

POLYPHENOLIC-RICH PRODUCTS MADE WITH GEORGIA-GROWN
RABBITEYE BLUEBERRIES

by

KATHRYN KITCHEN

(Under the Direction of William L. Kerr, PhD)

ABSTRACT

Blueberries contain fiber and bioactives, and therefore are a possible avenue for value-added products. One product, blueberry wine, is produced worldwide and associated with considerable anthocyanin and phenolics contents. However, these compounds may change during bottle storage. Total monomeric anthocyanin (TMA) content, total phenolics content (TPC), antioxidant activity, and color qualities of blueberry wine were investigated during six months of bottle storage. After 6 months storage, monomeric anthocyanins were almost complete gone, antioxidant activity slightly decreased, and phenolics content slightly rose at two months. Color density reduced and percent polymeric pigment increased. Monomeric anthocyanins likely polymerized with other compounds to create stable pigments. Blueberry juice processing, like wine-making, produces large amounts of waste (pomace) that contains fiber and polyphenols. Powder would be a more preferred form of blueberry pomace as a nutritional ingredient. Powders were produced using vacuum belt drying (VBD) and the effect of deseeding was investigated. VBD powders had similar TMA and TPC as freeze-dried

control powder when process at temperatures below 120°C but in much less time. Likely, this is because VBD allows drying at temperatures below 100°C. For both VBD and freeze-dried powders, deseeding decreased TMA and TPC. This is likely due to the loss of larger blueberry skin particles during the deseeding process. VBD pomace powder with seeds can be added into other products, such as baked goods, cereals and snacks, to improve the dietary profile. Extruded sorghum and pomace powder snacks were darker and more purple compared to the lighter, yellow control snack. The color is due to the anthocyanins naturally found within the pomace powder. Extruded sorghum and blueberry pomace snacks were less expanded, more dense, and harder than sorghum control, but still equally preferred by consumers. The snack with pomace powder had more total dietary fiber, anothcyanins, phenolics and radical scavenging capabilities when compared with control. The control snack had very little phenolics content and contained no anthocyanins. Overall, VBD can produce blueberry nutraecutical ingredients from wine and juice processing waste that can be used to improve the nutrition of food products.

INDEX WORDS: Blueberries, Blueberry Pomace, Blueberry Wine, Juice Processing Waste, Vacuum Belt Drying, Extruded Pomace

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RABBITEYE BLUEBERRIES

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DEDICATION

I dedicate this work to my husband, my mother, and my father for all the support they have given to me through this process.

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The importance of my parents' support cannot be described. My mother has always described me as the different one in my family. As a child, I never asked for permission since I simply investigated what I wanted, created a plan, and informed my parents of my decision. Patty and Manuel may not have always thought it the best, but they always supported me as I jumped head first into the next thing. My graduate studies

were no different. I have never been shy or embarrassed to proclaim how proud I am to be their daughter. I'd like to think I've made them proud.

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CHAPTER 1

INTRODUCTION

Blueberry and blueberry products are sources of both dietary fiber and polyphenols, which may help prevent chronic diseases states, such as cardiovascular disease, diabetes, obesity and cancer (Cho et al., 2013; Del Rio et al., 2013). Dietary fiber is the non-digestible carbohydrates and lignin intrinsic to and intact within plants. These include celluloses, hemicelluloses, pectins, gums, oligosaccharides and polysaccharides (Chawla & Patil, 2010). Phenolics encompass ~8000 compounds including flavonoids, phenolic acids, and procyanidins, and contain at least one hydroxylated aromatic ring (Robbins, 2003; Fennema, 1985). Because of these health promoting compounds, blueberries may be used to improve the nutritive profile of processed foods (Wang et al., 2012; Seeram, 2008).

The following research focuses on understanding the effects of storage on blueberry wine polyphenols, as well as the viability of dried blueberry pomace as a nutritional ingredient. This work is divided into seven chapters, including this introduction as chapter one. The second chapter is a wide-ranging literature review, highlighting blueberry polyphenols and dietary fiber, their health benefits, and processing effects. It also expounds upon research that used vacuum belt drying or investigated extruded snacks with pomace.

The third chapter explores the effects of short term bottle aging on blueberry wine. Blueberry wine has been shown to be a source of anthocyanins and phenolics and is

currently being produced worldwide (Johnson & Gonzalez de Mejia, 2012; Ortiz et al., 2013; Yang et al., 2012). Like red wine, the polyphenolic profile of blueberry wine changed in bottle storage. Monomeric anthocyanins decreased or completely disappeared while polymerized colored pigments and phenolics increased.

After producing wine, the authors noted a large percent of waste material composed of blueberry skins and seeds. Juice waste or pomace can account for as much as one third of the original fruit weight. Blueberry pomace has been reported to retain 15-55% of the original whole berry anthocyanin content and can be source of phenolic acids and procyanidins (O'Shea et al., 2012; Laroze et al., 2010; Brownmiller et al., 2008). The fourth chapter examines using vacuum belt drying to produce blueberry pomace powder as a possible nutraceutical ingredient. Fresh pomace is not feasible as an ingredient so a dried powder is preferable. Vacuum belt drying has produced apple pomace and muscadine pomace powders with similar anthocyanins and phenolics content as freeze-dried powder in considerable less time (Yan & Kerr, 2013; Vashisth et al., 2011). As seen with other pomaces, vacuum belt drying produced blueberry pomace powder with high retention of anthocyanins and total phenolics. Deseeding should be avoided as it reduced the anthocyanin and total phenolics content of the pomace powder.

In chapters five and six, the authors investigated the effectiveness of the vacuum belt dried blueberry powder as a nutraceutical ingredient in an extruded snack. There has been much interest in the addition of fruit and vegetable wastes in extruded products (Altan et al., 2008; Dar et al., 2008; Karkle et al., 2012; Khanal et al., 2009). Specifically, in chapter five, the physical affects and consumers' preferences for a sorghum and blueberry pomace extruded snack were evaluated. Pomace generally reduced expansion,

increased density, and made a harder product. The color went from yellow to more blue to purple, implying pomace powder may be a natural colorant. All extruded snacks were equally preferred and the color was considered a positive attribute to the participants.

Chapter six studied changes in the nutritional profile of the sorghum blueberry pomace extruded snack. Apple pomace successfully added dietary fiber to an extruded cereal, while cranberry pomace was a source of anthocyanins and procyanidins in an extrudate (Karkle et al., 2012; White et al., 2010) Blueberry pomace increased dietary fiber, anthocyanins, total phenolics content and antioxidant activity of the extruded snack compared to a sorghum only control. Vacuum dried blueberry pomace, not only was a naturally colorant, but became a source of polyphenols for the extruded snack.

The last chapter of this work reviews important findings of this dissertation and makes conclusions based on results in the other chapters. Also, there are suggestions for further research and ideas to continue work in this area.

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CHAPTER 2

LITERATURE REVIEW

Rabbiteye Blueberries

The genus *Vaccinium* is characterized as shrub plants that produce edible fruit, and includes cranberries, bilberries, lingonberries, and highbush, lowbush, and rabbiteye blueberries (*Vaccinium ashei*) (Johnson et al., 2010). Many commercial cultivars of rabbiteye blueberries (>20) have been developed from four native selections (Krewer & NeSmith, 2006; Aruna et al., 1993). These varieties are divided into three categories depending on time of ripening: early season, mid-season, or late season (NeSmith & Krewer, 2012). Two mid-season cultivars, ‘Tifblue’ and ‘Brightwell’ were selected for this investigation. ‘Tifblue’ was originally bred by crossing native selections ‘Ethel’ and ‘Clara,’ while ‘Brightwell’ was bred from crossing cultivars ‘Tifblue’ and ‘Menditoo,’ (Aruna et al., 1993). ‘Tifblue’ is one of the most popular cultivars produced in Georgia. ‘Tifblue’ consistently produces good crop yield with good berry size over multiple harvests. ‘Brightwell’ is considered the standard for testing, and is one of the more consistent cultivars for production (NeSmith, 2003; Ehlenfeldt & Kramer, 2012; Austin, 1994). Because of successful breeding efforts and marketing, rabbiteye blueberries have become a major commercial crop for the southeastern United States.

Market

Blueberry production has become a substantial United States (US) small fruit industry. In 2011, the US exported 115.5 million pounds of berries, which accounts for

about 15% of the total US production (Anon, 2013a). Georgia produces 12.7% of the total US blueberry production (Figure 2.1), and consists of three major species: northern highbush (*Vaccinium corymbosum*), southern highbush (*Vaccinium darrowii*), and rabbiteye varieties (Anon, 2013b; Krewer & NeSmith, 2006).

In Georgia, blueberries are native and have become a viable market commodity with rabbiteye accounting for more than 90% of the states acreage in 2000. Blueberries are Georgia's highest dollar market fruit, worth ~250 million annually, and accounts for 28% of Georgia's fruit and nut market. Since the 1950s, Georgia has experienced a steady increase in rabbiteye blueberry production for both fresh consumption and further processed products (Scherer & Krewer, 2003; Fonsah et al., 2011; Haire, 2013).

According to Fonsah et al. (2005), there are four major reasons for this explosive growth: (1) well-suited rabbiteye cultivars bred by the University of Georgia (UGA) breeding programs, (2) formation of the Georgia Blueberry Association Cooperation and first large-scale planting and packing facility in the 1970s, (3) expansion into the Michigan Blueberry Growers Association Cooperative and into domestic and export markets during the 1980s and 90s, and (4) development of new early-season southern highbush varieties (a cross between northern highbush and southern natives) in the mid-1990s. The market is continuing to grow as blueberries have been linked with improved health due to the presence of fiber and phytochemicals.

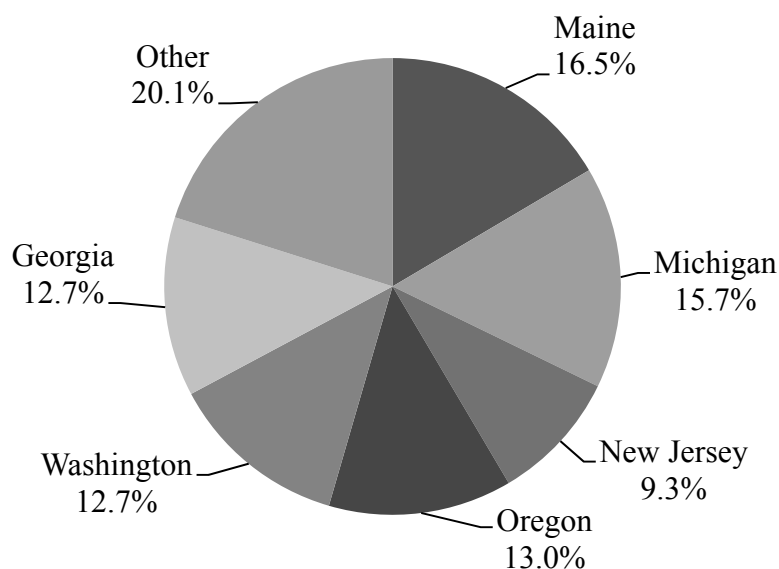


Figure 2.1: U.S. blueberry market by state

Fiber and Phytochemicals of Blueberries and Blueberry Pomace

Rabbiteye blueberries are rich in fiber and polyphenolics. Depending on the cultivar, research has found the presence of resveratrol, phenolic acids, organic acids, procyanidins and flavonoids (Wang et al., 2012; Seeram, 2008). Rabbiteye blueberries major phenolics include gallic acid, *p*-coumaric acid, ferulic acid, and ellagic acid. ‘Brightwell’ contained 3-4 mg/100 g fresh weight (FW) for gallic, *p*-coumaric, ferulic acids and 6 mg/100 g FW of ellagic acid. ‘Tifblue’ contained 260 mg/100 g FW gallic acid, 17 mg/100 g FW ferulic acid (Sellappan et al., 2002). Rabbiteye organic acid profile consisted of 4 major acids: citric, malic, succinic, quinic. Malic acid accounted for 68% of the total organic acid profile (Ehlenfeldt et al., 1994). Blueberries contain multiple flavonoids, such as epicatechin, quercetin, kaempferol and anthocyanins (Dulebohn et al., 2008).

Dietary Fiber

According to the Institute of Medicine (Anon, 2001), total fiber is a combination of added fiber and dietary fiber. Added fiber is isolated, non-digestible carbohydrates which may be beneficial to human health. Dietary fiber is non-digestible carbohydrates and lignin intrinsic to and intact within plants. These include celluloses, hemicelluloses, pectins, gums, and other oligosaccharides and polysaccharides associated with plants (Chawla & Patil, 2010). Depending on the fiber's solubility in water, the carbohydrates are separated into soluble or insoluble designations (Misner et al., 2013). Cellulose, some hemicelluloses and lignin are insoluble, and pentosans, pectins, gums and mucilage are soluble (Esposito et al., 2005)

Blueberries contain both soluble and insoluble fiber. Fresh blueberries contain 2.4 g/100 g FW total dietary fiber according to the USDA Database (2011), but larger amounts of total dietary fiber have been reported. Culled lowbush blueberries contained 3.67-5.04 g/100 g FW dietary fiber, with 0.27-0.54 g/100 g FW of it being pectin (Chen & Camire, 1997). Pectin is found within cell wall material and is made up of galacturonic acid-rich polysaccharides (Willats et al., 2001). Pectin can be either insoluble or soluble, but soluble pectin tends to decrease during maturity of blueberries due to the pectin methylesterase activity (Chen & Camire, 1997). Other dietary fiber found within the cell walls includes lignin, celluloses, and hemicelluloses (Marshall et al., 2006). Blueberry pomace, made up of seeds, skins and pulp, had 40.8 g/100 g dry weight (DW) dietary fiber. Dietary fiber polysaccharides accounted for 26.4 g/100 g of the total fiber content, and lignin was the other 14.4 g/100 g DW (Branning et al., 2009).

Phenolic Acids and Procyanidins

Blueberries contain phenolic acids and procyanidins. Phenolics, in the simplest form, are compounds containing a hydroxylated aromatic ring. There are ~8000 of these compounds occurring in nature (Robbins, 2003; Fennema, 1985). Blueberries vary in the total phenolics content (TPC) depending on cultivar and location. Commercial Korean blueberries contained 9.03 mg/g phenolics (Jeong et al., 2008). Moyer et al. (2001) determined Oregon rabbiteye blueberries had a TPC of 7.17-9.61 mg GAE/g. For rabbiteye blueberries grown in Nanjing, China, whole blueberries had a TPC of 26.94 mg GAE/g, while the pomace contained more phenolics by weight, 41.15 mg GAE/g (Li et al., 2013).

Phenolic acids are plant metabolites, and a specific group of phenolics containing a functional carboxyl group. Phenolic acids are formed from two main frameworks, hydroxycinnamic acid or hydroxybenzoic acid (Figure 2.2).

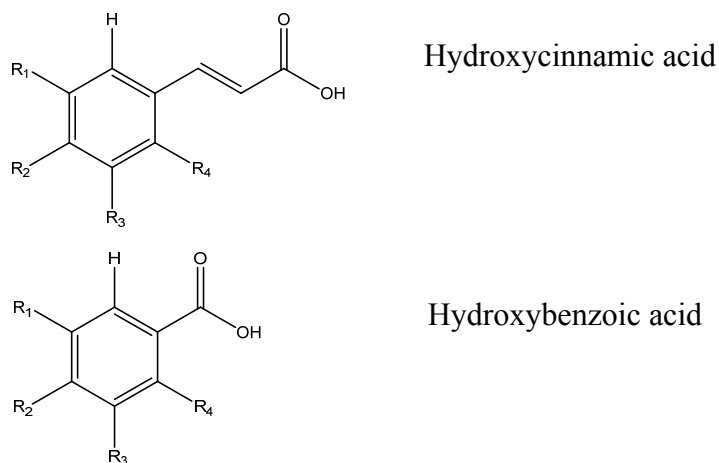


Figure 2.2: Basic structures of the two phenolic acid groups.

Common phenolic acids include cinnamic acid, *o*-, *p*-, and *m*-coumaric acid, ferulic acid, sinapic acid, caffeic acid, benzoic acid, vanillic acid, and gallic acid (Shahidi et al., 1992). For blueberries, the type of phenolic compounds and their concentration

varies with species, maturity and location. Northern highbush blueberries (*Vaccinium corymbosum*) were reported to contain caffeic acid (0.5-1.5 mg/100 g FW), ferulic acid (0.2-0.8 mg/100 g FW), and ellagic acid (<10.0 mg/100 g FW) (Häkkinen & Törrönen, 2000). One variety, *Vaccinium arctostaphylos* L., the most commonly found blueberry in Iran, contained high amounts of protocatechic acid (1456.4 ng/g FW), syringic acid (1350.4 ng/g FW), *p*-coumaric acid (3397.6 ng/g FW), and caffeic acid (3674.3 ng/g FW) (Ayaz et al., 2005).

Rabbiteye cultivars have been reported to have 10.3-68.6 mg/100 g FW chlorogenic acid, 1.53-258.9 mg/100 g FW gallic acid, 3.78-15.78 mg/100 g FW *p*-coumaric acid, and 3.02-16.97 mg/100 g FW. Some cultivars are also associated with *p*-hydroxybenzoic acid, caffeic acid, and ellagic acid. The cultivar 'Tifblue' was found to contain 21.9 mg/100 g FW chlorogenic acid, 258.9 mg/100 g FW gallic acid, and 16.97 mg/100 g FW ferulic acid, while 'Brightwell' had 20.3 mg/100 g FW chlorogenic acid, 4.03 mg/100 g FW gallic acid, 4.37 mg/100 g FW *p*-coumaric acid, 3.02 mg/100 g FW ferulic acid, and 6.02 mg/100 g FW ellagic acid (Wang et al., 2012; Sellappan et al., 2002).

While blueberries can be a high source of phenolic acids, they also contain other polyphenolics such as procyanidins. Procyanidins are members of the proanthocyanidins, or condensed tannins, class of polyphenolics (Fennema, 1985). Condensed tannins are formed by condensation reactions of flavanols to create carbon-carbon bonds between subunits (Schofield et al., 2001). Procyanidins are oligomers or polymers consisting of (+)-catechins and/or (-)-epicatechins units with either A-type linkages (C4-C8) or B-type linkages (C4-C8). Lowbush blueberries have been shown to contain procyanidins of

monomers, dimers, trimers, oligomers [4-10 degree of polymerization (DP)], and polymers (>10 DP) (Gu et al., 2002). Procyanidins are present mostly in the blueberry pulp and leaves (Riihinen et al., 2008). The rabbiteye cultivar 'Tifblue' was shown to contain 8 mg/kg DW total procyanidin content, which is greater than both lowbush (3 mg/kg DW) and northern highbush (6 mg/kg DW) varieties (Prior et al., 2001). Khanal et al. (2009b) discovered that in blueberry powder procyanidins were predominately polymers (~2508 mg/kg DW) while decamers and trimers were within 100-300 mg/kg DW. Monomers were present in the least amount (77 mg/kg DW). Blueberry pomace contained more monomer procyanidins (151 mg/kg DW) than whole blueberries (Khanal et al., 2009a).

