant capacity of elderberry tinctures throughout storage time. A moderate inverse correlation existed between IC_{50} and % inhibition of DPPH per 20 mg (r=-0.43), which was expected, due to both results demonstrating losses to the antioxidant capacity of elderberry tinctures throughout storage.

Table 56 IC_{50} (mg) of DPPH Radical Values of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	.	2.9±0.5*b*	3.8±0.6*ab*	4.4±0.9*a*
RME100	.	3.1±1.4*a*	3.3±0.6*a*	3.6±1.6*a*
RME200	.	3.0±1.0*a*	2.4±0.9*a*	3.2±0.4*a*
RME300	.	2.0±0.8*b*	3.0±1.4*ab*	4.0±0.3*a*
TA2.5	.	3.0±1.1*a*	3.6±0.6*a*	4.7±1.0*a*
TA5.0	.	3.1±0.4*b*	3.4±0.6*b*	5.6±0.4*a*
TA7.5	.	2.7±1.1*a*	2.8±0.9*a*	3.9±0.6*a*
BCC100	.	2.9±0.9*a*	2.6±1.1*a*	3.9±1.4*a*
BCC200	.	1.9±0.7*a*	2.6±0.8*a*	3.0±2.1*a*
BCC300	.	2.4±0.7*a*	1.6±0.1*a*	2.9±1.0*a*
PSPC100	.	2.3±0.3*a*	2.6±0.5*a*	3.9±1.4*a*
PSPC200	.	2.6±0.7*a*	3.2±1.8*a*	3.4±1.5*a*
PSPC300	.	2.1±0.6*a*	2.9±0.3*a*	2.8±1.4*a*
EMIQ25	.	1.8±0.3*a*	3.3±1.6*a*	3.8±1.0*a*
EMIQ50	.	1.6±0.5*b*	2.5±0.9*ab*	4.6±2.2*a*
EMIQ75	.	1.9±0.1*a*	1.8±0.2*a*	2.4±0.7*a*

Refer to Table 36 for treatment identification. IC_{50} of DPPH radical values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 3 analyses.

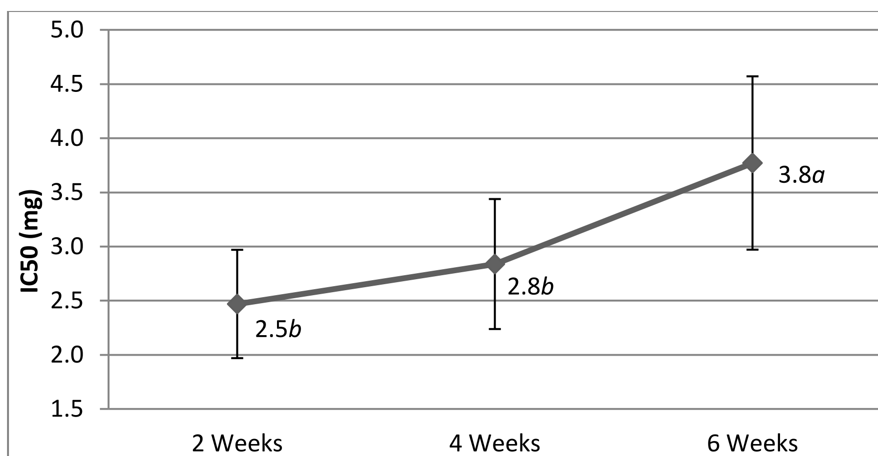


Figure 29 Mean IC_{50} (mg) of DPPH radical values among copigment treatments over storage time.

IC_{50} of DPPH radical values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=144 analyses (±SD).

In this study, average total antioxidants of elderberry tinctures was 40.1 micrograms of trolox equivalents per 100 g (µg TE/100 g), regardless of copigment treatment or weeks of storage. Interestingly, the control treatment contained the lowest amount of total antioxidants (29.3 µg TE/100 g), and treatments which contained copigment additives showed increases to total antioxidant levels as their concentration increased (Table 57). These results substantiate the fact that all of the copigment additives used in this study contained phenolic compounds, which contributed to the antioxidant capacity of elderberry tinctures. It is unlikely that these increases to antioxidant capacity were a result of effective copigmentation, as it was previously theorized that the high ethanol content (30.1%) of the tinctures disrupted intermolecular associations (Mazza and Brouillard 1990, Brouillard and others 1991, Gutiérrez 2003). Although there were observable differences among the total antioxidant levels of elderberry tinctures, many statistically significant ($p \leq 0.05$) effects

were not observed among copigment treatments due to relatively large variation in data. Regardless, it is believed that these data are presented with good accuracy and relatively good precision, due to the high amount of analyses among copigment treatments (*n*=18) and overall (*n*=448).

Table 57 Mean Total Antioxidant Values (µg TE/100 g) of Elderberry Tincture by Copigment Treatment

Copigment Treatment	Total Antioxidants (µg TE/100 g)
Control	29.3±6.8*cd*
RME100	35.3±15.5*bcd*
RME200	39.5±15.2*abcd*
RME300	42.0±23.2*abcd*
TA2.5	29.6±9.0*cd*
TA5.0	27.8±7.7*d*
TA7.5	36.9±15.7*bcd*
BCC100	38.0±16.9*abcd*
BCC200	49.5±19.3*ab*
BCC300	49.3±15.6*ab*
PSPC100	37.7±9.8*bcd*
PSPC200	41.6±23.4*abcd*
PSPC300	44.0±14.9*abc*
EMIQ25	40.8±16.5*abcd*
EMIQ50	46.9±23.9*ab*
EMIQ75	53.0±10.2*a*

Refer to Table 36 for treatment identification. Treatments not sharing the same letter are significantly different (p≤0.05) among copigment treatments based on one-way ANOVA. *n*=18 analyses (±SD).

Similar to the previous antioxidant results, significant (p≤0.05) losses of total antioxidant levels (µg TE/100 g) of elderberry tinctures were detected throughout storage, based on one-way ANOVA (Table 58). The total antioxidants of elderberry tinctures were 46.8, 41.9, and 31.6 µg TE/100 g at 2, 4, and 6 weeks of storage among all

copigment treatments (Figure 30). The majority of antioxidant loss within elderberry tinctures occurred between 2 and 4 weeks of storage, which was likely a result of one week of accelerated temperature (32° C) storage which occurred within that period of time. A strong inverse correlation was noted between total antioxidants and IC_{50} (r=-0.89), and moderate correlations were detected between total antioxidants and color, anthocyanin, and phenolic variables which were previously mentioned within this chapter.

Table 58 Total Antioxidant Values (µg TE/100 g) of Elderberry Tinctures by Copigment Treatment Over Storage Time

Copigment Treatment	0 Weeks	2 Weeks	4 Weeks	6 Weeks
Control	.	36.1±6.1*a*	27.6±3.8*ab*	24.2±4.7*b*
RME100	.	39.2±21.1*a*	31.6±5.3*a*	35.2±20.9*a*
RME200	.	37.1±10.4*a*	49.1±24.0*a*	32.4±4.4*a*
RME300	.	58.5±28.5*a*	41.7±23.1*a*	25.7±2.3*a*
TA2.5	.	36.7±11.2*a*	9.8±5.5*a*	22.4±4.2*a*
TA5.0	.	33.6±3.8*a*	31.2±5.2*b*	18.6±1.2*b*
TA7.5	.	44.2±23.2*a*	39.4±4.0*a*	27.1±4.5*a*
BCC100	.	38.2±11.5*a*	47.2±25.7*a*	28.5±9.5*a*
BCC200	.	59.5±19.0*a*	42.5±14.9*a*	46.6±25.8*a*
BCC300	.	44.8±13.4*a*	63.9±2.0*a*	39.3±17.0*a*
PSPC100	.	44.5±5.0*a*	40.3±7.8*ab*	28.4±9.4*b*
PSPC200	.	42.3±13.0*a*	48.1±40.9*a*	34.5±14.4*a*
PSPC300	.	52.9±18.1*a*	36.2±4.1*a*	42.8±18.1*a*
EMIQ25	.	58.1±9.6*a*	35.8±14.6*ab*	28.4±8.3*b*
EMIQ50	.	68.2±19.2*a*	46.3±21.3*ab*	26.0±11.1*b*
EMIQ75	.	54.4±1.7*a*	59.5±8.8*a*	45.1±13.3*a*

Refer to Table 36 for treatment identification. Total antioxidants values by weeks of storage not sharing the same letter are significantly different (p≤0.05) based on one-way ANOVA. Analysis at 6 Weeks includes one week of accelerated temperature storage (32° C). *n*=3 replicates (±SD), 3 analyses.

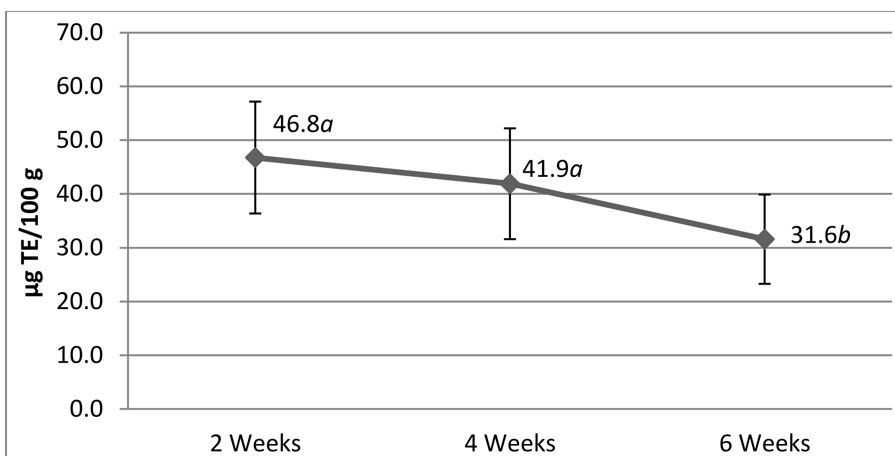


Figure 30 Mean total antioxidant values (µg TE/100 g) among copigment treatments over storage time.

Total antioxidant values by weeks of storage not sharing the same letter are significantly different ($p \leq 0.05$) based on one-way ANOVA. *n*=144 analyses (±SD).

Although the DPPH assay was effective for comparing the total antioxidants of elderberry tinctures among copigment treatments, caution is advised when comparing these data among research studies. Varying types and amounts of phenolic compounds occur within elderberry fruit based on the cultivar, growing conditions, degree of ripeness at harvest, and post-harvest storage and processing conditions of the fruit, which affects the reaction kinetics of the DPPH radical and can result in significant variability of data among studies (Brand-Williams 1995).

Conclusions

The elderberry tinctures which were tested in the previous study, entitled 'A Competitive Assessment of Commercial Elderberry (*Sambucus* sp.) Products', were identified as poor elderberry products, due to their high moisture/alcohol content, low phytochemical contents, poor color and nutrient stability, and relatively high costs.

Therefore, the major objective of this study was to evaluate the effectiveness of copigment additives on the color, phytochemical contents, and antioxidant capacity of elderberry tinctures throughout storage time. It was hypothesized that the copigment additives would complex with the natural elderberry anthocyanin pigments within the tinctures, resulting in enhanced color and phytochemical stability throughout storage. Although previous studies have evaluated the effectiveness of copigment additives on berry anthocyanins, no research has been conducted which evaluated copigmentation reactions within high alcohol anthocyanin systems, such as elderberry tinctures (Boulton 2001, Talcott and others 2003, Del Pozo-Insfran and others, Kammerer and others 2007).