Anthocyanins

The major class of flavonoids in rabbiteye blueberries is anthocyanins. In plants, anthocyanins act as red to blue pigments to attract pollinators and act as protectors from UV-B irradiation (Clifford, 2000). Anthocyanins are secondary metabolites produced from chalcone compounds (Springob et al., 2003). Like other flavonoids, the basic structure of an anthocyanin (Figure 2.3) consists of a diphenylpropane, C₆-C₃-C₆, with a positive oxygen ion at the C1 (Bravo, 1998; Castañeda-Ovando et al., 2009). The six major anthocyanidins found naturally in food are pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin (De Pascual-Teresa & Sanchez-Ballestra, 2008).

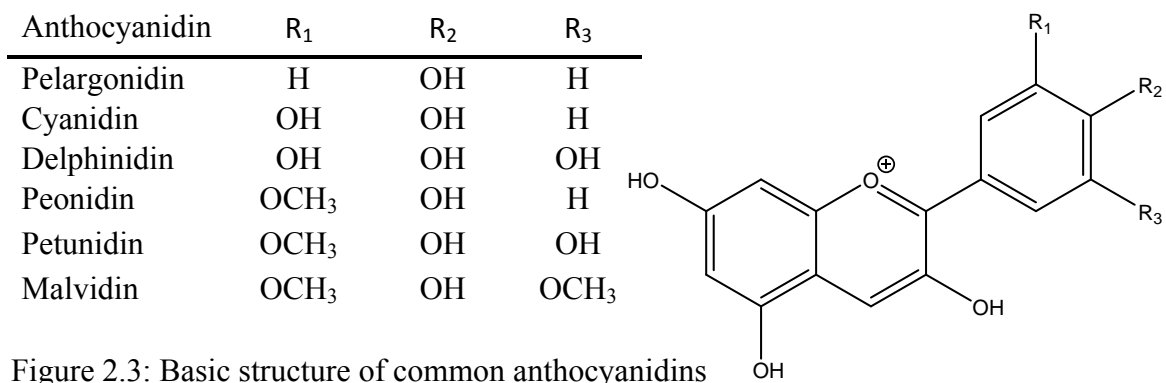


Figure 2.3: Basic structure of common anthocyanidins

The hydroxylated phenolic rings are responsible for anthocyanins' antioxidant capacities (Galleno et al., 2010). Antioxidants are compounds that inhibit or prevent the oxidation of materials by free radical species (Fennema, 1985). The phenolic hydrogens can act as hydrogen-donating radical scavengers to reduce reactive oxygen species. The anthocyanin radical formed after scavenging is stable (Rice-Evans et al., 1996). The antioxidant activity of anthocyanins is dependent on the number of hydroxyl groups around the benzene ring, with more groups resulting in greater antioxidant activity.

The structure is also important to the observed color of the anthocyanin. The pH of the environment dictates the ionic form of the anthocyanin, and thus the color exhibited. At pH 1.0, anthocyanins are in the flavylium cation form and display red colors. As pH increases to 4.5, the anthocyanins become either the carbonyl base or chalcone, which are colorless. As the pH further increases to 7.0, these compounds exist as the quinoidal base and are blue (Giusti & Wrolstad, 2001). Loss of color in elevated pH environments is prevented by anthocyanins forming complexes with other organic copigments (Wong, 1989).

Co-pigmentation occurs when pigments create molecular associations with other organic molecules, including proteins, phenolic acids, tannins, other flavonoids, and

polysaccharides within solution. Co-pigmentation complexes have been shown to increase the intensity of the exhibited color compared to the anthocyanin alone. The actual effect is dependent on the pigment, the co-pigment, the ratio between the two compounds, and the pH of the environment (Boulton, 2001; Fennema, 1985). For blueberries, co-pigmentation with aluminum is responsible for the characteristic blue color. When complexed with Al^{3+} , anthocyanins pass from the red flavylium form to the blue quinoidal form, while still being in a low pH (2-5) environment (Dangles et al, 1994; Elhabiri et al., 1997). Metal co-pigmentation only occurs with the glucosides of cyanidin, delphinidin, and petuidin as these anthocyanidins contain vicinal phenolic hydroxyl groups (two hydroxyl groups bonded to two adjacent carbon groups on the phenol ring) (Fennema, 1985). Glucosides of pelargonidin, peonidin, and malvidin complex with other co-pigment compounds such as flavonoids or phenolic acids (Osawa, 1982).

Blueberries have been shown to contain substantial amounts of anthocyanins, but variability exists between species, cultivars and locations. Blueberry anthocyanins are usually found in glycosylated forms, usually being acylated with glucosides, galactosides, or arabinosides (Li-Qiong et al., 2012). Georgia grown rabbiteye blueberry cultivars contained 12.70-197.34 mg/100 g FW total anthocyanin content. ‘Tifblue’ averaged 108.62 g/100 g FW and ‘Brightwell’ averaged 87.38 g/100g FW total anthocyanin content (Sellappan et al., 2002). Blueberries were recorded to have over 380 mg/g FW of anthocyanins with delphinidin (121 mg/g FW) and malvidin (131 mg/g FW) accounting for over 65% of the total anthocyanin content (Wu et al., 2006). Distribution of the individual anthocyanidin-glucosides tends to vary with species, cultivar and location as well. Rabbiteye blueberries grown in New Jersey contained 30.9-188 μ g/g FW

delphinidin-3-galactoside, 41.2-196 µg/g FW delphinidin-3-glucoside, 0.3-185 µg/g FW cyanidin-3-galactoside, 35.8-400 µg/g FW delphinidin-3-arabinoside, and 1.3-99.3 µg/g FW cyanidin-3-glucoside (Wang et al., 2012). Further work with 'Tifblue' cultured cells showed malvidin glycosides accounted for the greatest contribution to the anthocyanins content with 21% being malvidin-3-galactoside, 14% being malvidin-3-glucoside, and 15% being malvidin-3-arabinoside.

Health Benefits of Blueberries

Blueberries and blueberry products are a source of macronutrients, including fiber, and micronutrients such as phytochemicals and minerals (Ochmian et al., 2002). Being a source of fiber and phytochemicals has prompted interest in the health benefits associated with the consumption of blueberries and blueberry products. Blueberry consumption has been connected to reducing the risk of many disease states, such as cancer, cardiovascular disease (CVD), and type 2 diabetes (Muraki et al., 2013). Blueberries have also been associated with improved brain function (Renderiro et al., 2012).

Dietary Fiber

Dietary fiber (DF) may reduce the risk of cancer, CVD, type-2 diabetes, and obesity (Cho et al., 2013). Adequate fiber intake decreases low density lipoproteins (LDL) and total cholesterol levels, improves and regulates laxation, and promotes satiety (Marlett et al., 2002).

The Food and Drug Administration (FDA) has approved a health claim that consuming high fiber foods, including grains, fruits and vegetables, in conjunction with a low-fat diet may reduce the risk of some types of cancers (Anon, 2009). DF binds bile

acids and other potential toxins therefore diluting possible carcinogens within the gut, and also is the substrate for the production of short-chain fatty acids by gut microflora (Jacobs, 1986). The short-chain fatty acid, butyrate, in particular, has been linked with prevention of colon cancer by promoting cell death of cancer-type cells (Wong et al., 2006). DF is also connected with reduced risk for other cancers. For example, a meta-analysis of ten studies concluded high intake of DF reduced the risk of esophageal adenocarcinoma by 30% (Coleman et al., 2013).

The breakdown of soluble fiber into short chain fatty acids has led to the FDA denoting another fiber health claim. The consumption of soluble fiber, as part of a low cholesterol and low saturated fat diet, may reduce the risk of heart disease (Anon, 2009). It has been suggested that propionate, a metabolic product of fiber fermentation, is taken in by the liver and inhibits cholesterol synthesis, thereby reducing blood cholesterol levels and the risk of CVD (Wong et al., 2006; Law et al., 2003). According to a cohort study of Finnish males, consuming three more grams of DF a day reduced coronary death by 27%, with water soluble fibers having more effect than insoluble fibers (Pietinen et al., 1996).

Metabolic disease is another risk factor for CVD, and DF is thought to reduce the risk of type 2 diabetes. An epidemiological study following 35,988 Iowa women with a 6 year follow-up supported the hypothesis that the consumption of whole grains (a source of DF), and other sources of DF over time would be protective against developing type 2 (non-juvenile) diabetes in older women (Meyer et al., 2000). In a clinical study with diabetic patients, participants who were prescribed less than 40 units of insulin no longer needed medicine to regulate their diabetes after two weeks of a high fiber diet (Kiehm et

al., 1976). One theory of the effectiveness of DF is by aiding in weight management (obesity) and increasing satiety. However, there is mixed results in clinical studies. Thompson and others (2005) found no significant differences in weight loss between a high fiber/low glycemic index consumption group and a high dairy consumption group. In this study, meals were not strictly regulated so it was possible the high dietary fiber group was not truly consuming a low glycemic index diet.

Blueberry fiber, specifically, may be beneficial to intestinal health and reduce the risk of CVD. Gluglielmetti et al. (2013) found the consumption of beverages containing wild blueberry powder increased the concentration of *Bifidobacteria longum* subspecies *infantis* in the fecal material of the participants. Similarly, Vendrame et al. (2011) reported an increase in *Lactobacillus acidophilus* and *Bifidobacterium* spp. in fecal material when participants consumed blueberry-enhanced drink for six weeks.

Bifidobacteria and *Lactobacillus* spp. are part of the larger probiotics groups. Probiotic species are connected with promoting intestinal health by inhibiting pathogenic species that cause gastroenteritis, as well as stimulating immune functions (Ötles et al., 2003). Animal studies have also shown positive effects for a blueberry fiber diet. Combining probiotics and blueberry husks decreased the number of abnormal colonic and rectal lesions and ulcers, lowered acetic acid levels, increased propionic acid levels, and reduced the incidence of bacterial translocations to the liver in female Sprague-Dawley rats (Håkansson et al., 2009; Håkansson et al., 2012). Male Sprague-Dawley rats fed a combination of a high fructose and a 3% blueberry pomace diet had lower cholesterol (87.5-88.2 mg/dL) and triglycerides (90.6-105.8 mg/dL) levels compared to rats fed the control high fructose diet (114.6 mg/dL cholesterol, 145.0 mg/dL triglycerides) (Khanal

et al., 2012). Blueberry fiber may bind bile thereby reducing the absorption of cholesterol. Freeze dried blueberry DF bound 4.82 $\mu\text{mol}/100\text{ mg}$ bile acid in an *in vitro* bile acid binding procedure (Kahlon & Smith, 2007).

Antioxidants

Blueberries and blueberry products provide health-promoting procyanidins, phenolics, flavonoids and anthocyanins. Blueberry polyphenolics have been associated with reducing the risk of cancer and CVD, as well as improving brain health. *In vitro* research has supported the anticancer properties of blueberry polyphenolics. For example, Smith et al. (2000) found that bioactive fractions of spray dried wild blueberry extract, consisting of flavanols, anthocyanins, proanthocyanins, and phenolic acids, could be a potential inhibitor of the initiation stage of carcinogenesis. Similarly, polyphenolic extract from blueberries reduced breast cancer cell proliferation over 50% compared to the untreated control. The extract also attracted the cancerous cells and limited their migration, thereby preventing breast cells from becoming metastatic and spreading to other organs (Faria et al., 2010). Unlike *in vitro* studies, animal studies have had mixed results. Whole blueberry powder was fed as part of the diet to female nude mice at 5 and 10%. Breast tissue tumor volume was 75% lower than control for the mice fed 5% blueberry powder, and the tumor volume was 60% lower than control for mice fed a 10% blueberry powder diet (Adams et al., 2011). In contrast, an animal study using male and female offspring from Sprague-Dawley rats who were fed a 10% dried blueberry powder diet had no less azoxymethane-induced tumors (29.7-41.1 tumors for males, 9.5-16.2 tumors for females) within the colon than the control offspring group (Simmen et al.,

2009). The differences observed between the animal studies suggest blueberry polyphenolics are not equally effective towards all types of cancers.

Blueberries have also been shown to reduce risk factors of CVD. CVD is influenced by several factors such as blood lipid levels, blood pressure, diabetes type state, and obesity (NIH, 2011). Individuals who smoked at least one pack of cigarettes a day are a high risk population for CVD. Participants from this population had a significant decrease in serum lipid hydroperoxides after consuming whole blueberries. By decreasing the lipid hydroperoxides, the consumption of blueberries may have also decreased oxidative stress in the body (McAnulty et al., 2005). Oxidative stress has been related to CVD issues including clot formation and stroke (Daien et al., 2013). In a single-blind crossover study, men who consumed freeze dried blueberry powder with a higher fat diet had higher levels of serum antioxidant levels in the blood compared to a control group (Kay & Holub, 2002; Mazza et al., 2002). Oxidative stress may be reduced by higher antioxidant status within the blood. Male *KK-A^y* mice who consumed blueberry extracts, a high anthocyanin source, had decreased serum glucose, increased insulin tolerance, and lower liver weight over a five week feed study compared control mice. Such results could have significant implications for the prevention of type 2 diabetes (Takikawa et al., 2010).

In animal studies, an interesting relationship has been shown between blueberries and improved mental health, specifically for memory. Renderio et al., (2012) fed 8-week old Lister hooded rats a blueberry supplementation diet (2% blueberry powder) over 7 weeks. Rats who consumed the supplemented diet made more correct choices, had a faster speed of learning and took less time to complete tasks than the control rats (those

without a supplemented diet). The supplemented group also had elevated levels of hippocampal response proteins. The hippocampus is the area of the brain that creates long-term memory. Memory formation declines with age, and antioxidants may ameliorate some of these issues. Aged Fischer 344 rats fed a blueberry extract-enriched diet exhibited similar NMDA receptor-dependent long-term potentiation (LTP) in the hippocampus, which is associated with memory formation, as young rats (Coultrap et al., 2008).

Processing and Antioxidants

Blueberries are not always consumed fresh, but instead are used as ingredients in many processed products. As polyphenolics may have health-promoting capabilities, thermal and non-thermal processing effects on these compounds have become a major area of research. Flavonoids, such as anthocyanins in blueberries, have been found to be heat sensitive; thereby making non-thermal processing preferable for antioxidant preservation (Van der Sluis et al., 2005). Wine production is done at ambient temperatures (20-25°C) so is considered protective of heat labile compounds, but changes may occur in the phenolics profile during storage. Processes such as drying and extrusion cooking use heat, which may cause them to be detrimental to antioxidants within the food matrix.

Wine Production

Wine making is traditionally a non-thermal method, relying on low pH and ethanol for shelf stability, which tends to be a positive environment for phytochemicals. Often, wine is stored for long periods of time (>1 year) before consumption, so polyphenolics initially present in the wine may reduce or change during bottle aging.

Traditional wine made from grapes (*Vitis vinifera*) is associated with high antioxidant content. Analysis has determined red wine has a combination of anthocyanins and phenolic acids. Major anthocyanins include delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside, while major phenolic acids include caffeic, *p*-coumaric, ferulic, vanillic, benzoic, gallic and protocatechic acids (Baldi, 1997).

Total phenolics content has been shown to increase in wines during storage. Depending on the processing method, red wines have been associated with 1661-2391 mg GAE/L of phenolics after pressing and 1952-2503 mg GAE/L of phenolics after 1 year of bottle aging (Baiano et al., 2009). Like traditional red grape wines, fruit wines are coming into focus as a source of antioxidants. Fruit wines are produced much the same as traditional wine as a must is formed and then fermented. Fruit wines come from a variety of sources, and therefore may be a source of a variety of flavonoids or other phytochemicals. Comparatively, commercially available elderberry, blueberry, or black current wines were found to have no significant difference in total phenolics compared to commercially available red wines (Rupasinghe & Clegg, 2007).

Blueberry wine may be a high anthocyanin source alternative to traditional grape wine. Commercial blueberry wines from Illinois contained 966.7-2510.8 mg EAE/L of phenolics and 12.72-37.29 mg/L of total anthocyanins, while blueberry wine (*Vaccinium floribundum* Kunth.) produced in Ecuador contained 1090 mg GAE/L total phenolics and 8 mg/L total anthocyanins (Johnson & Gonzalez ed Mejia, 2012; Ortiz et al., 2013). Blueberry wines produced in New Jersey had 14.7-162.2 mg C3G/L anthocyanins, and 600-1860 mg GAE/L phenolics (Sánchez-Moreno et al., 2003). Southern highbush

blueberry wine contain 929 mg GAE/L phenolics, 60.62 mg C3G/L anthocyanins, and 1233 mg CE/L flavonoids (Yang et al., 2012). After fermentation with skins, blueberry wine (9.96 mg ACY/100ml) contained about twice as much anthocyanins content as fresh blueberry juice (4.52 mg ACY/100 ml) and 28.3 mg TPH/100 ml more total phenolics content. The larger concentration of polyphenolics can be attributed to larger contact with the skins (Su & Chien, 2007). As observed with other wines, bottle storage may change the polyphenolic profile of blueberry wine.

Bottle aging has been shown to be significant in the bioactive profile of traditional and fruit wine. Bottle aging affects both wine color intensity and antioxidant activity. Non-polymerized compounds in the wine, such as monomeric anthocyanins, have been found to decrease, while polymerized compounds increase during storage (Burin et al., 2011). A 72% reduction of anthocyanins and a 42% increase in procyanidins have been observed in red wines during one year of storage (García-Falcón et al., 2007). Reduction of monomeric anthocyanins was likely due to interactions with other compounds. Anthocyanins in red wine complex with polymeric phenolic tannins, which stabilizes color (Singleton & Trousdale, 1992). Fruit wines also exhibited loss of phytochemical compounds during bottle aging. After three years of bottle aging in the dark at room temperature, elderberry wine experienced a significant reduction in total phenolics (21%) and total anthocyanins (94%), as well as chlorogenic acid (52%), quercetin-rutinoside (26%), and quercetin-glucoside (41%) (Schmitzer et al., 2010). Litchi wine also experienced almost complete or complete loss in volatiles, including acetates and acids, after only 5 months of bottle storage (Wu et al., 2011).