Unfortunately, there are no results within this study which justify the effective copigmentation of elderberry tinctures by any of the copigment additives used in this study. However, some notable effects were observed among some copigment treatments. The additions of either black carrot color or purple sweet potato color at the 200 or 300 mg/100 mL levels resulted in significant ($p \leq 0.05$) effects to the L* color, b* color, monomeric anthocyanins, color density, and polymeric color of the elderberry tinctures. These two copigment additives were observed as dark purple in color, and likely contained substantial amounts of anthocyanins, which contributed to the lower L* and b* color values, higher anthocyanin content, and greater red and brown color depth of the elderberry tinctures. These effects are not attributed to effective copigmentation, and both black carrot color and purple sweet potato color were utilized between one and three times greater than their upper recommended usage levels (10-100 mg/100

mL) within juice products. Additionally, it was observed that all of the copigment additives (rosemary extract, tannic acid, black carrot color, purple sweet potato color, EM isoquercitrin) contributed to increased phenolic contents and antioxidant activities of the elderberry tinctures. The control treatment consistently displayed the lowest total phenolics and antioxidant values among the treatments, and few differences were observed among the tinctures which contained copigment additives. These results were expected considering that all of the copigment additives used in this study were phenolic compounds, and are typically effective copigments within low alcohol, anthocyanin-rich beverages. Unfortunately, these results do not signify the successful copigmentation of elderberry tinctures by any of the copigment additives, thus these additives were not utilized effectively within the tinctures. It is likely that the high ethanol content of the tinctures (30.1%) prevented, or dissociated, intermolecular complexation between the copigment additives and the elderberry fruit anthocyanins.

Many notable effects were observed to the dependent variables of the elderberry tinctures throughout 6 weeks of storage. Regardless of copigment treatment, significant ($p \leq 0.05$) losses of a* color (33% loss), monomeric anthocyanins (23% loss), total phenolics (38% loss), and total antioxidants (32% loss), and significant gains of color density (14% gain), and polymeric color (46% gain) were observed within the elderberry tinctures throughout 6 weeks of storage. These results demonstrated the susceptibility of anthocyanic pigmentation throughout storage, and indicate that copigment additives were not effective for enhancing the stability of elderberry tinctures. These results also showed an inverse relationship between anthocyanic

pigmentation and polymeric color, which signified that brown colored anthocyanin-tannin products formed as red colored anthocyanin pigments degraded. Furthermore, it is theorized that anthocyanins degraded into colorless products prior to converting into brown colored products, due to the lack of effects to a* color, b* color, color density, and polymeric color between 2 and 4 weeks of storage, despite the linear degradation of monomeric anthocyanins. Interestingly, elderberry tinctures which contained either black carrot color or purple sweet potato color at the 200 or 300 mg/100 mL levels consistently displayed the lowest % polymeric color throughout storage, compared to all of the other treatments tested in this study. These results indicate that these treatments had greater resistance to anthocyanin degradation, which is likely due to the acylated structure of the anthocyanins present within the black carrot and purple sweet potato color additives (Bąkowska-Barczak 2005). Additionally, the results of this study demonstrated the susceptibility of phenolics to high temperature storage. Notable effects were observed during the one week of accelerated temperature (32° C) storage, which occurred between 5 and 6 weeks, to the total phenolics, antioxidants, color density, and polymeric color of the elderberry tinctures. It is believed that the higher temperature storage accelerated the degradation of phenolic compounds, which subsequently lowered the antioxidant activity and increased the color depth of the tinctures.

Overall, the elderberry tinctures produced in this study were representative of retail elderberry products. Unfortunately, effective copigmentation of elderberry tinctures was not observed among any of the copigment additives, and tinctures cannot

be recommended as a valuable product form for the delivery of elderberry phytonutrients. It is theorized that alcohol prevents copigmentation, and promotes hydrolytic and oxidative degradation of anthocyanins due to low surface tension. Future research should investigate the use of copigment additives within low alcohol elderberry products, such as syrups, juices, or concentrates.

CHAPTER 4. OVERALL CONCLUSIONS

Elderberry (*Sambucus* sp.) fruit is renowned for its healthfulness and has been identified among previous research studies to aid in the prevention or treatment of illnesses such as influenza, rheumatism, malaria, respiratory syncytial virus, type 1 herpes, bladder or kidney infections, fever, edema, cancer, angiogenesis, and high cholesterol (Burge and others 1999, Youdim and others 2000, Barak and others 2001, Zakay-Rones and others 2004). Elderberry fruit has high antioxidant activity, and contains relatively large proportions of anthocyanins and other phenolic compounds, which contribute to its value as a natural food colorant in lieu of synthetic dyes or pigments. The healthfulness of elderberry fruit has been the major incentive for the production of value-added elderberry products, such as syrups, tinctures, concentrates, capsules, lozenges, dried elderberries, and powders. It was the objectives of the present study to analytically evaluate commercial elderberry products throughout accelerated temperature (32° C) storage, and to evaluate the effectiveness of copigment additives on the color and nutrient stability of elderberry tinctures throughout storage.