Drying

Drying is the removal of water from the food matrix to a desired moisture content and/or water activity. The effect of drying on antioxidant chemicals is dependent on the type of drying, material being dried, as well as drying conditions. Typically, drying is destructive of phenolic compounds due to heat delivered at relatively high temperatures. One exception is freeze-drying, which operates at fairly low temperatures, and thus is considered the best method to retain polyphenolics in plant material while producing a high quality dried material. Chlorophyll, ascorbic acid, niacin, riboflavin and carotenoids were all significantly reduced in tea during hot-air drying, but freeze-dried material retained higher values of each phytochemical compared to hot-air dried material (Mahanom et al., 1999). Destruction of phytochemicals during drying has also been observed in fruits and vegetables. Freeze-dried raspberries retained significantly higher anthocyanins, phenolics, and antioxidant activity compared to air dried raspberries (Sablani et al., 2011). Freeze-dried persimmons had similar ferulic, gallic, protocatechuic, vanillic, and *p*-coumaric acids as fresh persimmons (Jung et al., 2005). Freeze drying is also considered the gold standard for dried blueberries because it retains the highest total phenolics and anthocyanin contents, while hot-air is considered the most detrimental to both groups of compounds (Mejia-Meza et al., 2008; Lohachoompol & Sczednicki, 2004).

As with whole fruits or vegetables, there is growing interest in methods for preserving fruit and vegetable processing waste. Processing wastes includes seeds, skins, peels and rinds, which may contain beneficial phytonutrients for human health. By drying these materials, the industry may be able to form novel functional ingredients. As seen

with whole fruits and vegetables, drying has been detrimental to the polyphenolic profile of pomaces. Air dried grape peels from wine making processed at 60°C for over 8 h contained similar polyphenols and condensed tannins as the freeze-dried grape pomace, but when processed at 100°C or 140°C for 3.5 and 3 h, respectively, the air-dried pomace contained 18.6-32.6% less polyphenols and 11.1-16.7% less condensed tannins than the freeze-dried material (Larrauri et al., 1997). Blueberry pomace had a 52% decrease in anthocyanins and lower levels of procyanidins when heated to temperatures in excess of 100°C (Khanal et al., 2010). Low temperature becomes vital for polyphenolic preservation when drying pomace, but may be less efficient as drying times become lengthy.

Vacuum Belt Drying

Continuous vacuum belt drying (Figure 2.4) produces food products with good physical and nutritional properties. Vacuum belt dryers consist of a stainless steel chamber with one or more internal conveying belts. The belt(s) conveys material over multiple heating zones, and at least one cooling zone, into a collection vessel. The temperatures of the heating zones can each be set individually so multiple profiles can be used. Heating is by conduction, but additional radiation heat sources can be employed. Vacuum is produced by a pump system and the dryer is typically designed for 5-40 mbar vacuum (Burmester & Eggers, 2012). While using similar operating pressures, vacuum belt drying (VBD) differs from traditional vacuum drying in that product can be continuously run through the system, while the latter operates in a batch process (Liu et al., 2009; Burmester, 2012). In addition, combinations of temperatures and belt speed can be employed to better optimize the drying process.

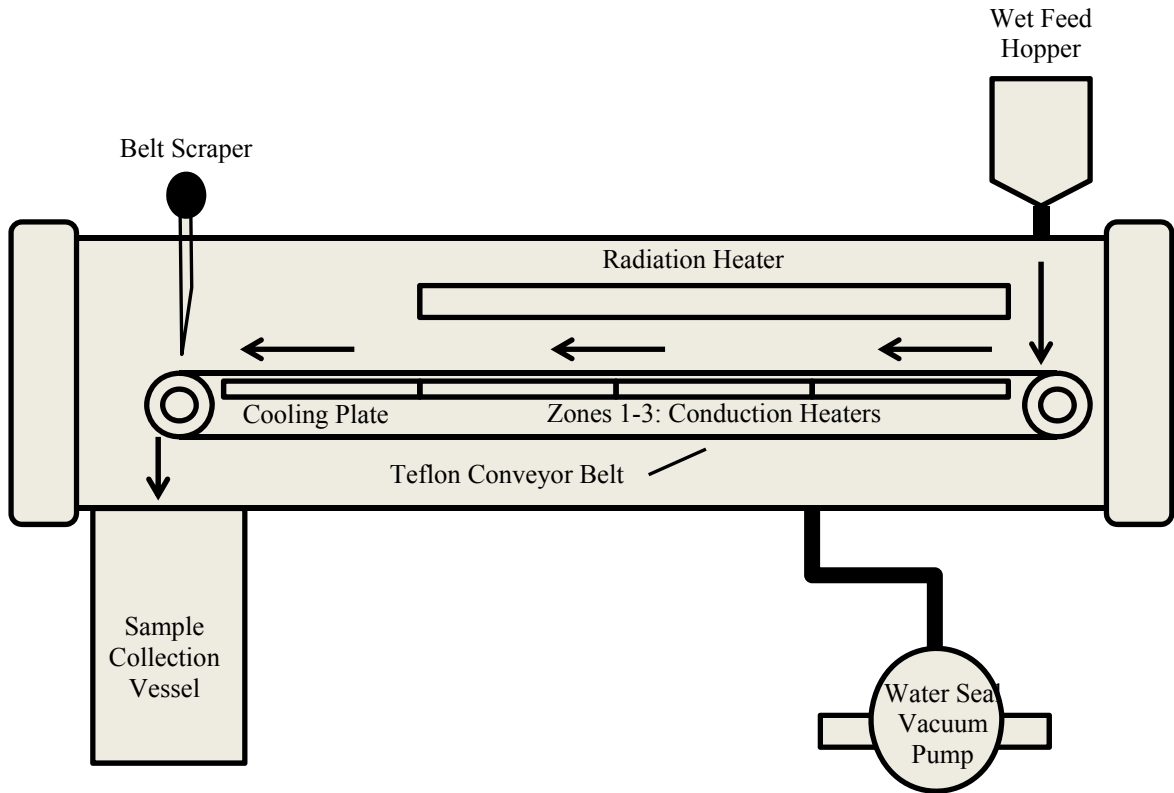


Figure 2.4: A pilot scale continuous vacuum belt dryer. A belt within a stainless steel chamber conveys material over multiple heating zones and a cooling zone into a collection vessel. A radiation heater may also be used to increase drying rate.

VBD combines conductive and radiant heating to dry heat sensitive foods of high viscosity in a short period of time (Hayashi et al., 1983). The decreased pressure creates low moisture potential in the surrounding air, increasing the driving force for moisture transfer away from the product. In some cases, the pressure may be low enough that water in the product may begin to boil. The relatively rapid phase change of liquid water to vapor can help keep the product temperature low through evaporative cooling, at least in initial stages of drying. In addition, lower drying temperatures are possible to drive the transformation. One drawback is that the rarified air does not contribute much to

convective heating. In some cases, this can make it harder to get uniform heating across a product.

VBD research has focused on liquid foods, fruits, vegetables and food extracts. This drying method has been suggested for making juice powders because it makes comparable products to freeze-drying with lower costs, and without the need for additives (Monzini & Maltini, 1990). VBD has also been shown to have a high drying rate when creating extracts (Zeng et al., 2006). Dried *Panax notoginseng* extract was made with multiple drying techniques, including vacuum belt drying, spray drying, freeze drying and traditional vacuum drying, and the products were analyzed for saponin retention and antioxidant activity. The vacuum belt dried extract had no statistical differences in saponin concentration compared to all other drying methods but showed the highest hydrogen peroxide scavenging activity (Liu et al., 2008). VBD has also been used to dry whole fruit, specifically whole blueberries. The VBD blueberries dried at 90°C had a total anthocyanin content (9.9-13.9 mg C3G/g DW), total phenolics content (30.2-35.1 mg GAE/g DW) and antioxidant activity (432-451 µmol TE/g DW) like the freeze-dried control (Pallas, 2011).

VBD has also gained some interest as a method for drying juice processing waste into ingredient powders as well. Although limited information is available for vacuum belt dried fruit pomace powders. VBD apple pomace powder had good flowability and acceptability color when dried at temperatures >100°C, while still being a high dietary fiber source (442-484 g/kg). In addition, apple pomace powder dried at temperatures >100°C had total phenolics content (47.9-51.9 mg GAE/kg) and total monomeric anthocyanin content (65-74.0 mg C3G/kg) comparable to the freeze-dried control apple

pomace phenolics (51.1 g GAE/kg) and anthocyanins (67.0 mg C3G/kg) contents (Yan, 2012; Yan & Kerr; 2013). As observed with apple pomace, VBD blueberry pomace had good powder physical properties. The blueberry pomace powders had good flowability, good color with no perceivable signs of browning, and did not readily absorb water (Kim, 2012). The polyphenolic profile of the blueberry pomace is unknown, but VBD has had success with preserving the bioactives of muscadine pomace. VBD muscadine pomace powders had high phenolics content (453-642 $\mu\text{mol GAE/g DW}$) similar to the freeze-dried control (583-608 $\mu\text{mol GAE/g DW}$) but in a considerable shorter time. The muscadine pomace powder was dried in 60 or 90 min while the freeze-dried material took 14-16 h (Vashisth et al., 2011).

VBD may also be an option to manufacture healthier snack foods. For tortilla chips, vacuum belt dried low-fat tortilla chips contained significantly less oil (1.57-1.82 g oil/100 g) compared to a traditional deep fat fried tortilla chip (33.37-34.80 g oil/100 g) and still had an acceptable sensory profile (Xu & Kerr, 2012). VBD also produced sweet potato chips with good quality orange color and high β -carotene content (Xu & Kerr, 2013).

One concern with vacuum belt drying has been the retention of flavor and aroma compounds. During drying of a liquid food such as fruit juice, volatiles go through chemical changes as well as being physically stripped away from the dried material. Di Cesare and Nani (1995) observed variable changes in peach juice volatiles. The most important volatile compounds to the peach aroma, 1 & 2-hexenal and benzaldehyde had only 24-37% retention after processing. VBD may not retain but cause the breakdown of volatile compounds. Esters are the most important compounds in banana aroma. Freeze

dried banana powder contained differing ester profiles when compared to both vacuum belt dried banana powder and air dried banana powder. The loss of volatiles observed by the vacuum belt dried and air dried banana powders is likely due to the elevated drying temperatures, which destroyed some of the original esters and produced other breakdown products (Wang et al., 2007).

Extrusion

Extrusion has become a mainstay in the food industry for production of both ready-to-eat snack foods and ready-to-eat breakfasts, and for food ingredients such as pre-gelatinized starch (Harper, 1981). By definition, extrusion is the operation of pushing a plastic or dough-like material through a die (Riaz, 2000). Extrusion in the food industry is most commonly performed with high temperature, short time (HTST) equipment, in which solid and liquid feeds are combined under heat and high pressure through a shaping die (Moscicki & Zuilichem, 2011). The addition of fruit and vegetables affects physical and chemical properties of the extruded material. Important physical characteristics for extrusion include expansion, density, color, texture and sensory evaluation. Expansion and bulk density are indicators of the puffing the material undergoes as it exits the extruder (Altan et al., 2008a). Puffing of a material that contains high fiber tends not to be as great because fiber particles rupture cell walls of the extruded material before gas bubbles can reach full size (Liu et al., 2000).

Recently, interest in formulating snack products with fruit pomaces has increased. Some of these projects are summarized in Table 2.1. Inclusion of fruit pomace generally reduced expansion, increased bulk density, changed the color and created a harder product, while changing the nutritional profile of the product. Apple pomace added to a

cornmeal extruded snack decreased expansion 80% and increased density 67-78%. Apple pomace also reduced the average cell size from 1.88 mm, to 0.51-0.36 mm, when added at 17-28 g/100 g. The smaller cell size created a harder product increasing the crushing force from 30.9 N for the control to 79.5-144.4 N for snacks containing pomace. Even though physical properties changed, apple pomace did successfully increase fiber content within the extruded snack. Total fiber increased from 3.0 g/100 g to 17.0, 21.3, and 26.4 g/100 g at an inclusion of 17, 22, and 28 g/100 g, respectively (Karkle et al., 2012a; Karkle et al., 2012b).

As well as being a fiber source, fruit pomaces may provide polyphenolics such as anthocyanins and procyanidins in extruded snacks. Extrusion negatively affected the anthocyanin content of a cranberry and cornstarch extrudate, reducing the content 46, 61, and 64% when processed at 150°C, 170°C and 190°C, respectively (White et al., 2010). At 170°C, extrusion increased procyanidin monomers by 157% and dimers by 164%, while decreasing larger procyanidins (>3 DP). It was suggested that this could increase the bioavailability of these molecules. The authors hypothesized that the increase in low molecular weight procyanidins is a combination of changes in the cell wall material caused by extrusion facilitating greater extraction and the breakdown of larger molecular weight procyanidins into monomers and dimers. Increases of low molecular weight procyanidins were also observed in extruded blueberry pomace and white sorghum. The total anthocyanin content of a freeze-dried blueberry pomace and sorghum (30:70 ratio) mixture was reduced 33-42% in extrusion, but the amount of monomer, dimer, and trimer procyanidins were increased because of extrusion processing (Khanal et al., 2009a).

Table 2.1: Effects of including fruit or vegetable processing wastes into extruded products.

Pomace Blends	Source	Effects from Inclusion of Pomace and Extrusion
Apple	Hwang et al., 1998	Increased water soluble polysaccharides (WSP) in pomace, from 11.94-19.75% with 53.09 to 59.27% of the WSP being composed of anhydrogalacturonic acid, (backbone of pectin)
Apple/Yellow Corn	Karkle et al., 2012a; Karkle et al., 2012b	Decreased specific mechanical energy, total digestible starch, expansion, and starch (18-30%); increased crushing force and fiber 5 to 9-fold
Blueberry/White Sorghum	Khanal et al., 2008b; Khanal et al., 2009a	Increased monomer, dimer and trimer procyanidins by 83, 51, and 90%, respectively. Total anthocyanin content reduced by 33-42%
Carrot/Rice/ Gram	Upadhyay et al., 2010	Decreased expansion, increased bulk density; improved color to consumers but decreased overall acceptability at incorporation >5%
Carrot/Rice	Kumar et al., 2010	Decreased expansion and bulk density, increased hardness; acceptable to consumers; optimal level of usage is 16.5% pomace
Carrot/Rice/Pigeon Pea	Dar et al., 2012	In 6 months storage: extrudates darkened, became 70% harder and 45% less crispy, 53% reduction of β -carotene, and 50% less Vitamin C
Cassava/Corn Grit	Dischsen et al., 2013	Increased dietary fiber from 4.43 g/100 g to 17.09 g/100 g; increased the crispness and overall acceptability

Cauliflower/Wheat	Stojceska et al., 2008	Increased fiber (7.6-11.6 g/100 g) compared with control (5.6 g/100 g); decreased expansion and increased bulk density; acceptable to consumers at levels <10%. Extrusion increased phenolics and antioxidant activity
Cranberry/Corn starch	White et al., 2010	Decreased anthocyanins 46-64% and increased total flavonol content 30-34% compared with unextruded pomace processed at 150-190°C; increase monomers 119-157% and dimers 164% for procyanidins. For 170°C and 190°C, antioxidant activity increased 16% and 30%, respectively
Grape/Barley	Altan et al., 2008a; Altan et al., 2009	Decrease in expansion; increased bulk density; increased hardness and crispness; was dominate in total change of color; higher antioxidant activity at higher temperatures; reduced β -glucan levels
Grape/White Sorghum	Khanal et al., 2009c	Increased monomer, dimer and trimer procyanidins by 123, 124, and 64%, respectively. Total anthocyanin content reduced by 53%
Guava/Rice	Tangirala et al., 2012	Increased bulk density, decreased hardness; found overall acceptable by consumers and optimum amount was 20.7% pomace
Pea hulls/Corn Semolina	Rzedzicki et al., 2004	Addition from 20-80% increased total dietary fiber form 15.66-54.19%
Pumpkin/Corn grit	Norfezah et al., 2011	Decreased expansion and increased bulk density and hardness
Tomato/Barley	Altan et al., 2008b; Altan et al., 2009	Decreased expansion; increased hardness and sensory preference for color; reduced β -glucan levels

While whole fruit and pomaces may have some impact on expansion and bulk density, the inclusion of juice concentrates produced less change in the extruded product. Blueberry concentrate increased expansion ratio (2.05) and decreased bulk density (0.18 g/cc), while grape control had similar expansion (1.96) and lower bulk density (0.23 g/cc) than a cornmeal control (expansion = 1.9, bulk density = 0.33 g/cc). Extrusion decreased the total anthocyanins (10.8-11.9 mg/100 g DW) and color density (1.7-1.9) of extruded corn-based cereals containing blueberry or grape concentrate, as compared to unextruded control (34.9-36.7 mg/100 g DW total anthocyanins, 4.3-4.4 color density) (Camire et al., 2002; Chaovanalikit et al., 2003). The loss of anthocyanins in the extruded is due to the high temperature in the extruder

2.4.5 Sorghum

In extrusion, the starch source is important for product quality. Sorghum has a high starch content (48.61-58.89 g/100 g) and is therefore useful in producing extruded snacks (Jadhav & Annapure, 2013). Sorghum (*Sorghum bicolor*) is one of the major worldwide grain crops. The US is one of the largest producers with an expected production of 358.89 million bushels in the 2013/2014 market year (Capehart et al., 2013). Traditionally associated as a feed grain, there is a renewed interest in sorghum as a gluten-free grain for those suffering from Celiac disease and other gluten sensitivities. Celiac disease is a combination of malabsorption of nutrients and an abnormal immune response to gluten (NIDDK, 2008).

Sorghum can also be a source of phenolic acids, tannins and anthocyanins. There are four broad classes of sorghums based on their polyphenolic profile. White sorghum has no detectable amounts of tannins or anthocyanins and very low phenolics content. Red sorghums have little tannins but high levels of phenolics. Black sorghums contain significantly high amounts of anthocyanins. Lastly, brown (sumac) sorghums contain high levels of tannins (Awika & Rooney,

2004). In this study, white sorghum was used in extrusion and so provided a small contribution of phenolic acids. Major phenolic acids present in sorghum are ferulic, *p*-coumaric, syringic, vanillic, and *p*-hydroxybenzoic (Guenzi & McCalla, 1966). While white sorghum may provide little to no phenols (0.8 mg GAE/g dry basis), the minimal color and antioxidant contribution allowed the effects of adding pomace powder to be displayed without interference (Awika et al., 2005).

Fiber and Phytochemical Assays

Specialized assays have been developed to quantify fiber and phytochemicals. Fiber can be measured as total dietary fiber, soluble dietary fiber and insoluble dietary fiber, acid detergent fiber and neutral detergent fiber or crude fiber (Van Soest et al., 1991). Also, many methods are available to characterize phytochemicals and antioxidant activity of polyphenolics (Table 2.2). To fully appreciate why these methods are appropriate, the basic chemistry of the assay needs to be understood.