The majority of the commercial elderberry products tested in this study contained appreciable amounts of anthocyanins and other phytonutrients, which were generally in greater proportions than observed within raw elderberry fruit. However, very low levels of phytonutrients, and/or poor nutrient and color stability were observed within some of the value-added products. The elderberry tinctures tested in this study contained low levels of anthocyanins, proanthocyanidins, and sugars, high levels of moisture/alcohol, appreciable amounts of organic acids, and displayed poor

nutrient and color stability characteristics. Manufacturers of elderberry tinctures should reevaluate their processing techniques for elderberry fruit, and future research should be dedicated towards improving the tinctures' nutrient and color stability characteristics. Elderberry syrups represented a substantially better product compared to the tinctures tested in this study. The majority of the elderberry syrups displayed favorable nutrient and color stability characteristics, however, some of the syrups displayed poor characteristics, such as low phytochemical content or poor color. Although, the market for elderberry syrup is considered mature, these results signify that some of the leading commercial elderberry syrups could be improved upon. It may be beneficial for elderberry syrup manufactures to investigate enzymatic clarification, filtration, or encapsulation technologies to counter anthocyanic degradation effects. The elderberry capsules and lozenges tested in this study generally contained higher levels of phytonutrients, and displayed better nutrient and color stability than the elderberry syrups or tinctures. Additionally, the ability of the elderberry capsules to utilize pomace by-product, and the effective encapsulation of elderberry phytonutrients by the capsules and lozenges distinguishes both product forms for their value. The two elderberry products tested in this study which contained the highest concentrations of phytochemicals and anthocyanic pigmentation were Kerr Elderberry Concentrate and NP Nutra® Elderberry P.E. 10:1 powder. Both Kerr Elderberry Concentrate and NP Nutra® Elderberry P.E. 10:1 powder have great value-added potential as natural food colorants and phytochemical enhancers within wholesale food markets. Future product

development endeavors should develop value-added foods with the intention of exploiting the rich phytochemical contents of these two similar products.

Based on the poor performance of the elderberry tinctures which were analyzed in the first part of this research, it was the objective of the second study to evaluate the use of copigment additives (rosemary extract, tannic acid, black carrot color, purple sweet potato color, EM isoquercitrin) for the enhancement of phytochemical and color stability of elderberry tinctures. Unfortunately, the results of the second study did not demonstrate effective copigmentation among any of the copigment additives utilized within elderberry tinctures. It is believed that the high ethanol content (30.1%) of the tinctures prevented the pigments from forming a complex, which should have occurred between the copigment additives and the elderberry anthocyanins (Mazza and Brouillard 1990). Although, previous research studies have demonstrated effective copigmentation within berry wines, which typically contain 9%-21% ethanol, there are no studies which have evaluated copigmentation reactions within higher alcohol, anthocyanin-rich, beverage systems (Brouillard and others 1991, Gutiérrez 2003). Interestingly however, all of the copigment additives contributed to increased phenolic contents and antioxidant activity within the elderberry tinctures, and both black carrot color and purple sweet potato color additives affected the L*a*b* color values, monomeric anthocyanins, color density, and polymeric color of the tinctures. Additionally, this study provided insight into the degradation kinetics of elderberry tinctures throughout storage time. It is theorized that elderberry anthocyanins first degraded into colorless products prior to converting into brown tannin pigments, due to

the lack of effects on a*and b* color values, color density, and polymeric color between 2 and 4 weeks of storage, despite the linear degradation of monomeric anthocyanins which was observed throughout the entire 6 weeks of storage. Although, the elderberry tinctures produced in this study initially displayed positive nutrient and color characteristics, their decrease in phytochemical content and lack of color stability throughout storage signified an insufficient product form for the utilization of elderberry fruit. Future research studies should investigate the effectiveness of copigment additives within elderberry tinctures which contain lower ethanol contents (i.e. 26%-30%), and/or evaluate the effects of enzymatic clarification on copigmentation reactions.

Overall, this research is beneficial to consumers who desire to make informed elderberry product purchases, as well as to food or beverage processors who are looking to utilize elderberry or similar fruit crops. With the known healthfulness of elderberry fruit and the low market competitiveness of elderberry fruit products, there is great opportunity for the introduction of new elderberry products within the general consumer market (Cernusca and others 2011). Additionally, this research provides insight into the reaction kinetics of anthocyanin copigmentation, and should be beneficial to researchers who are attempting to develop anthocyanin-rich products with enhanced nutrient and color stability characteristics. Future research should be dedicated toward the development of a wider array of elderberry products and determining their acceptability by consumers.

REFERENCES

Anderson ØM, Jordheim M. 2006. The anthocyanins. In: Anderson ØM, Markham KR. (Eds.) Flavonoids Chemistry, Biochemistry and Applications. Boca Raton, FL: CRC Press. pp 471-551.

Anonymous. 2008. Noncitrus fruits and nuts 2007 summary. United States Department of Agriculture National Agricultural Statistics Service. pp 1-80. Available from: (http://usda.mannlib.cornell.edu/usda/nass/NoncFruiNu//2000s/2008/NoncFruiNu-07-08-2008_revision.pdf). Accessed November 2012.

Anonymous. 2009. Elderberry, raw. Department of Nutrition, National Food Institute, Danish Food Composition Databank – edition 7.01. Available from: (http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=0096). Accessed November 2012.

AOAC. 2005. Official methods of analysis. 18th ed. Gaithersburg, MD: AOAC International.

Arslan D. 2015. Effects of degradation preventative agents on storage stability of anthocyanins in sour cherry concentrate. Agronomy Research. 13(4):892-899.

Asen S. 1976. Known factors responsible for infinitive flower color variation. Acta Horticultura. 63:217-223.

Bagchi D, Sen CK, Bagchi M, Atalay M. 2004. Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. Biochemistry (Moscow). 69(1):75-80.

Bąkowska-Barczak A. 2005. Acylated anthocyanins as stable, natural food colorants - a review. Polish Journal of Food and Nutrition Sciences. 14/55(2):107-116.

Barak V, Halperin T, Kalickman I. 2001. The effect of Sambucol, a black elderberry-based, natural product, on the production of human cytokines: I. Inflammatory cytokines. European Cytokine Network. 12(2):290-296.

Barak V, Birkenfeld S, Halperin T, Kalickman I. 2002. The effect of herbal remedies on the production of human inflammatory and anti-inflammatory cytokines. Israel Medical Association Journal. 4:919-922.

Blochwitz M. 1677. Anatomia sambuci, or, The anatomy of the elder cutting out of it plain, approved, and specific remedies for most and chiefest maladies:, confirmed ... in Latine by Dr. Martin Blochwich .... EEBO Editions, Proquest. pp 1-264.

Borchers AT, Gershwin E, Keen CL, Stern JS. 2000. Inflammation and Native American medicine: The role of botanicals. American Journal of Clinical Nutrition. 72:339–347.

Borges G, Mullen W, Crozier A. 2010. Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. Food and Function. 1:73-83.

Boulton R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. American Journal of Enology and Viticulture. 52(2):67-87.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie. 28:25-30.

Brouillard R. 1982. Chemical structure of anthocyanins. In: Markakis P. (Ed.) Anthocyanins as food colors. London: Academic Press, Inc. pp 1-38.

Brouillard R. 1983. The in vivo expression of anthocyanin colour in plants. Phytochemistry. 22:1311-1323.

Brouillard R, Mazza G, Saad Z, Albrecht-Gary AM, Cheminat A. 1989. The copigmentation reaction of anthocyanins: a microprobe for the structural study of aqueous solutions. Journal of the American Chemical Society. 11(7):2604-2610.

Brouillard R, Wigand MC, Dangles O, Cheminat A. 1991. pH and solvent effects on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine derivatives. Journal of the Chemical Society, Perkin Transactions 2. 1235-1241.

Brown PN, Shipley PR. 2011. Determination of anthocyanins in cranberry fruit products by high-performance liquid chromatography with ultraviolet detection: single laboratory validation. Journal of AOAC International. 94(2):459-466.

Burge B, Mumcuoglu M, Simmons T. 1999. The effect of Sambucol on flu-like symptoms in chimpanzees: prophylactic and symptom-dependent treatment. International Zoo News. 46:16-19.

Byers PL, Thomas AL, Cernusca MM, Godsey LD, Gold MA. 2012. Growing and marketing elderberries in Missouri. The Center for Agroforestry, University of Missouri. pp 1-12. Available from: (http://www.centerforagroforestry.org/profit/ElderberryGuide.pdf). Accessed October 2012.

Cao G, Prior R. 1999. Anthocyanins are detected in human plasma after oral administration of an elderberry extract. Clinical Chemistry. 45(4):574-576.

Carlsen C, Stapelfeldt H. 1997. Light sensitivity of elderberry extract. Quantum yields for photodegradation in aqueous solution. Food Chemistry. 60(3):383-387.

Casati CB, Sánchez V, Baeza R, Magnani N, Evelson P, Zamora MC. 2012. Relationships between colour parameters, phenolic content and sensory changes of processed blueberry, elderberry and blackcurrant commercial juices. International Journal of Food Science and Technology. 47:1728-1736.

Cernusca M, Gold M, Godsey L. 2011. Elderberry market research: report based on research performed in 2009. The Center for Agroforestry, University of Missouri. pp 1-45. Available from: (http://www.centerforagroforestry.org/profit/elderberrymarketreport.pdf). Accessed October 2012.

Charlebois D. 2007. Elderberry as a medicinal plant. In: Janick J, Whipkey A. (Eds.) Issues in New Crops and New Uses. Alexandria, VA: ASHS Press. pp 284-292.

Chen L, De Borba B, Rohrer J. 2013. Determination of organic acids in fruit juices and wines by high-pressure IC. Thermo Fisher Scientific Application Note 1068. pp 1-10. Available from: (http://www.dionex.com/en-us/webdocs/114937-AN1068-IC-Organic-Acids-Fruit-Juices-Wines-AN70753_E.pdf). Accessed February 2016.

Cliff MA, King MC, Schlosser J. 2007. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. Food Research International. 40:92-100.

Clifford MN. 2000. Anthocyanins - nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture. 80:1063-1072.

Conn EE. 1979. Biosynthesis of cyanogenic glycosides. Naturwissenschaften. 66:28-34.

Cox RJ, Brokstad KA, Ogra P. 2004. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. Scandinavian Journal of Immunology. 59:1-15.

Cunningham DG, Vannozzi S, O'Shea E, Turk R. 2002. Analysis and standardization of cranberry products. In: Ho CT, Zheng QY. (Eds.) Quality Management of Nutraceuticals. Washington, DC: American Chemical Society. pp 151-166.

D'Souza N. 2006. Effect of dietary soybean meal and its components on the quality of rainbow trout fillets: isoflavone deposition and lipid oxidative status (Doctoral dissertation, University of Maine). 176 pp.

Del Pozo-Insfran D, Del Follo-Martinez A, Talcott ST, Brenes CH. 2007. Stability of copigmented anthocyanins and ascorbic acid in muscadine grape juice processed by high hydrostatic pressure. Journal of Food Science. 72(4):S247-S253.

Denev P, Ciz M, Ambrozova G, Lojek A, Yanakieva I, Kratchanova M. 2010. Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. Food Chemistry. 123:1055-1061.

Eiro MJ, Heinonen M. 2002. Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. Journal of Agricultural and Food Chemistry. 50:7461-7466.

Elisia I, Hu C, Popovich DG, Kitts DD. 2007. Antioxidant assessment of an anthocyanin-enriched blackberry extract. Food Chemistry. 101:1052-1058.

Feliciano RP, Shea MP, Shanmuganayagam D, Krueger CG, Howell AB, Reed JD. 2012. Comparison of isolated cranberry (*Vaccinium macrocarpon* Ait.) proanthocyanidins to catechin and procyanidins A2 and B2 for use as standards in the 4-(dimethylamino)cinnamaldehyde assay. Journal of Agricultural and Food Chemistry. 60(18):4578-4585.