Total Dietary Fiber

Total dietary fiber (TDF) assay is a combination of enzymatic and gravimetric procedures. In this method, dried, fat-extracted foods are broken down through a series of enzymatic reactions: a heat stable α -amylase, a protease, and an amyloglucosidase. Then the material is filtered and rinsed with a series of solvents (Anon, 2005). This method has been considered robust due to its repeatability and attention to certain details. For example, enzymes were chosen to ensure that only food components normally digested in the human system are broken down by the enzymes and not the digestion-resistant components of the sample. This helps ensure consistency to the definition of dietary fiber. Later modifications to this method were made to separate dietary fiber into soluble and insoluble fractions. As the definition of

dietary fiber has increased to include other polysaccharides, the method no longer quantifies all substances considered dietary fiber. This method, while still used, does not necessary account for food components such as resistant starch, fructans, polydextrose, and resistant maltodextrins (McCleary et al., 2010, DeVries et al.,1999).

Total Monomeric Anthocyanins

Anthocyanin pigments are important to food quality as a natural colorant, and as health promoting constituents. A common assay is the total monomeric anthocyanins (TMA) method. This is a pH-differential spectrophotometric method based on the color-structure relationship (Figure 2.5) of anthocyanins (Lee et al., 2005; Wrolstad et al., 2005). To manipulate this relationship, samples are combined with two buffers at pH = 1 and pH = 4.5 as to promote the formation of the flavylium cation and the chalcone or hemiketal form, respectively. The flavylium cation expresses the orange to purple colors associated with anthocyanins, while the chalcone or hemiketal forms are colorless (Giusti & Wrolstad, 2001). Concentrations are determined by Beer's Law (assuming a 1 cm pathway) using the following equation:

(Equation #1)

Eq. 1

$$\frac{(A_{\lambda_{\max}} - A_{700})_{pH1.0} - (A_{\lambda_{\max}} - A_{700})_{pH4.5}}{(\epsilon \times 1)}$$

where

$$A = (A_{\lambda_{\max}} - A_{700})_{pH1.0} - (A_{\lambda_{\max}} - A_{700})_{pH4.5}$$

MW = molecular weight of selected compound

DF = dilution factor

ϵ = molar absorptivity of selected compound

As seen in the Eq. 1, quantification of the total concentration is based on a specific compound, for example cyanidin-3-glucoside. This can cause issues as the assumption is that all anthocyanins have similar molecular weight and molar absorptivity. Actually, when reporting as different compounds, the differences in molecular weight and molar absorptivity between anthocyanins and the influence of solvents can distort calculated values, and leads to discrepancies when comparing results with other research (Dai & Mumper, 2010).

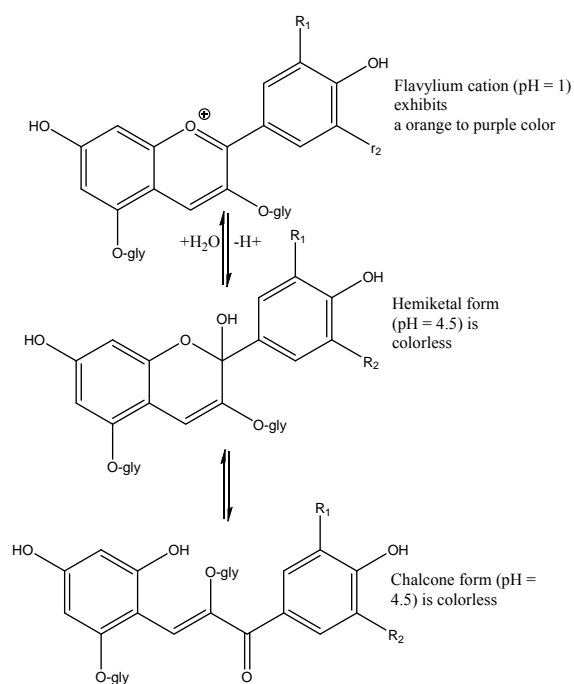
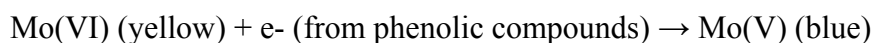


Figure 2.5: The pH-dependent forms of anthocyanins and the color exhibited used by the total monomeric anthocyanins assay.

Total Phenolics Content

Total phenolics content (TPC) is based on a spectrophotometric method that measures the total concentration of soluble phenolic compounds in a given sample. The TPC assay originated from a modified Lowry protein assay. This colorimetric method detected and quantified total proteins by measuring the content of tyrosine and tryptophan using the Folin-Denis reagent (FD) (Krohn, 2001). The Folin-Ciocalteu (FC) reagent method displaced the FD method as the standard for total phenolics quantification as it was more sensitive and reproducible than the FD method (Sanchez-Rangel et al. 2013, & Folin & Denis 1912).

The TPC method is based on the reduction of the FC reagent in the presence of phenols in a basic environment, thereby exhibiting an intense blue color (Waterhouse, 2001). The FC reagent is produced by combining sodium tungstate and molybdate with phosphoric acid and concentrated hydrochloric acid to create the active phosphomolybdic/phosphotungstic acid complex (Peterson, 1979). The basic conditions (pH ~ 10) produce phenolate ions, which are able to reduce the molybdates in the FC reagent, thereby causing a color change (Huang et al., 2005). The reaction proceeds with the transfer of an electron from the phenolic compound:



Using Beer's law, the measured absorbance is linearly related to the concentrations of phenolics within the sample. Quantification of phenolics is done by producing a standard curve using a phenolic acid common to the sample of interest. For blueberries, the common phenolic acid used in the literature is gallic acid. The FC is reproducible and has become standard for the measurement of total phenolics content, but as with any assay the method does have some

limitations. The FC assay is actually more of a test of the ability of compounds to reduce the FC reagent, which can then be compared to the standard. As the FC does not actually measure the concentration of phenolics, the reduction of the FC reagent is not specific to phenolics. Other compounds may interfere with the reading, such as ascorbic acid and reducing sugars (glucose and fructose) (Huang et al., 2005; Sanchez-Rangel et al., 2013).

DPPH

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) scavenging method is a common antioxidant assay that is based on the ability of antioxidants to quench the DPPH[•] stable free-radical (Sharma & Bhat, 2009). During the reaction (Figure 2.6), a hydrogen atom is transferred between the compounds to reduce DPPH[•] to DPPHH and the antioxidant becomes a stabilized radical (Bondet et al., 1997). The transferring of the hydrogen causes a loss of violet color, and leaves behind a residual yellow color from the still present picryl group (Molyneux, 2003).

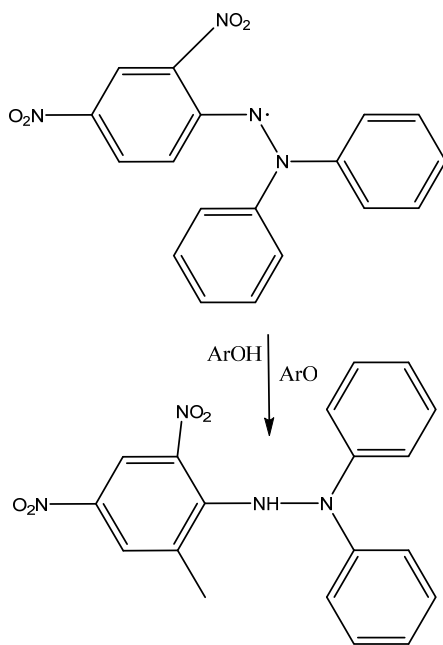


Figure 2.6: Hydrogen atom transfer from an antioxidant to DPPH[•] to form DPPHH.

Even though DPPH[•] method is one of the most widely used assay, it has been criticized because it does not employ a reactive oxygen species and therefore may not be biologically significant. Also, the risk of potential interference is high because DPPH[•] reacts with a variety of compounds other than phenolic compounds such as ascorbic acid, aromatic amino acids, and α -tocopherol (Craft et al., 2012).

Table 2.2: Description of assays for characterizing polyphenols.

Method	Type	Source(s)	Basic Concepts	Advantages	Limitations
Total Monomeric Anthocyanins (TMA)	Quantification	Giusti & Wrolstad, 2001	Anthocyanin concentration determined by pH-differential due to change from flavylum cation (orange to purple) to chalcone or hemiketal form (colorless)	Accurate and rapid even if interfering compounds and polymerized pigments are present	Have to estimate concentration on one anthocyanin even though samples contains a mixture of anthocyanins
Total Phenolics Content (TPC)	Quantification	Waterhouse, 2002	Chemical reduction of the Folin-Ciocalteu reagent (mixture of tungsten and molybdenum oxides) that exhibits blue color	Equivalent response to different phenols	Not specific to phenols so responds to sulfur dioxide and sugar
Total Flavonoids Content [Al(III) method]	Quantification	Papoti et al., 2011	Colorimetric method based on the ability of flavonoids to chelate metals to form aluminum-flavonoid complexes	Simple, rapid, inexpensive and useful for multiple applications	Aluminum is not always completely soluble and may make complexes with compounds structurally similar to flavonoids present in the sample

Reverse Phased High Performance Liquid Chromotography (RP-HPLC)	Identification and quantification	Gratzfeld-Hüsgen & Schuster, 2001	Using solubility to breakdown mixtures into individual compounds that are identified and quantified by detectors	Suitable for thermally labile compounds as done at room temperature; highly sensitive and depending on detector, may be used to separate fractions	Limitations in detection due to response of compounds; accuracy and precision highly dependent on quality of column and therefore needs consistent maintenance
2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH') Scavenging Method	Antioxidant activity	Kitagaki & Tsugawa, 1999; Bondet et al., 1997	Colorimetric method to determine ability of compound to scavenge DPPH' stable free radical	Suitable for thermally labile compounds as done at room temperature; easy and rapid method for antioxidant activity	Time dependent, which may influence data; reaction mechanism is structure dependent so multiple kinetics may exist; interference possible
Ferric Reducing Ability of Plasma (FRAP)	Antioxidant activity	Benzie & Strain, 1999; Carlsen et al., 2010	Ferric ion is reduced to ferrous ion forming a colored ferrous-tripyridyltriazine complex	Relatively fast and reproducible for a single antioxidant and mixtures of antioxidants	Unable to detect small molecular weight thiols and molecules containing sulfur

Oxygen-Radical Absorbance Capacity (ORAC) Assay	Antioxidant activity	Cao et al., 1993; Prior et al., 2005	Fluorescence assay where protection from radical species by antioxidants is measured by area under the curve and compared to standard	Controlled source of radicals that model activity of both hydrophilic and hydrophobic antioxidants	Temperature sensitive so differences between cells will affect analysis
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CHAPTER 3
CHANGES IN RABBITEYE BLUEBERRY WINE BIOACTIVES AND COLOR
DURING BOTTLE STORAGE

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ABSTRACT

Blueberry wine has been described as a source of anthocyanins and phenolics, but no information exists about the effects of storage on these compounds. Changes in anthocyanins, total phenolics, percent polymeric color, and antioxidant activity of blueberry wine were monitored during a six-month period. Total monomeric anthocyanins content of the unfermented juice was 312 mg GAE/L. The anthocyanins in the wine decreased from 120 mg C3G/L to 37 mg C3G/L in 6 months of storage. Total phenolics content of the blueberry juice was 1620 mg GAE/L. After two months of storage, phenolics increased (1310 mg GAE/L) compared to wine bottling day (1133 mg GAE/L). Color density of the blueberry wine decreased while in storage (14.88 to 7.17) and percent polymeric color increased during storage (38.71 to 72.43%). Antioxidant activity also decreased slightly between bottling day (87.41%) and 6 months of storage (82.80%). The large decrease in anthocyanins and increase in percent polymeric color during storage implies anthocyanins complexed with other compounds to create new stable pigments.

INTRODUCTION

Red wine, when consumed in moderation, has been associated with health benefits derived from the polyphenolics it contains. Red grape wines are a source of gallic acid, anthocyanins, resveratrol, catechins, epicatechins, quercetin, and proanthocyanidins (Das et al., 1999; Frankel et al., 1995). Anthocyanins are the major class of phytochemicals in red wine responsible for the characteristic red color, and also contribute to the antioxidant activity (Lapidot et al., 1998). Mature red wines have been

found to contain 113.5-356.4 mg/L of anthocyanins (Cristino et al., 2013). Other phenolic compounds also contribute to the antioxidant capacity of wine.

The total phenolics concentrations in wine can vary depending on the type of grape used, growing region and processing procedures. California white wines have been reported to have 165-331 mg GAE/L, while California red wines contain 1800-4059 mg GAE/L. The concentration of these compounds is also dependent on time allowed for maceration (contact with seeds and skins during initial fermentation) to occur. For red wines, total phenolics more than doubled, from 1332 to 2771 mg/L, and total tannins quadrupled, from 887 to 3216 mg/L, over a maceration period of nine days (Jordão et al., 2012).

While most traditional wines are made from *Vitis vinifera* grapes, other fruit wines have become popular due to their unique flavors. They are also being studied for their potential health benefits. Some of these wines can even be produced from bruised fruits or fruit otherwise unsuitable for the fresh market (Wells, 2012). There has been particular interest in the various phytochemicals these wines contain. For example, spine grape wines (*Vitis davidii* Foex) have been reported to have higher anthocyanin content (184.8-494.0 mg/L) than traditional red wine (Meng et al., 2012). Berry wines, such as those made from blueberry, blackberry or strawberry, have similar compounds as red grape wines as they usually contain anthocyanins and phenolics. Concentrations of phenolics can vary quite a bit depending on source. In studies on 44 different types of wine (including for example black current, cowberry, cranberry and cherry wines), total phenolics ranged from 125-1820 mg of GAE/L (Heinonen et al., 1998).

The color, polyphenolic profile and antioxidant activity of grape and fruit wines often changes during bottle aging. During storage of Cabernet Sauvignon, the concentration of anthocyanins and non-polymerized phenolics decreased, while the antioxidant activity and concentration of polymerized compounds increased (Burin et al., 2011). In studies on Mencia and Brancellao wines, anthocyanin content decreased by 72% in 1 year of storage, while procyanidin content increased by 42% (García-Falcón et al., 2007). The reduction of monomeric anthocyanins was traced to their degradation and interactions with other compounds. During storage, anthocyanins complex with polymeric phenolic tannins to a large extent, creating a heterogeneous group of compounds that stabilizes color (Singleton & Trousdale, 1992). Some of these stable pigments, referred to as pyranoanthocyanins, compounds that have an additional pyran ring between the C-4 and the hydroxyl group in the 5 position on the aglycon (Schwarz et al., 2003; Nave et al., 2010). For example, Bakker & Timberlake (1997) determined links were formed between the C-4 and the 5-hydroxyl groups in malvidin-3-glucoside, through a cycloaddition process and ethylenic bond provided by pyruvic acid. This results in a fourth ring structure and a more stable compound. This compound, carboxypyranomalvidin-3-*O*-glucoside, has been studied for its unique effects on health (Oliveira et al., 2013). Another stable pigment forms between anthocyanins and 4-vinylphenol groups, which involves a cyclisation between C-4 and the C-5 and the vinylphenol double bond (Fulcrand et al., 1996). These polymeric compounds can also give older wines a characteristic “tawny” color. More importantly, the pyranic ring acts as a protectant against shifts in pH and nucleophilic attack of water that would lead to color loss (Rentzsch et al., 2007). The volatile compounds of wine also change during

storage. After one year of bottle aging, Falanghina wine had lesser amounts of ethyl esters (ethyl hexanoate, ethyl octanoate, and ethyl decanoate), acids (hexanoic, octanoic, and decanoic), and acetates (3-methylbutyl acetate and 2-phenylethyl acetate) (Moio et al., 2004).

While the effects of bottle storage have been studied in red grape wines, less is known about the changes in fruit wine during aging. After three years of bottle aging in the dark at room temperature, elderberry wine experienced a 21% reduction in total phenolics content and a 94% reduction in total anthocyanins content. Chlorogenic acid (-52%), quercetin-rutinoside (-26%), and quercetin-glucoside (-41%) levels were also less in the aged elderberry wine (Schmitzer et al., 2010). Specific storage effects tend to vary amongst different fruit wines. After 2 months of storage, red raspberry wine had less anthocyanins, lower color density and higher percent polymeric material (Rommel et al., 1990). Strawberry wine experienced similar changes in anthocyanins and percent polymeric material after storage of only 6 weeks (Pilando et al., 1985).

Like other berry wines, blueberry wine has been shown to be a source of both anthocyanins and phenolics. Canadian commercial blueberry wines contained 1676 mg GAE/L total phenolics and had an antioxidant capacity of 1655 mg AAE/L, while commercial wines from Illinois contained 12.72-37.29 mg/L anthocyanins and 966.7-2510.8 mg EAE/L total phenolics (Rupasinghe & Clegg, 2007; Johnson & Gonzalez de Mejia, 2012). Southern highbush blueberry wine contained 929 mg GAE/L phenolics, 60.62 mg C3G/L anthocyanins, and 1233 mg CE/L flavonoids (Yang et al., 2012). Even though no information is available for blueberry wine, effects on storage on blueberry juice has been studied. Blueberry juice stored for less than a year contained less

anthocyanins and increased color due to polymeric compounds but did not show a change in antioxidant activity (Brownmiller et al., 2008).

While the anthocyanins and phenolics content of young blueberry wine has been described, as well as the effects of storage on blueberry juice, little is known about the role of bottle aging on the polyphenolics of blueberry wine. Thus, the objective of this study was to measure changes in anthocyanins, phenolics content, color density and antioxidant activity during bottle storage of blueberry wine stored in ambient conditions.

MATERIALS AND METHODS

Chemicals

Methanol and acetonitrile were purchased from Fisher-Scientific (Thermo Fisher Scientific Inc., Waltham, MA). Potassium chloride, sodium acetate, hydrochloric acid, and phosphoric acid were purchased from J. T. Baker (Avantor Performance Materials, Inc., Center Valley, PA). Folin-Ciocalteu reagent, sodium carbonate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and gallic acid were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO). Dephinidin and cyanidin were purchased from Extrasynthese (69726 Genay Cedex, France). Potassium metabisulfite was purchased from Crosby & Baker, Ltd. (Westport, MA).