Fernández-López JA, Angosto JM, Giménez PJ, León G. 2013. Thermal stability of selected natural red extracts used as food colorants. Plant Food for Human Nutrition. 68(1):11-17.

Ferreira D, Slade D, Marais JPJ. 2006. Flavans and proanthocyanidins. In: Anderson ØM, Markham KR. (Eds.) Flavonoids Chemistry, Biochemistry and Applications. Boca Raton, FL: CRC Press. pp 553-616.

Fitzgerald W, James KJ, Waibel BJ. 1999. Precision high pressure control assembly. U.S. Patent 5,888,050. Filed August 25, 1998. Issued March 30, 1999.

French J. 1651. The art of distillation—book I. Richard Cotes (Ed.), London.

Galić A, Dragović-Uzelac V, Levaj B, Bursać Kovačević D, Pliestić S, Arnautović S. 2009. The polyphenols stability, enzyme activity, and physio-chemical parameters during producing wild elderberry concentrated juice. Agriculturae Conspectus Scientificus. 74(3):181-186.

Garofulić IE, Ganić KK, Galić I, Dragović-Uzelac V, Savić Z. 2012. The influence of processing on physico-chemical parameters, phenolics, antioxidant activity and sensory attributes of elderberry (*Sambucus nigra* L.) fruit wine. Croatian Journal of Food Technology, Biotechnology and Nutrition. 7(Special Issue):9-13.

Giusti MM, Wrolstad RE. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Current Protocols in Food Analytical Chemistry. F1.1-F1.2.

Giusti MM, Wrolstad RE. 2003. Acylated anthocyanins from edible sources and their applications in food systems. Biochemical Engineering Journal. 14(3):217-225.

Gómez-Míguez M, González-Manzano S, Escribano-Bailón MT, Heredia FJ, Santos-Buelga C. 2006. Influence of different phenolic copigments on the color of malvidin-3-glucoside. Journal of Agricultural and Food Chemistry. 54(15):5422-5429.

González-Molina E, Gironés-Vilaplana A, Mena P, Moreno DA, García-Viguera C. 2012. New beverages of lemon juice with elderberry and grape concentrates as a source of bioactive compounds. Journal of Food Science. 77(6):C727-C733.

Gourdin GT, Richheimer SL, Tempesta MS, Baily DT, Nichols RL, Daugherty FJ, Freeberg DR. 2005. Efficient method for producing compositions enriched in total phenols. U.S. Patent 2005/0266104 A1. Filed July 12, 2005. Issued December 1, 2005.

Gu L, Kelm MA, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, Prior RL. 2002. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. Journal of Agricultural and Food Chemistry. 50:4852-4860.

Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL. 2004. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. The Journal of Nutrition. 134(3):613-617.

Gutiérrez IH. 2003. Influence of ethanol content on the extent of copigmentation in a Cencibel red wine. Journal of Agricultural and Food Chemistry. 51:4079-4083.

Hammerstone JF, Lazarus SA, Mitchell AE, Rucker R, Schmitz HH. 1999. Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. Journal of Agricultural and Food Chemistry. 47:490-496.

Han X, Shen T, Lou H. 2007. Dietary polyphenols and their biological significance. International Journal of Molecular Science. 8:950-988.

He F, Mu L, Yan GL, Liang NN, Pan QH, Wang J, Reeves MJ, Duan CQ. 2010. Biosynthesis of anthocyanins and their regulation in colored grapes. Molecules. 15:9057-9091.

He F, Liang N, Mu L, Pan Q, Wang J, Reeves MJ, Duan C. 2012. Anthocyanins and their variation in red wines I. monomeric anthocyanins and their color expression. Molecules. 17:1571-1601.

Howell AB, Reed JD, Krueger CG, Winterbottom R, Cunningham DG, Leahy M. 2005. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. Phytochemistry. 66(18):2281-2291.

Hubbermann EM, Heins A, Stöckmann H, Schwarz K. 2006. Influence of acids, salt, sugars and hydrocolloids on the colour stability of anthocyanin rich black currant and elderberry concentrates. European Food Research and Technology. 223:88-90.

Inami O, Tamura I, Kikuzaki H, Nakatani N. 1996. Stability of anthocyanins of *Sambucus canadensis* and *Sambucus nigra*. Journal of Agricultural and Food Chemistry. 44:3090-3096.

Jackobek L, Šeruga M, Medvidović-Kosanović M, Novak I. 2007. Anthocyanin content and antioxidant activity of various red juices. Deutsche Lebensmittel-Rundschau. 103(2):58-64.

Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM. 2008. Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. Journal of Agricultural and Food Chemistry. 56:9391-9398.

Johansen O, Andersen Ø, Nerdal W, Aksnes D. 1991. Cyanidin 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from fruits of *Sambucus canadensis*. Phytochemistry. 30(12):4137-4141.

Kaack K, Fretté X, Christensen L, Landbo AK, Meyer AS. 2008. Selection of elderberry (*Sambucus nigra L.*) genotypes best suited for the preparation of juice. European Food Research and Technology. 226:843-855.

Kammerer DR, Schillmőller S, Maier O, Schieber A, Carle R. 2007. Colour stability of canned strawberries using black carrot and elderberry juice concentrates as natural colourants. European Food Research and Technology. 224:667-669.

Kelm MA, Hammerstone JF, Schmitz HH. 2005. Identification and quantitation of flavanols and proanthocyanidins in foods: How good are the datas? Clinical and Developmental Immunology. 12(1):35-41.

King JW, Grabiel RD, Wightman JD. 2003. Subcritical water extraction of anthocyanins from fruit berry substrates. Proceedings of the 6th International Symposium on Supercritical Fluids - Tome 1, Versailles, France.

Kolodziej H. 1992. In: Hemingway RW, Laks PE. (Eds.) Plant Polyphenols: Synthesis, Properties, Significance. New York, NY: Plenum Press. pp 295-320.