Processing

Wine Fermentation and Bottling

Frozen rabbiteye blueberries were donated from the Blueberry Research and Demonstration Farm (Alma, GA). Thirty pounds of the frozen blueberries were mashed using a pilot-scale food processor (R10DVV, Robot Coupe U.S.A., Inc., Jackson, MS)

for 1 min and then transferred to a 38 L metal open top fermenter. The mash was heated to 40°C before Pectinex Ultra SP-L (Novozymes, Bagsvaerd, Denmark) was added (0.26 ml/kg), and the mash was allowed to incubate for 1.5 h. After incubation, sugar was added to a final Brix of 23°-25°, and water was combined with the mash to a final must volume of 37.9 L. To prevent fermentation by wild yeast, potassium metabisulfite (15 g/L) was added to the mash and the mixture allowed to sit at 4°C for 24 h. Ten grams of Red Star Pasteur Champagne yeast (Lesaffre Yeast Corporation, Milwaukee, WI) was used for fermentation. Initial fermentation occurred in an open top fermenter with seeds and skins. The seeds and skin wine “cap” was pressed down into the must for five min twice each day. After seven days, wine was racked from the seeds and skins into a covered glass carboy, which was then sealed with an airlock. Fermentation continued in the covered carboy for 1 month until the alcohol level reached 13-15% (by volume). After fermentation, wine was bottled into 355 ml brown glass bottles and capped with a metal top. Wine was stored at room temperature (25°C) for 6 months. Wine was sampled at bottling day (day 0), 2 months, 4 months, and 6 months.

Freshly pressed single-strength juice and diluted juice were used as control samples. Single-strength juice was used as a control to determine starting values of phenolics. Total phenolics content, total monomeric anthocyanins and color values were determined for diluted juice to give an equivalent comparison with wine after fermentation and storage. Values for diluted juice were made by dividing single-strength juice measurements by 3.5, which is the same dilution that occurred during must production.

Chemical Analysis

Total Phenolic Content

Total phenolics content (TPC) was measured using the Folin-Ciocalteu method as described by Amarowicz et al. (2004). Each sample was diluted (0.1 ml sample into 5 ml total) with deionized (DI) water and, then two milliliters of diluted sample were combined with 6.5 ml of DI water in a test tube. After mixing, 0.5 ml aliquot of Folin-Ciocalteu phenol's reagent and 1 ml of saturated sodium carbonate solution were added. The test tubes were vortexed for 15 s. The samples were allowed to react at room temperature (25°C) for 40 min. Afterwards, absorbance readings were taken at 750 nm using a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA). Gallic acid was used to prepare a standard curve for quantification. Readings were done in triplicate and results reported in mg GAE/L.

Total Monomeric Anthocyanins

Total monomeric anthocyanins (TMA) content was analyzed using the pH differential method as described by Giusti and Wrolstad (2001). A 0.5 ml aliquot of wine was combined with 4 ml 0.025 M potassium chloride buffer and 4 ml of 0.4 M sodium acetate buffer in separate test tubes and allowed to equilibrate for 15 min. Absorbance was read at wavelengths of 520 nm and 700 nm with a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA). All measurements were done in triplicate. Calculations were made using Equation 1 and reported as mg C3G/L:

$$TMA = \frac{(A \times MW \times DF \times 100)}{(\epsilon \times l)} \quad \text{Eq (1)}$$

where

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$$

MW = molecular weight of cyanidin-3-glucoside

DF = dilution factor

ϵ = molar absorptivity of cyanidin-3-glucoside

Antioxidant Activity

Free-radical scavenging potential was measured using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) procedure as described by Su and Chien (2007). To determine the antioxidant activity (in % inhibition), 0.05 ml of wine was added to 5 ml of DPPH solution (0.025 g/l) and allowed to react in the dark for 30 min at ambient temperature (25°C). Absorbance was read at 515 nm using as a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA), and antioxidant activity was calculated as percentage of DPPH discoloration as compared to control (methanol):

$$\% \text{ DPPH Inhibition} = 100 \times \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \quad \text{Eq 2}$$

Polymeric Color Analysis

Color density, polymeric color, and percent polymeric color were measured using the method described by Hager and others (2008). This approach has been useful for tracking color changes in anthocyanin-containing products. The assay takes advantage of the fact that anthocyanin monomers combine with bisulfite to form colorless sulfonic adducts, while polymeric anthocyanins do not. Using 0.25 M potassium buffer (pH = 1), 0.1 ml of wine samples were brought up to a volume of 5 ml. After mixing, 2.8 ml of the diluted samples was pipetted into two cuvettes. Next, 0.2 ml of water was pipetted into one cuvette, and 0.2 ml of potassium metabisulfite solution (0.2 g/ml) was pipetted into the other. The solution was allowed to equilibrate for 15 min. Absorbance was recorded at 420, 520, and 700 nm using as a Spectronic Genesys 2 spectrophotometer

(ThermoFisher Scientific, Waltham, MA). The color density was first calculated using measured absorbances for the unbleached samples (those without added bisulfite):

$$\text{Color density} = [(A_{420nm} - A_{700nm}) + (A_{520nm} - A_{700nm})] \times DF \quad \text{Eq (2)}$$

where

A = absorbance at the specified wavelength

DF = dilution factor

Polymeric color was calculated using absorbance values for the bleached samples (those with added bisulfite):

$$\text{Polymeric color} = [(A_{420nm} - A_{700nm}) + (A_{520nm} - A_{700nm})] \times DF \quad \text{Eq (3)}$$

The percent polymeric color was calculated as:

$$\% \text{ Polymeric color} = (\text{Polymeric color} / \text{Color density}) \times 100 \quad \text{Eq (4)}$$

HPLC Separation of Anthocyanins

Anthocyanin separation and identification by reverse phase separation was performed with a HP 1090 Series II HPLC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a UV diode array detector. A Waters Spherisorb (Waters Corporation, Milford, MA) 5 μm ODS (2) (250 X 4.6 mm) column was used for separation. Separation of polyphenols was done as described by Lee and others (2002). The solvent system used a linear gradient of 94% solvent A (4% phosphoric acid) and 6% solvent B (acetonitrile)

to 75% solvent A and 25% solvent B in 55 min. Detection was taken at 520 nm for anthocyanins. Wine samples were filtered through a 0.45 µm Phenex nylon filter (Phenomenex, Torrance, CA) before HPLC injection. Anthocyanins were identified by standards elution time and spectral analysis. Anthocyanins were quantified using delphinin as an external standard and reported as mg del/L.

Statistical Analysis

Wine processing was replicated twice and each assay was performed in triplicates. One-way analysis of variance and Pearson's correlation were performed using JMP (SAS Institute Inc., Cary, NC). Tukey's post hoc testing was used to determine different means.

RESULTS AND DISCUSSION

Total Phenolic Content

The total phenolics content (TPC) for blueberry juice and wines aged up to 6 months is shown in Table 3.1. Single-strength blueberry juice contained more phenolics (1620 mg GAE/L) than wine (1224-1310 mg GAE/L). The dilution of phenolics is expected as the mash was diluted ~3.5:1 with water to produce the must. Rough calculations show that the undiluted mash would contain ~4100 mg GAE/L. When compared to juice that was diluted (463 mg GAE/L), blueberry wine contained greater phenolics content. The process of making red wines increases the TPC as the wine must is allowed to sit for 24 days with the skins and seeds, effectively allowing the liquid to extract more pigmented compounds. Also, the punch down of the cap process allows for greater extraction from the skins and seeds by the ethanol that is produced via fermentation. Some of this increase is lost, however, due to dilution with water. Studies

on elderberry wine also showed they had less phenolics (5136.75-7401.71 mg GAE/L) compared to fresh elderberry (Garofulić et al., 2012). Initial TPC values for the wine are in keeping with values reported for other wines. Rupasinghe & Clegg (2007) found that TPC values ranged from 250 to 2005 GAE/L for 14 categories of wine, with lowest values for Riesling and pear wines, and highest values for elderberry and cabernet grape wines.

Blueberry wine that had been aged in the bottle for 2 months had slightly higher levels of phenolics (1310 mg GAE/L) than wine sampled on bottling day (1133 mg GAE/L). TPC levels did not change after 2 months. The observed increase in TPC is consistent with changes reported for other wines. Red 'Primitivo' wine experienced an increase in TPC during 1 year of aging (Baiano et al., 2009). Red wines have a variety of phenolic acids, including caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, *p*-OH-benzoic acid, gallic acid, and protocatechic acid (Baldi, 1997), and several of these (ferulic, caffeic, and *p*-coumaric acids) have been found to increase during storage of the wine. Kallithraka et al. (2009) hypothesized that enzymatic and non-enzymatic oxidative processes changed the phenolic composition during fermentation. Oxygen in the system has shown to increase polymerization and condensation reactions as reactive oxygen species produce phenolic radicals which then polymerize through a radical-radical coupling reaction. Thereby, a greater portion of the total phenolics content is due to larger molecular weight phenolics (Castellari et al., 2000; Eker et al., 2009). These changes may be responsible for elevated phenolics content observed in the blueberry wine during storage compared to bottling day.

Total Monomeric Anthocyanin

Blueberry wine total monomeric anthocyanin (TMA) content decreased during bottle storage (Table 3.1). Single-strength juice TMA was larger (312 mg C3G/L) and diluted juice (89 mg C3G/L) was less than the wine at bottling (120 mg C3G/L). As observed for phenolics, while more anthocyanins are extracted in the mash, dilution with water brings the levels below that observed for single-strength juice, but the extraction by ethanol during the first stage of fermentation increased the TMA compared to diluted juice. During storage, TMA values decreased from 120 to 37 mg C3G/L. This may be due to oxidation, as some oxygen was dissolved in the mash when the cap was pressed down during the first 7 days of fermentation. Oxygen is known to destabilize anthocyanins during processing (Kalt et al., 2000). Su & Chein (2007) found that fermenting blueberry juice, or juice with pomace, increased the anthocyanin levels compared to unfermented juice. In that investigation, little to no oxygen was introduced to the must throughout fermentation, which limited destruction of anthocyanins during the wine making process.

TMA decreased 69% in the wine by 6 months of storage. This is consistent with TMA changes found in other berry wines. Strawberry wine retained only 3-9% of the original anthocyanin content after 6 weeks of storage (Pilando et al., 1985). Reduction of TMA in raspberry wine was not as great, as the wine retained 78.1% of the original TMA content after 6 months of bottle storage (Rommel et al., 1990). As seen with berry wines, anthocyanins in red wine have been found to decrease during bottle aging, usually experiencing a logarithmic reduction during storage (Dallas et al., 1995). In studies on 32 vintage red wines, there was almost a complete destruction of monomeric anthocyanins

within a year, while polymerized material increased, thereby stabilizing the color (Somers & Evans, 1986).

Antioxidant Activity

Antioxidant activity is an important property of phytochemicals, and has been touted as one of the fundamental mechanisms by which these compounds influence cardiovascular and other diseases (Cook & Samman, 1996). Blueberry juice had slightly higher antioxidant activity (90.18% inhibition of DPPH) as compared to wine at month 0 (87.41% inhibition) (Table 3.1). No significant changes in antioxidant activity occurred until the sixth month of storage, at which point the antioxidant activity had decreased to 82.80%. As antioxidant activity was correlated with anthocyanin content ($r = 0.644$, $R^2 = 0.415$), it is reasonable it would mimic the reduction of anthocyanins at 6 months. Antioxidant activity was also negatively correlated with percent polymeric color ($r = -0.814$, $R^2 = 0.662$). As anthocyanins polymerize, the antioxidant activity of the compounds is also affected. The antioxidant activity of red wine has been attributed to several phenolic compounds, although the role of each compound and their interactions are not completely understood. Arnous et al. (2001) showed that monomeric flavonols and proanthocyanidins had a significant effect on the antioxidant properties of red wines, while the role of monomeric anthocyanins was less certain. In contrast, Poiana et al. (2008) found that monomeric anthocyanin content was highly influential in the total antioxidant capacity of Cabernet Sauvignon wines, while the polymeric anthocyanins were responsible for the increase in free radical scavenging ability during storage. These findings are not consistent for all red wines; however, as Primitivo wine experienced a reduction (13%) in DPPH inhibition after one year in storage. They attributed this to the

polymerization of due to monomeric anthocyanins, resulting in compounds that had less antioxidant ability (Baiano et al., 2009).

Polymeric Color Analysis

Color density, polymeric color and percent polymeric color were all affected by bottle storage time (Table 3.2). The starting single-strength juice material had greater color density (28.73) as compared to the wine at bottling (14.88). The color density of diluted juice (8.19) was lower than all other treatments except wine sample at 6 months. The lower color density of diluted is expected as the concentration of the major color components, anthocyanins, were also diluted. During bottle storage, color density in the wine decreased from 14.88 to 7.17, a reduction of 43% overall. As previously noted, the color density is a measure of the bleachable monomeric anthocyanins. The color density was relatively high compared to some reported values. For example, Ortiz et al. (2013) found that blueberry wines from Ecuador had an average color density of 4.5. Anthocyanins are the major contributors to color of the blueberry wine, and as expected, color density and TMA values were positively correlated ($r = 0.703$, $R^2 = 0.494$). Color density is an important wine quality attribute and can be an indicator of the organoleptic properties in young wines. When compared by wine experts, wines with deep red color, or greater color density, tended to be rated higher quality wines (Somers & Evans, 1974). Color density is a combination of both monomeric anthocyanins and polymerized material, and these compounds are associated with the astringency and flavor profile of wines. Therefore, color intensity can be used to describe overall quality of the wine (Somers, 1978.) Color density has been found to decrease in Cabernet Franc, Merlot, and

Pinot Noir wines during 1 year storage (Mazza et al., 1999). The decreased color density was thought to be due to polymerization of anthocyanins into large tannin compounds.

Polymerized color is an indicator of how much of the color pigments are resistant to bleaching. Polymerized color was lowest for diluted juice (1.34) compared to single-strength juice (4.68) and wine. Polymerized color changed slightly during wine storage with increases at 2 months, followed by decreases at 4 months. Similar results have been found in red grape wines. For example, (Gómez-Plaza et al., 2000) found that the polymeric color increased from 0.90 to 1.26 during 1 year storage of a young Spanish wine.

Percent polymeric color (the ratio between polymerized color and color density) is an indicator of the relative contribution of color from polymerized material (non-monomeric anthocyanins) or tannins (Wrolstad, 1993). The percent polymeric color was lowest for single-strength juice (16.29%) and diluted juice (16.31%) while wines stored for 6 months (72.43%) had the highest percent polymeric color. The similarity in percent polymeric color of the juice values was expected as polymeric color had the same impact on color. Throughout storage, small differences were observed in the polymeric color, while greater reductions were observed in the color density, thereby, allowing the polymeric pigments to have a greater impact within the wine, creating the more tawny color, than the monomeric anthocyanin component. The percent polymeric color was higher than previously reported for blueberry juice (6.2%-10.2%), which is probably due to polymerized material produced by heating the mash needed for the enzyme reaction to increase juice yield (Brownmiller et al., 2008). Color density and percent polymeric color were strongly negatively correlated ($r = -0.938$, $R^2 = 0.879$). Percent polymeric color and

TMA was also strongly negatively correlated ($r = -0.814$, $R^2 = 0.662$). The increased percent polymeric color and decreased TMA has been associated with co-pigmentation or the formation of complexes that occur during storage (Hager et al., 2008). Anthocyanins form co-pigments with other organic molecules including proteins, phenolic acids, tannins, other flavonoids, and polysaccharides within solution to create stable pigments (Fennema, 1985).

HPLC

Blueberry juice and wine samples were also analyzed by HPLC, and the peaks identified as shown in Figure 3.1. Blueberry juice had the most peaks, while wine samples held for 6 months had the least. The elution order for juice was delphinidin-3-galactoside, delphinidin-3-glucoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-galactoside, petunidin-3-arabinoside, peonidin-3-glucoside, malvidin-3-galactoside, malvidin-3-glucoside, malvidin-3-arabinoside, and one unidentified compound, which is in agreement with others (Wang et al., 2012; Hamamatsu et al., 2004). The unidentified compound was only detected in the juice and may be either an acylated anthocyanin or polymerized material detected by the HPLC (Lee & Wrolstad, 2004). The number of anthocyanins detected, as well as the concentration of each was highest for juice compared to wine samples (Table 3.3). Other than malvidin-3-galactoside, all anthocyanidin-galactosides were only detected within juice. This may be due to hydrolysis of the galactosides, possibly due to the glycosidase activities of enzymes active during fermentation (Hermosin-Gutierrez et al., 2011). Overall, all anthocyanins decreased in fermentation and bottle storage. This concurred with the observation that TMA levels in the wine decreased with storage time.

During in-bottle storage there was a great decline of all anthocyanin-glycosides. After six months of storage, only peonidin-3-glucoside (0.67 mg/L), malvidin-3-glucoside (0.80 mg/L), and malvidin-3-arabinoside (1.28 mg/L) were detectable, and each of those showed a >78% reduction in concentration. As this coincided with an increase in percent polymerized color, it is likely that the anthocyanins formed stable pigments with other material. In red grape wines, it was found that anthocyanins link by vinyl groups with phenolic acids (coumaric or caffeic), catechins, or pyruvate in storage (Wang et al., 2003). Overall, malvidin glycosides were present at greater levels than other anthocyanins in both the juice and wine, which is typical for blueberry products. Even though some small amounts of both malvidin-3-glucoside and malvidin-3-galactoside were still in the blueberry wines stored for 6 months, there was still a 94% reduction compared to initial levels. Malvidin has been shown to polymerize with (epi)catechin or (epi)gallocatechin (Lee et al., 2002; Alcalde-Eon et al., 2007). It is likely that after 6 months of storage, much of the malvidin-glycosides were converted into dimeric anthocyanins by complexation with other flavanols.

CONCLUSIONS

Blueberry wine is a source of both anthocyanins and phenolics directly after fermentation. Fermentation increased the polyphenolic profile due to extraction by ethanol. Bottle aging changed the concentration of these compounds as well the wine polyphenolic profile. Monomeric anthocyanins almost completely disappeared during the six month storage, with just a small concentration of peonidin-3-glucosides and malvidin-glycosides detectable by HPLC. Anthocyanins likely polymerized with other organic

compounds such as phenolic acids and flavanols as color density decreased and percent polymerized color increased in storage. Blueberry wine still contained high levels of phenolics and had radical inhibition above 80% after six months of storage.

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Table 3.1: Total phenolics content (TPC), total monomeric anthocyanins (TMA), and antioxidant activity (AA) of single-strength blueberry juice, diluted blueberry juice, and blueberry wine during storage.¹

	Months ³	TPC (mg GAE/L)		TMA (mg C3G/L)		AA (% Inhibition)	
		Mean	SD	Mean	SD	Mean	SD
Juice ²	0	1620 ^a	123	312 ^a	17.3	90.18 ^a	0.85
Diluted Juice	0	463 ^d	32	89 ^c	4.9	----	----
Wine	0	1133 ^c	96	120 ^b	14.6	87.41 ^b	1.53
	2	1310 ^b	64	68 ^d	6.2	87.94 ^b	1.44
	4	1225 ^{bc}	125	51 ^e	10.8	86.68 ^b	2.13
	6	1224 ^{bc}	84	37 ^f	14.4	82.80 ^c	0.61

¹Means in the same column followed by the same letter are not significantly different ($p < 0.5$).