Kühnau J. 1976. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Review of Nutrition and Dietetics. 24:117–191.

Kyle JAM, Duthie GG. 2006. Flavonoids in foods. In: Anderson ØM, Markham KR. (Eds.) Flavonoids Chemistry, Biochemistry and Applications. Boca Raton, FL: CRC Press. pp 219-262.

Lapornik B, Prošek M, Wondra AG. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering. 71:214-222.

Lazarus SA, Hammerstone JF, Adamson GE, Schmitz HH. 2001. High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in food and beverages. Methods in Enzymology. 335:46-57.

Lee J, Durst RW, Wrolstad RE. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International. 88(5):1269-1278.

Lee J, Finn C. 2007. Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. Journal of the Science of Food and Agriculture. 87:2665-2675.

Losey RJ, Stenholm N, Whereat-Phillips P, Vallianatos H. 2003. Exploring the use of red elderberry (*Sambucus racemosa*) fruit on the southern northwest coast of North America. Journal of Archaeological Science. 30:695-707.

Malien-Aubert C, Dangles O, Amiot MJ. 2001. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. Journal of Agricultural and Food Chemistry. 49:170-176.

Marais JPJ, Deavours B, Dixon RA, Ferreira D. 2006. The stereochemistry of flavonoids. In: Grotewold E. (Ed.) The Science of Flavonoids. New York, NY: Springer Science + Business Media, Inc. pp 1-46.

Martin CO, Mott SP. 1997. American elder (*Sambucus canadensis*) section 7.5.7, U.S. Army Corps of Engineers wildlife resources management manual. Department of the Army. Technical Report EL-97-14:1-18.

Mazza G, Brouillard R. 1990. The mechanism of co-pigmentation of anthocyanins in aqueous solutions. Phytochemistry. 28(4):1097-1102.

Merica E, Lungu M, Balan I, Matei M. 2006. Study on the chemical composition of *Sambucus nigra* L. essential oils and extracts. NutraCos. 5(1):25-27.

Mohebalian PM, Cernusca MM, Aguilar FX. 2012. Discovering Niche Markets for Elderberry Juice in the United States. HortTechnology. 22(4):556-566.

Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE. 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus*, and *Ribes*. Journal of Agricultural and Food Chemistry. 50:519–525.

Muldrew K, McGann LE. 1994. The osmotic rupture hypothesis of intracellular freezing injury. Biophysics Journal. 66(2):532-541.

Murugesan R. 2010. Enhancement of the antioxidant content of elderberry (*Sambucus nigra*) fruit by pulsed ultraviolet light followed by spray drying of the elderberry juice. A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Bioresource Engineering at McGill University, Ste Anne de Bellevue, Quebec. pp 1-129.

Murugesan R, Orsat V. 2011. Spray drying of elderberry (*Sambucus nigra* L.) juice to maintain its phenolic content. Drying Technology: An International Journal. 29(14):1729-1740.

Murugesan R, Orsat V, Lefsrud M. 2012. Effect of pulsed ultraviolet light on the total phenol content of elderberry (*Sambucus nigra*) fruit. Food and Nutrition Sciences. 3:774-783.

Nafisi-Movaghar K. 1991. Method of making dried fruits. U.S. Patent 5,000,927. Filed June 21, 1989. Issued March 19, 1991.

Nielson S. 2010. Food Analysis. Springer Science + Business Media. Springer, New York.

Özgen M, Scheerens JC, Reese RN, Miller RA. 2010. Total phenolic, anthocyanin contents and antioxidant capacity of selected elderberry (*Sambucus canadensis L.*) accessions. Pharmacognosy Magazine. 6(23):198-203.

Pérez-Jiménez J, Arranz S, Saura-Calixto F. 2009. Proanthocyanidin content in foods is largely underestimated in the literature data: An approach to quantification of the missing proanthocyanidins. Food Research International. 42:1381-1388.

Plank DW, Szpylka J, Sapirstein H, Woollard D, Zapf CM, Lee V, Liu RH, Tsao R, Düsterloh A, Baugh S. 2012. Determination of antioxidant activity in foods and beverages by reaction with 2,2'-diphenyl-1-picrylhydrazyl (DPPH): collaborative study first action 2012.04. Journal of AOAC International. 95(6):1562-1569.

Prior RL, Gu L. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry. 66:2264-2280.

Prior RL, Fan E, Ji H, Howell A, Nio C, Payne MJ, Reed J. 2010. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. Journal of the Science of Food and Agriculture. 90:1473-1478.

Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis P, Sinanoglu VJ. 2013. Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants. 2:11-22.

Rein M. 2005. Copigmentation reactions and color stability of berry anthocyanins (Doctoral dissertation, University of Helsinki). 88 pp.

Richmond ML, Brandao SCC, Gray JI, Markakis P, Stine CM. 1981. Analysis of simple sugars and sorbitol by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 29(1):4-7.

Rimpapa Z, Toromanovic J, Tahirovic I, Šapčanin A, Sofic E. 2007. Total content of phenols and anthocyanins in edible fruits from Bosnia. Bosnian Journal of Basic Medical Sciences. 7(2):119-122.

Rodríguez-Saona LE, Giusti MM, Wrolstad RE. 1999. Color and pigment stability of red radish and red-fleshed potato anthocyanins in juice model systems. Journal of Food Science. 64(3):451-456.

Rothwell JA, Pérez-Jiménez J, Neveu V, Medina-Ramon A, M'Hiri N, Garcia Lobato P, Manach C, Knox K, Eisner R, Wishart D, Scalbert A. 2013. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Available from: (http://www.phenol-explorer.eu/reports/39). Accessed October 2015.

Samee W, Engkalohakul M, Nebbua N, Direkrojanavuti P, Sornchaithawatwong C, Kamkaen N. 2006. Correlation analysis between total acid, total phenolic and ascorbic acid contents in fruit extracts and their antioxidant activities. The Pharmaceutical and Health Science Journal. 1(3):196-203.

Schmitzer V, Veberic R, Slatnar A, Stampar F. 2010. Elderberry (*Sambucus nigra* L.) wine: a product rich in health promoting compounds. Journal of Agricultural and Food Chemistry. 58:10143-10146.

Seabra IJ, Braga M, Batista M, Sousa H. 2010a. Fractioned high pressure extraction of anthocyanins from elderberry (*Sambucus nigra L.*) pomace. Food and Bioprocess Technology. 3:674-683.

Seabra IJ, Braga M, Batista M, Sousa H. 2010b. Effect of solvent (CO2/ethanol/H2O) on the fractionated enhanced solvent extraction of anthocyanins from elderberry pomace. Journal of Supercritical Fluids. 54:145-152.

Sesso HD, Gaziano JM, Liu S, Buring JE. 2003. Flavonoid intake and the risk of cardiovascular disease in women. The American Journal of Clinical Nutrition. 77:1400-1408.

Stevens M. 2001. Plant guide for common elderberry (*Sambucus nigra* L. ssp. *Canadensis* (L.) R. Bolli. USDA-National Resources Conservation Service, National Plant Data Center, Baton Rouge, LA. Available from: (http://plants.usda.gov/plantguide/pdf/cs_sanic4.pdf). Accessed June 2012.

Steyn WJ, Wand SJE, Holcroft DM, Jacobs G. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytologist. 155(3):349-361.

Stintzing FC, Carle R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends in Food Science and Technology. 15:19-38.

Strang J. 2012. Elderberry. University of Kentucky-College of Agriculture Cooperative Extension Service. Available from: (http://www.uky.edu/Ag/CDBREC/introsheets/elderberry.pdf). Accessed June 2012.

Talcott ST, Brenes CH, Pires DM, Del Pozo-Insfran D. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. Journal of Agricultural and Food Chemistry. 51:957-963.

Tay PY, Tan CP, Abas F, Yim HS, Ho CW. 2014. Assessment of extraction parameters on antioxidant capacity, polyphenol content, epigalocatechin gallate (EGCG), epicatechin gallate (ECG), and iriflophenone 3-C-*β*-glucoside of agarwood (*Aquilaria crassna*) young leaves. Molecules. 19:12304-12319.

Thole J, Kraft TB, Sueiro LA, Kang YH, Gills J, Cuendet M, Pezzuto J, Seigler D, Lila MA. 2006. A comparative evaluation of the anticancer properties of European and American elderberry fruits. Journal of Medicinal Food. 9(4):498-504.

Thomas AL, Byers PL, Gu S, Avery JD, Kaps M, Datta A, Fernando L, Grossi P, Rottinghaus G. 2015. Occurrence of polyphenols, organic acids, and sugars among diverse elderberry genotypes grown in three Missouri (USA) locations. Acta Horticulturae. 1061:147-154.

Turker N, Aksay S, Ekiz HI. 2004. Effect of storage temperature on the stability of anthocyanins of a fermented black carrot (*Daucus carota* var. L.) beverage: shalgam. Journal of Agriculture and Food Chemistry. 52:3807-3813.

Veberic R, Jakopic J, Stampar F, Schmitzer V. 2009. European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chemistry. 114(2):511-515.

Velioglu YS, Mazza G, Gao L, Oomah BD. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry. 46(10):4113-4117.

Vespalcova M, Grulichova H, Matejicek A, Kaplan J. 2011. Elderberry as a source of important flavonoid rutin. Vědecké Práce Ovocnářské. 22:243-251.

Waknine-Grinberg J, El-On J, Barak V, Barenholz Y, Golenser J. 2009. The immunomodulatory effect of Sambucol on leishmanial and malarial infections. Planta Medica. 75:581-586.

Waibel BJ, Morton WC, Cope SM. 2008. Dense gas means for extraction of a solute from solids. U.S. Patent 2008/0251454 A1. Filed March 11, 2008. Issued October 16, 2008.

Wallace TC, Giusti MM. 2010. Evaluation of Parameters that Affect the 4-Dimethylaminocinnamaldehyde Assay for Flavanols and Proanthocyanidins. Journal of Food Science. 75(7):C619-C625.

Whiting GC. 1958. The non-volatile organic acids of some berry fruits. Journal of the Science of Food and Agriculture. 9(4):244-248.

Wu X, Cao G, Prior R. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. The Journal of Nutrition. 132:1865-1871.

Wu X, Gu L, Prior RL, McKay S. 2004. Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. Journal of Agricultural and Food Chemistry. 52:7846-7856.

Wu X, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior R. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. Journal of Agricultural and Food Chemistry. 54:4069-4075.

Youdim K, Martin A, Joseph J. 2000. Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. Free Radical Biology and Medicine. 29(1):51-60.

Zakay-Rones Z, Thom E, Wollan T, Wadstein J. 2004. Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. The Journal of International Medical Research. 32:132-140.

BIOGRAPHY OF THE AUTHOR

Joseph A. Galetti was born in Quincy, Massachusetts in July of 1983. He was raised in Plymouth, Massachusetts and graduated from Plymouth North High School in 2001. Joseph attended Cape Cod Community College for a period of 2 years where he studied Liberal Arts. He then attended Johnson & Wales University where he received an Associate's degree in Culinary Arts in 2004 and a Bachelor's degree in Culinary/Nutrition in 2006. In 2008, Joseph enrolled at The University of Maine for graduate studies and obtained a Master's degree in Food Science and Human Nutrition in 2010. He has extensive employment experience within the fields of culinary arts, product development, quality assurance, research sciences, and analytical chemistry. Joseph is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from The University of Maine in May 2016.