²Single-strength juice before must production and fermentation

³Months are months stored in bottles with Month 0 being bottling day.

Table 3.2: Color density, polymerized color, and percent polymeric color of single-strength blueberry juice, diluted blueberry juice, and blueberry wine during storage.¹

	Months ³	Color Density		Polymerized Color		Polymeric color (%)	
		Mean	SD	Mean	SD	Mean	SD
Juice ²	0	28.73 ^a	0.62	4.68 ^c	0.16	16.29 ^e	0.76
Diluted Juice	0	8.19 ^e	0.19	1.34 ^d	0.05	16.31 ^e	0.82
Wine	0	14.88 ^b	0.38	5.76 ^{ab}	0.10	38.71 ^d	1.28
	2	12.27 ^c	0.43	5.92 ^a	0.68	48.25 ^c	4.97
	4	10.17 ^d	1.43	5.73 ^{bc}	0.52	56.71 ^b	3.87
	6	7.17 ^e	0.94	5.16 ^{bc}	0.65	72.43 ^a	7.04

¹Means in the same column followed by the same letter are not significantly different ($p < 0.5$).

²Single strength juice before must production and fermentation

³Months are months stored in bottles with Month 0 being bottling day.

Figure 3.1: HPLC-DAD chromatogram (520 nm) of (a) single-strength juice, (b) wine at bottling day, (c) two months of storage, (d) four months of storage, and (e) six months of storage. The juice absorbance is from 1-100 mAU, while the wine samples' absorbances are from 1-25 mAU. The elution order was: (1) delphinidin-3-galactoside, (2) delphinidin-3-glucoside, (3) cyanidin-3-galactoside, (4) delphinidin-3-arabinoside, (5) cyanidin-3-glucoside, (6) petunidin-3-galactoside, (7) cyanidin-3-arabinoside, (8) petunidin-3-glucoside, (9) peonidin-3-galactoside, (10) petunidin-3-arabinoside, (11) peonidin-3-glucoside, (12) malvidin-3-galactoside, (13) malvidin-3-glucoside, (14) malvidin-3-arabinoside, (15) unidentified.

Absorbance
(mAU)

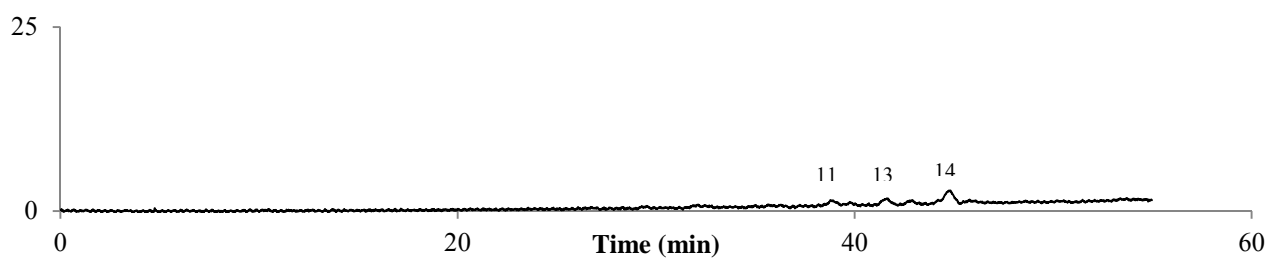
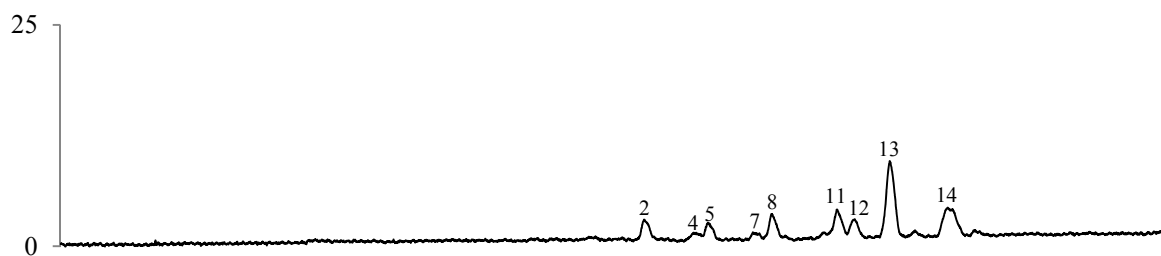
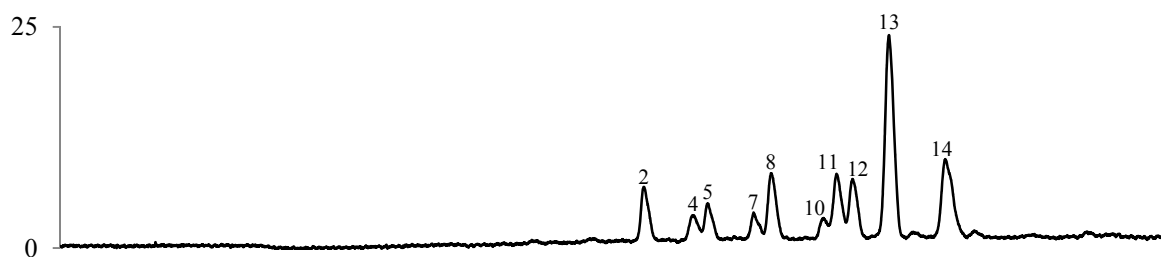
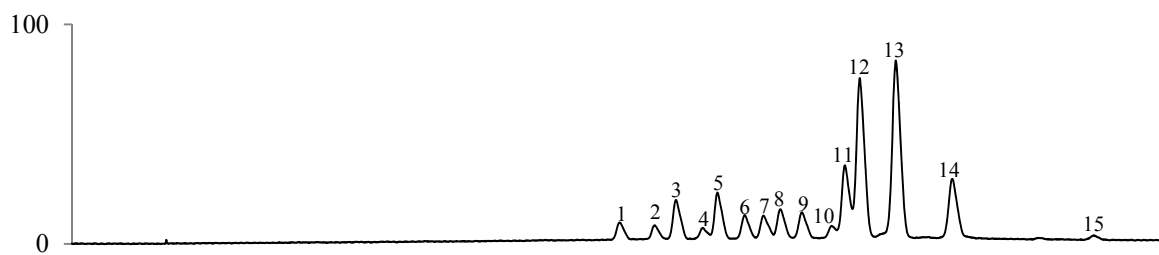


Table 3.3: Anthocyanins (mg del/L) as determined by HPLC-DAD in single-strength blueberry juice and blueberry wine during storage.¹

Anthocyanin	Single-strength Juice	Month ² ₀	Month 2	Month 4	Month 6
Delphinidin-3-galactoside	4.27				
Delphinidin-3-glucoside	3.44 ^a	3.29 ^a	1.54 ^b	0.78 ^c	
Cyanidin-3-galactoside	8.23				
Delphinidin-3-arabinoside	2.80 ^a	1.46 ^b	0.76 ^b	0.52 ^b	
Cyanidin-3-glucoside	9.33 ^a	1.85 ^b	1.22 ^{bc}	0.63 ^c	
Petunidin-3-galactoside	5.42				
Cyanidin-3-arabinoside	5.17 ^a	1.84 ^b	0.70 ^c		
Petunidin-3-glucoside	6.58 ^a	4.09 ^b	1.59 ^c	0.75 ^d	
Peonidin-3-galactoside	5.74				
Petunidin-3-arabinoside	2.71 ^a	1.45 ^b			
Peonidin-3-glucoside	15.19 ^a	4.10 ^b	1.86 ^c	0.85 ^d	0.67 ^d
Malvidin-3-galactoside	34.96 ^a	3.72 ^b	1.37 ^c	0.62 ^c	
Malvidin-3-glucoside	39.84 ^a	12.38 ^b	4.56 ^c	1.78 ^d	0.80 ^d
Malvidin-3-arabinoside	13.67 ^a	5.83 ^b	2.55 ^c	1.49 ^{cd}	1.28 ^d
Unidentified	1.28				

¹Means followed by same letter in the same row are not significantly different.

²Month 0 is bottling day of blueberry wine.

CHAPTER 4

EFFECTS OF DRYING TEMPERATURE AND DESEEDING ON THE
ANTHOCYANINS AND TOTAL PHENOLICS OF VACUUM BELT DRIED
BLUEBERRY POMACE POWDER

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ABSTRACT

Vacuum belt drying is a low temperature and high efficiency drying process that may be an alternative method to freeze drying. Dried blueberry pomace powder was made using a continuous vacuum belt dryer at 80°C for 100 min, 100°C for 70 min, and 120°C for 45 min to attain a water activity between 0.20-0.25. The total monomeric anthocyanins (TMA), total phenolics content (TPC), and HPLC characterization of anthocyanins of dried pomace powder, with and without seeds, was compared to freeze dried pomace powder. Processing in the vacuum belt dryer was no more than 100 min compared to 72 h in the freeze drier. Vacuum belt dried pomace powder processed at 100°C or less had no difference in TMA (11.54-15.37 mg C3G/g DS) and TPC (33.41-44.66 mg GAE/g DS) as compared to freeze dried powder. This is likely because the actual product temperature did not increase above 90°C. Drying the pomace at 120°C significantly reduced anthocyanins and total phenolics. Deseeding of the pomace powder significantly reduced both anthocyanins and phenolics.

INTRODUCTION

The consumption of fresh blueberries and blueberry juice has increased, in part because of the health benefits derived from nutrients and phytochemicals they contain. For example, blueberries have relatively high antioxidant capacity compared to other fruits, with ORAC values on the order of 45-55 μ mole TE/g FW (You et al., 2011). The juice waste streams, or pomace, also contain phytochemicals, but are not currently used by the food industry. The pomace consisting of peels, seeds, and pulp is a source of flavonoids, procyanidins, and phenolics (Laroze et al, 2010; Khanal et al 2009; Gao et al.,

2010). Blueberry pomace has been shown to contain upwards of 1100 mg/kg DW anthocyanins, and can account for 15 to 55% of the original whole berry anthocyanin content (Laroze et al., 2010; Brownmiller et al., 2008). For consumers' benefit, it may be preferable to incorporate the antioxidants into food without removing the compounds from the fruit matrix (Saura-Calixto, 1998). To create an ingredient for value-added products, dried pomace powder may be more desirable for both storage and ease of use compared to fresh pomace.

Depending on the drying method, antioxidant loss and energy usage may be substantial during drying of fruit pomace. For blanched apple pomace, total polyphenol content decreased from ~1500 mg/100 g DW to ~600 mg/100 g DW and total flavonoid content decreased from ~550 mg/100 g DW to ~450 mg/100 g DW when air dried at 50-80°C (Heras-Ramírez et al., 2012). Likewise, grape skins dried at 100°C and 140°C contained 19% and 33%, respectively, less total polyphenolics compared to freeze dried grape pomace peels (Larrauri et al., 1997). As seen with other fruit pomaces, thermal processing has been reported to negatively affect blueberry pomace phytochemical content. Khanal et al. (2010) found that when blueberry pomace was heated to temperatures above 60°C, there were decreases in total anthocyanin content and total procyanidin content. As drying is often done at elevated temperatures, a method for lower temperature drying is needed to preserve antioxidant quality. Freeze drying is one approach but has relatively long drying times. Vacuum belt drying is a potential alternative.

Vacuum drying has been associated with producing products with high quality physical and sensory attributes, but is sometimes destructive of bioactives (Sigg & Koch,

1995). Vacuum dried pomegranate peels had less total phenolics than fresh or freeze dried peels, and there was a significant reduction of flavonoids in vacuum dried strawberries when compared with fresh and freeze dried strawberries (Al-Rawahi et al., 2013; Wojdyło et al., 2009). Vacuum drying has been combined with microwave heating to improve dried fruit quality (Sagar & Kumar, 2010). Unlike traditional vacuum drying, microwave-vacuum produced dried strawberries had similar quercetin (12.2-22.4 mg/100 g DW) and kaempferol (4.1-4.3 mg/100 g DW) levels as fresh and freeze dried strawberries (Wojdyło et al., 2009). Microwave-vacuum drying was also found to be less destructive of anthocyanins compared to convective drying for cranberries (Ruse et al., 2011). Blueberries dried by a microwave vacuum oven contained ~19 g/100g polyphenol glycosides while blueberries dried by hot air only contain ~8 g/100g sample, a 58% difference, and vacuum dried blueberries were associated with redder color than forced air dried blueberries (Mejia-Meza et al., 2008; Yang & Atallah, 1985).

Another modification to vacuum drying is vacuum belt drying, which is a continuous form of dehydration. Continuous vacuum belt drying has relatively higher energy efficiency and larger throughput, which would make it a superior choice to traditional vacuum drying (Wang et al., 2007a). Unlike conventional vacuum drying, manipulation of temperature, feed speed and belt speed can be used to increase efficiency while maintaining proper dehydration of food stuffs (Liu et al., 2009). Vacuum belt drying uses both radiation and conduction heating which increase the thermal efficiency 52-74% compared to traditional vacuum drying (Hayashi et al., 1983).

Research has focused on the utilization of vacuum belt drying as a novel way to dry extracts, fruits, fruit purees, and juices. Liu et al. (2010) investigated drying *Panax*

notoginseng extract. Vacuum belt drying produced powders with the same saponin glycosides quantity as freeze dried samples, but produced samples in 30 min compared to the 24 h period needed for freeze drying. Muscadine pomace dried by a vacuum belt dryer dried in less time (60-90 min) when compared to freeze drying (14-16 h) with similar total phenolics content, 465-642 $\mu\text{mol GAE/g DW}$, and antioxidant activity, 1.64-2.27 $\text{mmol Fe}^{2+} \text{E/g DW}$ (Vashisth et al., 2011). One issue with vacuum belt drying is it may change the volatile profile of fruit products when compared to freeze drying. For dried banana powders, freeze dried powders contained higher amounts of esters and alcohols while vacuum belt dried powders contained higher amounts of acids, ketones and benzenes. The difference in these volatile breakdown products may be due to the relatively higher temperatures associated with vacuum drying as compared to freeze drying (Wang et al., 2007b).

While vacuum dried blueberry pomace powders were found to have good color and flow properties (Kim, 2012), no studies have shown the effects of vacuum belt drying on blueberry pomace phytochemicals. The objective of this study was to determine the effects of drying temperature on the anthocyanins and total phenolics content of vacuum belt dried blueberry pomace powder. In addition, as removal of seeds allows a finer powder to be made, the effects of deseeding on these constituents were also studied.

MATERIALS AND METHODS

Chemicals

Acetone, methanol, and acetonitrile were bought from Fisher-Scientific (Thermo Fisher Scientific Inc., Waltham, MA). Potassium chloride, sodium acetate, hydrochloric

acid, and phosphoric acid were bought from J. T. Baker (Avantor Performance Materials, Inc., Center Valley, PA). Folin-Ciocalteu reagent, sodium carbonate, and gallic acid standard were bought from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO). Dephinidin and cyanidin was bought from Extrasynthese (69726 Genay Cedex, France).

Processing

Pomace Production:

Frozen rabbiteye blueberry (*Vaccinium ashei*) varieties 'Brightwell' and 'Tifblue' were donated from the Blueberry Research and Demonstration Farm (Alma, Ga). Frozen berries were crushed in a Hobart Model 98 grinder (Hobart Corporation, Troy, OH) and then heated in a steam kettle to 40°C. Pectinex Ultra SP-L (Novozymes, Basvaerd, Denmark) was added (0.26 ml/kg) to the mash and held for 1.5 h. Pectinex Ultra SP-L is a polygalacturonase used to break down pectin during juice production to increase yield. Mash was pressed in a Bucher HP 14 Laboratory Press (Bucher Industries AG, Murzlenstrasse 80, CH-8166 Niederweningen) yielding ~70% juice. Pomace was frozen and stored at -20°C.

Vacuum Belt Dried Pomace Powder Production:

Pomace was dried using a pilot scale continuous vacuum belt dryer (Model: Zwag LKM-101, Zschokke Wartman, Ltd., Bucher, Döttingen, Switzerland) with a 22 cm Teflon belt passing over 3 conduction heating plates (Figure 4.1). A 22.9 cm long radiation plate was set 4.7 cm above the three heating zones. During processing, all three heating zones and the radiation plate were set to the same temperature, and vacuum was held at 2.7-3.3 kPa (20-25 torr). Pomace was removed from the -20°C freezer and allowed to thaw for 24 h at 4°C. Pomace was placed directly onto the belt at 4-5 mm

thickness and dried until all samples reached a 0.20-0.25 water activity. Three drying temperatures were tested: 80°C for 100 min, 100°C for 70 min, or 120°C for 45 min. The temperature of material during drying was measured in three locations with type T thermocouples attached to a data logger (Model RDXL121-D, Omega Engineering, Inc., Stamford, CT), with readings taken every second. Dried pomace was removed from the drier and formed into powders in a grinder (Model KFP600, Kitchen Aid, St. Joseph, MI) for 1 min and sieved through a #40 sieve (420 µm).

Vacuum belt dried pomace powder was made with and without seeds. To remove seeds, pomace powder was sieved through a #70 sieve (210 µm) (Newark Wire Cloth Co., Newark, NJ). Grinding and sieving was repeated three times, and the resultant powder was pooled. Powders were stored in Pet/Al/PE laminate pouches and kept in -20°C freezer. All pomace powder processing was done in duplicate.

Pomace was also freeze-dried as a control using a Freezemobile 25 SL Unitop 600L freeze dryer (Virtis Company, Gardiner, NY), and dried for 48 h. Pomace was frozen with liquid nitrogen and placed into the freeze drier on previously chilled (-35°C) plates. When pomace temperature was in equilibrium with the plates, vacuum was pulled. After two hours, plate temperature was raised to 25°C to promote sublimation.

Chemical Analysis

Preparation of Crude Blueberry Pomace Powder Extracts

To determine effects of drying on the polyphenolic fraction of the pomace powders, a crude extract was created from the blueberry pomace powder, using a modified method from Srivastava et al. (2010). Seven and a half gram samples were mixed with 70% (v/v) acidified acetone (0.1% HCl) and blended using a Polytron PT

1200 homogenizer (Kinematica AG, CH-6014 Luzern) at 5000 rpm for 5 min. The slurry was vacuum filtered through #1 Whatman (Whatman International Ltd, ME14 2LE, UK) filter paper. Extraction and filtration was repeated three times. All filtrates were pooled, and acetone was evaporated under vacuum using a rotary evaporator. Extractions were freeze dried using a Freezemobile 25 SL Unitop 600L freeze drier (Virtis Company, Gardiner, NY) and stored at -40°C. Extractions of each sample were performed in triplicate.

Total Monomeric Anthocyanins

Total monomeric anthocyanins (TMA) content was measured by the pH differential method as described by Giusti & Wrolstad (2001). Freeze dried crude extract was dissolved in methanol (0.5 mg/ml). Methanolic dilutions were combined, in a 1:5 ratio, with 0.025 M potassium chloride buffer and 0.4 M sodium acetate buffer and allowed to equilibrate for 15 min. Solutions were measured with a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA) at wavelengths 510 nm and 700 nm. All measurements were done in triplicate. Concentrations were reported as cyanidin-3-glucoside determined by absorbance using Equation 1:

$$TMA = \frac{(A \times MW \times DF \times 100)}{(\epsilon \times l)} \quad (\text{Eq. 1})$$

where

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

MW = molecular weight of cyanidin-3-glucoside

DF = dilution factor

ϵ = molar absorptivity coefficient of cyanidin-3-glucoside

Values are reported as mg C3G/g DS (dry solids).

Total Phenolics Content

Total phenolics content (TPC) was determined colorimetrically by the Folin–Ciocalteu method as described by Amarowicz et al. (2004). Freeze dried crude extracts were dissolved in methanol (0.5 mg/ml), and two milliliters of the methanolic solution was pipetted into a test tube containing 6 ml of distilled water. After mixing, 0.5 ml of Folin–Ciocalteu phenol reagent and 1 ml of saturated sodium carbonate solution were added. The contents were vortexed for 15 s. The test tubes were then left to rest at room temperature (25°C) for 45 min. Afterwards, absorbance readings were taken at 750 nm using a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA). Gallic acid was used to prepare a standard curve. Readings were done in triplicate and results are reported in mg GAE/g DS (dry solids).

HPLC Determination of Anthocyanins

Reverse phase separation of anthocyanins was done as described by Lee et al. (2002) using an HP 1090 Series II HPLC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a UV diode array detector and a Waters Spherisorb (Waters Corporation, Milford, MA) 5 µm ODS (2) (250 x 4.6 mm) column. The solvent system used a linear gradient of 94% solvent A and 6% solvent B to 75% solvent A and 25% solvent B in 55 min, where solvent A was 4% phosphoric acid and solvent B was 100% acetonitrile. Detection was taken at 520 nm. Extracts were dissolved in 10 ml of methanol and filtered through a 0.45 µm Phenex nylon filter (Phenomenex, Torrance, CA) before HPLC injection. Anthocyanins were identified by elution time and spectral analysis of

delphinidin and cyanidin standards. Anthocyanins were quantified using delphinidin as an external standard and reported as mg del/g powder.

Hydrolysis of anthocyanins into anthocyanidins was done as described by Yi et al. (2005). Anthocyanin extracts were dissolved in 50% methanol with 2N HCl. Samples were capped and placed in a water bath at 80°C with constant shaking at 200 rpm for 2 h. Hydrolyzed samples were analyzed by HPLC as described above.

Statistical Analysis

Pomace powder production was replicated twice. Extractions were made in triplicate and all analyses were done in triplicate. Analysis of variance was performed using JMP (SAS Institute Inc., Cary, NC). Tukey's post hoc testing was used to determine difference amongst means.

RESULTS AND DISCUSSION

Temperature-Time Curves

Anthocyanins are heat labile and therefore it is important to know not only the temperature of the dryer, but also the product temperature during drying to understand the processing effects on the phytochemicals. Temperature-time curves are illustrated in Figure 4.2. During initial drying, the product experienced a drop in temperature due to evaporative cooling. At the low chamber pressure (2.7-3.3 kPa), rapid evaporation or even boiling can occur, and rapidly escaping water absorbs heat from the product, thereby decreasing the temperature of the product (Bisbee, 2007). After the initial 5-10 mins, the product temperature gradually rose but stayed well below the set temperatures of the heating plates. Pomace processed at 80°C had a final temperature of 65°C at the

end of drying and was the lowest of all three processing conditions. Pomace processed at 100°C had a final temperature of 88°C, and pomace processed at 120°C had a final temperature of 97°C.

Total Monomeric Anthocyanins and Total Phenolics Content

Total monomeric anthocyanins and total phenolics content are reported in Table 4.1. Both vacuum belt drying at temperatures >100°C and deseeding decreased anthocyanins in pomace powder compared to freeze dried control. Freeze dried pomace powders (with seeds) had TMA values of 17.3 mg C3G/g DS. Only samples dried at 120°C had significantly lower TMA values (12.2 mg C3G/g DS). After deseeding, the freeze-dried control powders contained 14.4 mg C3G/g DS, while those powders dried at 80°C and 120°C contained 11.5 and 9.5 mg C3G/g DS, respectively. Similar to blueberry pomace powders, it was found that apple pomace powder only had decreased TMA when vacuum belt dried above 100°C (Yan & Kerr, 2013). Again, as Figure 2 shows, at plate temperatures of 100°C the product temperature stayed below 85°C for most of the drying period, and while at plate temperatures of 80°C the pomace temperature stayed below 65°C.

While the differences in TMA compared to freeze-dried control were significant for pomace dried at 120°C, changes were still much lower than seen with other types of drying. Lohachoompol et al. (2008) reported almost a 50% reduction of anthocyanins in blueberries dried in a cabinet dryer at a reducing temperature profile (90°C for 90 min, then 70°C for 120 min, and then 50°C for 120 min). Comparatively, pomace dried in the vacuum belt drier at 80°C for 100 min, not only was dried in one third of the time, but with considerably less (~10%) anthocyanin loss.

For all temperatures, pomace with seeds contained higher total anthocyanins (12.2-17.3 mg C3G/g) than pomace without seeds (9.5-14.4 mg C3G/g). While the seeds contribute the least towards the total berry anthocyanin content (0.1 mg C3G/100 g berry), it is likely the reduction could be due to the deseeding process (Lee & Wrolstad, 2004). Skins contribute the largest portion of the anthocyanin content (~900 mg C3G/100 g skins), and during the deseeding process of the dried material, larger skin pieces (> 212 μ m) would have also been removed with the seed component (Wang et al., 2012a).

Unlike the anthocyanins, total phenolics in vacuum-dried products were not significantly different than in the freeze-dried material. Freeze dried powders with seeds contained 38.3 mg GAE/g DS, while those after deseeding contained 32.4 mg GAE/g DS. While vacuum-drying temperature was not a factor, the deseeding process did result in lower TPC values for both freeze-dried and vacuum-dried products at each temperature.

Blueberries are known to be a source of phenolic acids, 113 mg/100 g fresh weight, and proanthocyanins, >19 mg/g fresh weight (Gu et al., 2002; Häkkinen et al., 1999; Ayaz et al., 2005). Freeze drying has been demonstrated to maintain or even increase the polyphenols (750-900 mg/100 g DM) of blueberries, and therefore can be considered the most preferred drying method when trying to maintain polyphenols in dried blueberry products (Reyes et al., 2011). Freeze drying involves relatively long drying times, and even though energy consumption by the vacuum belt drier can be variable (42.92-188.21kWh/kg), the low processing time makes it more efficient when compared to freeze drying (Xu, 2012). There were no statistical differences between vacuum belt dried blueberry pomace powder total phenolics content (33.4-44.7 mg GAE/g DS) and freeze-dried pomace powder (32.4-38.3 mg GAE/g DS). Similarly, it

was reported that vacuum belt dried muscadine powder contained total phenolics content (453-642 $\mu\text{mol GAE/g DW}$) comparable to freeze dried powder (583 $\mu\text{mol GAE/g DW}$) (Vashisth et al., 2011). Temperatures above 60°C have shown to negatively affect the proanthocyanin content of blueberry pomace so it is possible that some reduction in proanthocyanin content has occurred since all products reached a temperature of at least 65°C during processing (Khanal et al., 2010).

HPLC Characterization and Quantification

The separation of anthocyanins by HPLC is shown in Figure 4.3. Fourteen distinct peaks were observed at 520 nm, which is in agreement with others (Lee et al., 2002; Lee & Wrolstad, 2004). Peaks were identified using standards' spectral analyses and retention times. The elution order was as follows: dephinidin-3-galactoside, delphinidin-3-glucoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-glucoside, petunidin-3-glucoside, cyanidin-3-arabinoside, petunidin-3-glucoside, peonidin-3-galactoside, petunidin-3-arabinoside, peonidin-3-glucoside, malvidin-3-galactoside, malvidin-3-glucoside, and malvidin-3-arabinoside. Hydrolysis of the crude blueberry extracts showed four major anthocyanidins: delphinidin, cyanidin, malvidin, and peonidin. This differs from Wang et al. (2012b) where petunidin glycosides have been found instead of peonidin glycosides in the rabbiteye cultivar 'Reade'. Large differences for flavonoids exist between cultivars of rabbiteye blueberries because of the variability in quantities of aglycones and glycosides (Lohachoompol et al., 2008).

Quantification of anthocyanins was done using the standard addition method with delphinidin (Table 4.2). A standard curve was created, giving an R-squared of 0.999. For rabbiteye blueberries, the anthocyanin composition consists of delphinidin glycosides,

cyanidin glycosides, petunidin glycosides, peonidin glycosides and malvidin glycosides, with malvidin glycosides being the most abundant (Hamamatsu et al., 2004). Factors that resulted in significant changes were the deseeding process and temperature. The pomace powder with seeds contained more of each anthocyanin than the pomace powder without seeds. There were no statistical differences in the two freeze dried controls. For pomace dried at both 80°C and 100°C, anthocyanidin glycosides levels were not significantly different than the freeze dried control.

Blueberry pomace powder dried at 120°C contained 22.5-36.9% less of various anthocyanidin glycosides compared to the freeze-dried. For vacuum belt dried pomace powder with seeds, delphinidin glycosides were the most different from the freeze-dried control (30.5%), followed by the malvidin glycosides (27.6%), cyanidin glycosides (24.4%), peonidin glycosides (23%) and petunidin glycosides (22.5%). When seeds were removed from the pomace powder, the percent difference from the freeze dried control increased for all the anthocyanidin glycosides. Malvidin glycosides had the greatest difference from control (36.9%), followed by petunidin glycosides (36%), delphinidin glycosides (31.6%), peonidin glycosides (29.8%) and cyanidin glycosides (29.6). During thermal degradation, anthocyanidin glycosides go through a deglycosylation step to the associated anthocyanidin, and the anthocyanidin is cleaved into further degradation products (Sadilova et al., 2006). For blueberries heated at temperatures above 100°C, it was determined that anthocyanidins did not degrade similarly as malvidin and petunidin were less thermally stable than cyanidin and delphinidin (Queiroz et al., 2009).

CONCLUSION

Dried blueberry pomace powder can be used as an ingredient by the food industry to incorporate color and phytochemicals into food products. While freeze-drying is protective of heat labile compounds, it is energy inefficient, taking up to 72 h to create a batch of pomace powder. Vacuum belt drying is a good alternative to freeze drying due to the short processing time. At temperatures below 100°C, vacuum belt drying produced powders with anthocyanin and total phenolics contents comparable to the freeze-dried powder in less than two h. While product temperature during vacuum belt drying was higher than found with freeze-drying, the product temperatures were still relatively low and for shorter times as compared to other forms of drying. However, removal of seeds can be detrimental to the phytochemicals present in the pomace powder due to loss of larger skin particles during the deseeding process.

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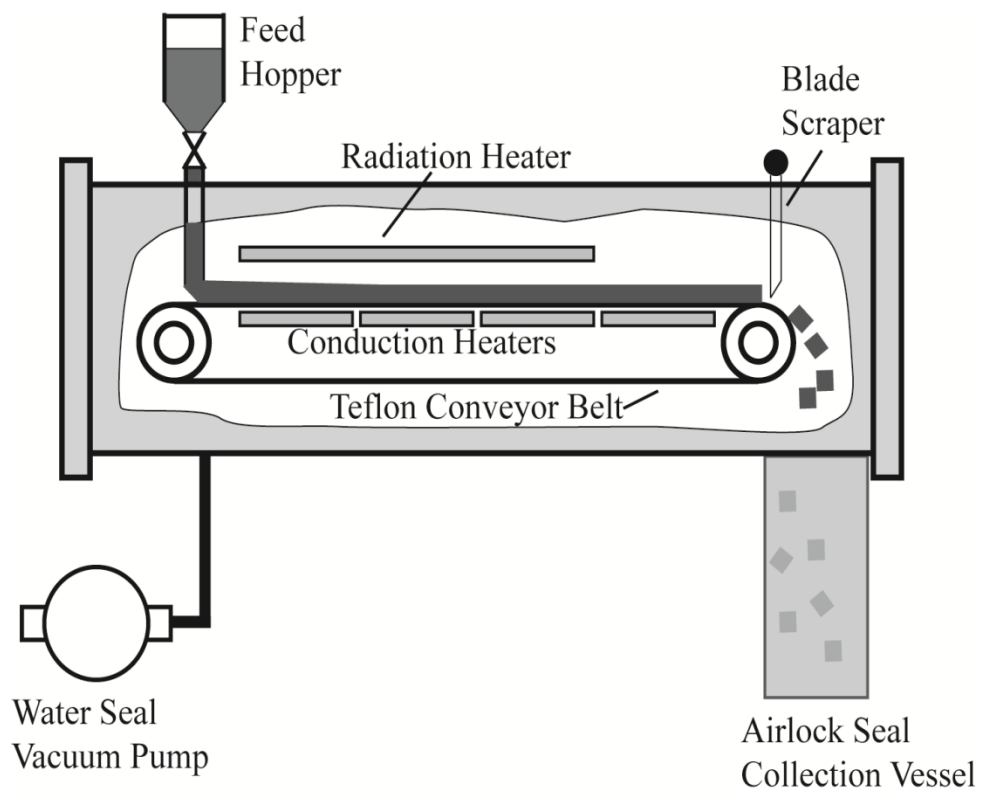


Figure 4.1: Schematic of a pilot scale continuous vacuum belt dryer.

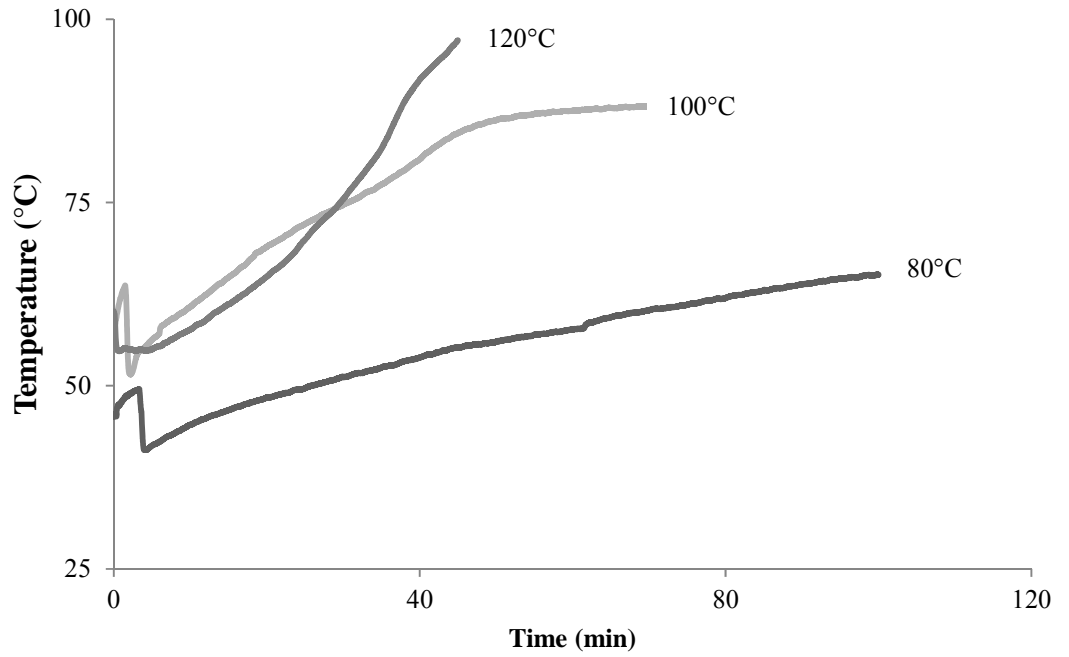


Figure 4.2: Product temperature during vacuum belt drying of blueberry pomace at plate temperatures of 80, 100, or 120°C

Table 4.1: Total monomeric anthocyanin (TMA) and total phenolics content (TPC) of freeze-dried (FD) and vacuum belt dried (VBD) blueberry pomace powders dried at three temperatures.^{1,2}

	TMA (mg C3G/g DS) ¹		TPC (mg GAE/g DS)	
	Mean	SE	Mean	SE
Pomace without seeds				
FD	14.4 ^{ab}	0.75	32.4 ^{abc}	3.61
VBD 80°C	11.5 ^c	0.38	33.4 ^{bc}	1.80
VBD 100°C	12.7 ^{bc}	0.44	33.8 ^{bc}	2.08
VBD 120°C	9.5 ^d	0.36	34.4 ^{bc}	1.80
Pomace with seeds				
FD	17.3 ^a	0.75	38.3 ^{abc}	3.61
VBD 80°C	15.4 ^a	0.34	44.7 ^a	1.54
VBD 100°C	14.9 ^a	0.29	40.8 ^a	1.24
VBD 120°C	12.2 ^{bc}	0.29	36.7 ^{bc}	1.36

¹TMA reported as cyaniding-3-glucoside equivalents and TPC reported as gallic acid equivalents

²Means in the same column followed by similar letters are not significantly different (p<0.05)

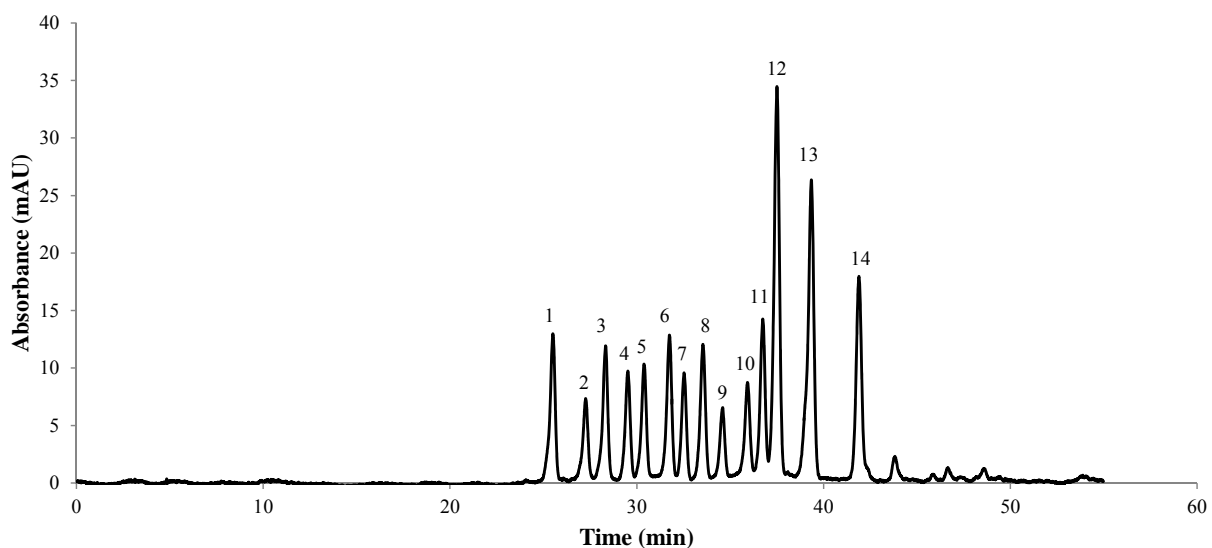


Figure 4.3: HPLC chromatogram of vacuum belt dried blueberry pomace powder. The elution order was (1) dephinidin-3-galactoside, (2) delphinidin-3-glucoside, (3) cyanidin-3-galactoside, (4) delphinidin-3-arabinoside, (5) cyanidin-3-glucoside, (6) petunidin-3-glucoside, (7) cyanidin-3-arabinoside, (8) petunidin-3-glucoside, (9) peonidin-3-galactoside, (10) petunidin-3-arabinoside, (11) peonidin-3-glucoside, (12) malvidin-3-galactoside, (13) malvidin-3-glucoside, and (14) malvidin-3-arabinoside.

Table 4.2: Anthocyanins (mg del/g powder) as determined by HPLC-DAD in freeze dried (FD) and vacuum belt dried blueberry (VBD) pomace powders dried at three temperatures.¹

Anthocyanin	Powder with Seeds				Powder with Seeds Removed			
	FD	VBD 80°C	VBD 100°C	VBD 120°C	FD	VBD 80°C	VBD 100°C	VBD 120°C
Delphinidin-3-galactoside	0.69 ^{ab}	0.75 ^a	0.71 ^{ab}	0.54 ^{abc}	0.51 ^{abc}	0.42 ^c	0.53 ^{bc}	0.36 ^c
Delphinidin-3-glucoside	0.39 ^{ab}	0.42 ^a	0.41 ^{ab}	0.30 ^{bc}	0.33 ^{abc}	0.25 ^c	0.29 ^{bc}	0.22 ^c
Cyanidin-3-galactoside	0.54 ^{abc}	0.63 ^a	0.62 ^{ab}	0.43 ^{bc}	0.41 ^{bc}	0.36 ^c	0.47 ^{abc}	0.33 ^c
Delphinidin-3-arabinoside	0.56 ^{ab}	0.42 ^a	0.41 ^{ab}	0.30 ^{bc}	0.33 ^{abc}	0.25 ^c	0.29 ^{bc}	0.22 ^c
Cyanidin-3-glucoside	0.53 ^{ab}	0.55 ^a	0.53 ^{ab}	0.40 ^{bc}	0.41 ^{abc}	0.34 ^c	0.41 ^{bc}	0.29 ^c
Petunidin-3-galactoside	0.68 ^{ab}	0.74 ^a	0.71 ^{ab}	0.53 ^{bc}	0.57 ^{abc}	0.43 ^c	0.53 ^c	0.36 ^c
Cyanidin-3-arabinoside	0.53 ^{ab}	0.55 ^a	0.49 ^{abc}	0.38 ^{bcd}	0.43 ^{abcd}	0.33 ^{cb}	0.4 ^{bcd}	0.26 ^d
Petunidin-3-glucoside	0.62 ^{ab}	0.68 ^a	0.67 ^{ab}	0.49 ^{bc}	0.51 ^{abc}	0.40 ^c	0.47 ^{bc}	0.35 ^c
Peonidin-3-galactoside	0.30 ^{ab}	0.34 ^a	0.34 ^a	0.25 ^{ab}	0.25 ^{ab}	0.21 ^b	0.27 ^{ab}	0.20 ^b
Petunidin-3-arabinoside	0.48 ^{ab}	0.50 ^a	0.51 ^{ab}	0.36 ^{abc}	0.42 ^{abc}	0.29 ^c	0.37 ^{bc}	0.25 ^c
Peonidin-3-glucoside	0.70 ^{ab}	0.75 ^a	0.72 ^{ab}	0.52 ^{bc}	0.59 ^{abc}	0.46 ^c	0.55 ^{bc}	0.39 ^c
Malvidin-3-galactoside	1.79 ^{ab}	2.00 ^a	1.85 ^{ab}	1.34 ^{bc}	1.46 ^{abc}	1.18 ^c	1.42 ^{bc}	0.97 ^c
Malvidin-3-glucoside	1.56 ^{ab}	1.69 ^a	1.58 ^{bc}	1.13 ^{bc}	1.31 ^{abc}	1.04 ^c	1.21 ^{bc}	0.83 ^c
Malvidin-3-arabinoside	1.03 ^{ab}	1.14 ^a	0.98 ^{abc}	0.70 ^{bcd}	0.86 ^{abcd}	0.67 ^{cd}	0.80 ^{bcd}	0.49 ^d

¹Means followed by same letter in the same row are not significantly different (p<0.05).

CHAPTER 5
PHYSICAL ATTRIBUTES AND SENSORY PREFERENCE FOR EXTRUDED
SORGHUM SNACKS INCORPORATING VACUUM BELT DRIED BLUEBERRY
POMACE

Kitchen KM, Xu S & Kerr WL. Submitted to *Food and Bioprocess Technology*

ABSTRACT

Extruded snacks were produced using white sorghum and 0-6 g/100 g dry feed (DF) of blueberry pomace powder produced by vacuum belt drying. Samples were extruded at three exit temperatures (110, 120, and 130°C). Addition of the pomace powder created a darker purple color due to the inclusion of the anthocyanins naturally found in blueberry. Higher extrusion temperature and pomace levels decreased expansion and increased bulk density compared to sorghum-only control. The control processed at 120°C had the greatest expansion (5.43:1) and lowest bulk density (4.0 g/100 ml), while the extruded snack with 6 g pomace/100 g DF processed at 130°C had the lowest expansion (3.49:1) and largest bulk density (8.3 g/100 ml). Pomace powder also produced a harder, crisper extruded snack compared to the control. Even though the pomace powder affected expansion, bulk density and texture, all levels were still equally preferred in a force-choice ranked consumer test.

INTRODUCTION

Snacks make up an increasingly large portion of the U.S. diet, accounting for an average of 580 extra calories per day (Duffey & Popkin, 2011). Extrusion is one important method for creating ready-to-eat cereals or puffed snacks with cheese or other flavored coatings (Frame, 1994). It is energy efficient due to its ability to use high temperature and short residence times in a continuous system. Single and twin-screw extruders have revolutionized the snack food industry with basic designs that have minimized cost, time, and energy while providing high production levels (Booth, 1990).

As consumers are becoming more aware of food and its role in health, the food industry is researching alternative ingredients for extruded snack foods. Current trends include the incorporation of gluten-free flours and fruit or vegetable-based ingredients, as well as the creation of more nutrient-dense snack foods. Gluten-free diets have become a lifestyle choice, not only for those who suffer from celiac disease, but also for other consumers who may have gluten sensitivities. This has resulted in an increase in US sales to \$2.5 billion for gluten-free products in 2010 (Sapone et al., 2012). Sorghum is a gluten-free ancient grain and its use as a flour for baked goods and other gluten-free products has increased. After wheat, rice, corn, and barley, sorghum is the fifth most important grain worldwide (Awika & Rooney, 2004). Sorghum contains a high percentage of starch (56-73 g/100 g), which makes it a good option for extruded puffed snacks. During extrusion, starch structural changes due to gelatinization are vital in creating networks associated with puffed snacks texture (Jadhav & Annapure, 2013; Lai & Kokini, 1991). Sorghum extruded snacks have been found to have high likeability, with scores of 7 to 8 on a 9-point hedonic scale; thus, it is a good option for gluten-free extruded snacks (Zamre et al., 2012).

In addition to concerns with celiac disease, food-for-health has come into focus as the quality of nutritional intake for children and adults affects the risk of chronic diseases, including type 2 diabetes, cardiovascular disease, and osteoporosis (Anon, 2007). One disadvantage to sorghum is that it provides little nutrition other than being a carbohydrate source. Therefore, creating value-added extruded snack products using fruit or vegetable based ingredients is a viable option for a market looking for more nutritious snacks. While the addition of fruits or vegetables into snacks may create a product with added

nutrients, fiber and phytochemicals, they may also affect the physical and sensory qualities of the extruded product. The addition of nutrient-rich ingredients, particularly those with fiber, has been shown to increase the density and hardness of some extruded products (Chanvrier et al., 2007). Fruit and vegetable pulp (up to 10% by weight) have been added to rice/maize extruded snacks, increasing the nutritional profile without affecting the physical properties (Jain et al., 2013; Tangirala et al., 2012). In contrast, extruded snacks formulated with different whole-fruit powders showed an increase of both soluble and insoluble fiber, while creating a denser, less expanded, and harder extruded product. However, such snacks were still found to be acceptable by consumers (Potter et al., 2013, Gamlath 2008).

Fruit and vegetable pomaces are the waste material left after juice processing and can account for as much as one third of the original weight of material (O'Shea et al., 2012). Fruit and vegetable pomaces are available in large amounts and can be a low-cost method to increase fiber and phytochemicals in extruded snack products (Kumar et al., 2010). For example, when grape and tomato pomaces were added to barley to increase fiber and nutrient content, researchers found that while the extruded snacks were harder than the non-pomace control, the products containing pomace were still acceptable to consumers (Altan et al., 2008a; Altan et al., 2008b).

Blueberry pomace is a promising ingredient for inclusion into snacks as it contains several nutrients, fiber and high levels of anthocyanins (Lee & Wrolstad, 2004). Due to the antioxidant and anti-inflammatory activity of anthocyanins, these flavonoids have been associated with many health benefits, such as reducing the risk of cardiovascular disease and cancer (Duthie et al., 2006). Some work has been done with

blueberry pomace as an ingredient for extruded snack products. Unfortunately, due to the heat treatment, extrusion was detrimental to anthocyanins within the pomace. Freeze-dried blueberry pomace used in extrusion experienced a 33 to 42% reduction in total anthocyanins (Camire et al., 2002). Even though extrusion reduces the anthocyanin content, the level of monomers, dimers and trimers of procyanidins have been found to increase in sorghum and blueberry pomace with extrusion (Khanal et al., 2009). The reduction in size of the oligomeric procyanidins may help increase their bioavailability.

While freeze-dried pomace retains high anthocyanin content, freeze drying can be relatively costly due to energy use and long drying times. An alternative drying option is continuous vacuum belt drying. Vacuum belt drying (VBD) has been shown to produce apple pomace powders with good flowability while preserving the phytochemicals and fiber in a manner similar to freeze-dried pomace (Yan & Kerr, 2013). VBD blueberry pomace powders have been shown to have good flowability and color retention but, no work has been done on the use of these powders in snack products (Kim, 2012). The objective of this study was to determine the effects of adding different levels of VBD blueberry pomace powders and extrusion temperature on the physical and sensory attributes of sorghum based extruded snacks. This included the characterization of color differences, moisture content and water activity, expansion and density, and textural properties related to hardness and crispness. In addition, consumer preference for the different products was also tested.

MATERIAL AND METHODS

Processing

Pomace Production:

Frozen rabbiteye blueberry (*Vaccinium ashei*) varieties 'Brightwell' and 'Tifblue' were contributed from the University of Georgia Blueberry Research and Demonstration Farm (Alma, GA). The frozen berries were crushed in a grinder (Hobart Model 98, Hobart Corporation, Troy, OH). The resultant mash was heated in a steam kettle to 40°C. Pectinex Ultra SP-L (Novozymes, Bagsvaerd, Denmark) was added (0.26 ml/kg mash) and held for 1.5 hr. The mash was pressed in a pilot scale juice press (Bucher HP 14 Laboratory Press, Bucher Industries AG, Murzlenstrasse 80, CH-8166 Niederweningen) yielding ~70% juice. The leftover pomace was frozen and stored at -20°C.

Vacuum Belt Dried Pomace Powder Production:

Pomace was removed from the -20°C freezer and allowed to thaw for 24 h at 4°C. Pomace was dried using a pilot scale Bucher Dryband continuous vacuum belt dryer (Bucher Unipektin AG, Switzerland) at 90°C for 60 min until all samples reached a 0.20-0.25 water activity. Pomace was placed directly onto a 22 cm Teflon belt that passed over 3 conduction heating plates (zones 1, 2, and 3). Also a 22.9 cm radiation plate was set 4.7 cm above zones 1, 2, and 3. All heating zones and the radiation were set at the same temperature during processing. Vacuum was held constant at 2.7-3.3 kPa (20-25 torr). Dried pomace was ground in a pilot scale food processor (R10DVV, Robot Coupe U.S.A., Inc., Jackson, MS) for 1 min to form a powder.

Extrusion

Harvest pearl white sorghum flour was donated from ADM (ADM Milling Co., Plainview, TX). Extrusion of the sorghum with dried blueberry pomace snack was done in a pilot scale 25:1 twin screw extruder (MPF30, APV Baker, Inc., Staffordshire, England). The screw profile configuration was as follows: (3) 1.5D twin lead feed screws, (1) 1D twin lead feed screw, (5) 30° forward peddles, (3) 1.5D twin lead feed screws, (2) 60° forward peddles, (3) 60° backward peddles, (3) 60° forward peddles, (3) 1.5D twin lead feed screws, (7) 60° forward peddles, (5) 30° forward peddles, and (1) 1D single lead discharge screw. The extrusion die included a 2 mm diameter hole. Calibration of solids feeder and water input was done before processing. Formulations of 0 g/100 g, 2 g/100 g, 4 g/100 g, and 6 g/100 g dry feed (DF) of pomace powder:sorghum were conveyed via a screw pump and combined with water to a moisture content of 17.5 g/100 g. Exit temperatures were set at 110, 120, or 130°C. Immediately following extrusion, samples were dried in an impingement oven at 90°C for 10 min. Samples were sealed in water- and air- impermeable bags.

Physical Properties

Color

Color was measured using a Chroma Meter CR-410 colorimeter (Minolta Co., Ltd., Osaka, Japan). Calibration was done using a standard white calibration tile. Samples were ground with a Model 111338 grinder (General Electric, Fairfield, CT) and arranged in a single layer on a petri dish. Color measurements were reported in L*, C*, h° and were done in triplicate.

Moisture Properties

Ground samples were also used for measurement of water activity and moisture content. Water activity was determined using an Aqualab CX-2 (Aqua Lab Technologies, Riverside, CA) water activity meter. Moisture content was determined on samples by a modified AOAC official method 925.04 (Anon, 2005). Samples were weighed and placed in a vacuum oven at 70°C and allowed to dry for 72 h. After 72 h, sample weight was checked to determine if it had stabilized. After equilibration in a desiccator for 24 h, samples were weighed again. Both moisture content and water activity were measured in triplicate.

Expansion and Bulk Density

Expansion ratio was determined by dividing the diameter of the extruded product by the diameter of the extruder die (2 mm). The diameter of the product was measured by digital calipers. The expansion ratio was measured for each treatment. Bulk density was measured by placing the extruded product into a tared 400 ml glass cylinder container without packing and then weighing. Bulk density measurements were measured six times.

Texture

Textural attributes were analyzed using a TA-XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY) with a 5 kg load cell. A penetrometer test was performed using a 3 mm ball probe with a test speed of 1.0 mm/s. Product hardness was measured as the peak force (N) as the probe descended. The gradient force was determined from the force/time ratio (N/s) from the origin to the first fracture, and gives a measure of the product brittleness. Assessments of crispness were done by analyses of the

“linear distance”. This routine characterizes the jagged response of force-distance curves for crisp products, measuring the total point-to-point distance from the origin to the maximum force. In general, as crisper products have more fractures over time, they have larger linear-distance measurements.

A three-point bend test was also performed as described by Altan et al. (2008a), and used as a measure of snapping force. A rounded blade was lowered at 2 mm/s and broke through extruded samples held on two supports 22 mm apart. Snapping force was taken as the maximum force. All texture data were collected using Texture Expert Exceed software.

Sensory

A forced-choice preference ranking consumer panel (n = 52) was performed to determine if consumers preferred one sample over others. Each panelist was given a control, 2, 4, and 6 g /100 g DF pomace snack processed at 120°C. Panelists were asked to rank samples 1 to 4, where 1 was the most preferred sample and 4 was the least preferred sample. Panelists were also given the option to leave remarks about each sample. Data were analyzed by computing the rank sum for each treatment group, and determining if the difference amongst groups was greater than the critical value (33.8) found from published tables (Basker, 1988).

Statistical Analysis

For all physical measurements, analysis of variance was performed using JMP (SAS Institute Inc., Cary, NC). Color measurements were done 3 times. Expansion ratio was measured 15 times. Bulk density and texture measurements were taken 6 times. Tukey’s post hoc testing was used to determine difference among means.

RESULTS AND DISCUSSION

Color

The effects of pomace addition and extrusion temperature on product color are shown in Table 5.1. The control (sorghum only) was the lightest ($L^* = 75.27-78.21$) of the extruded products. Samples were darker when greater levels of pomace powder were added, and extruded snacks with 6 g/100 g DF blueberry pomace powder were the darkest ($L^* = 25.34-29.18$). The color of the extruded product containing blueberry pomace was less saturated than control ($c^* = 14.87-15.60$), with 2 g/100 g DF having the least intense color ($c^* = 7.28-7.38$). The hue of the control was in the yellow to orange range ($h = 85-87^\circ$), and for all samples containing pomace powder, the hue was in the blue-purple to red region ($h = 346-361^\circ$). For the control, color was likely due to a combination of non-enzymatic browning reactions including Maillard reaction and caramelization of sugars (Lei et al., 2007). The color of extruded snacks produced with 6 g/100 g DF pomace powder was blue, while snacks with 2-4 g/100 g DF powder were more purple. Similarly, Camire et al. (2002) found that extruded cereals products made with blueberry concentrate were darker and bluer than control. Extrusion temperature did not play a role in final product color.

The increase in blue-purple color is due to the anthocyanins and co-pigments present in the blueberries. Anthocyanins are a natural colorant and are responsible for the reds, blues, and purples in fruits and flowers (Castañeda-Ovando et al., 2009). Camire et al. (2007) studied a variety of anthocyanin-containing fruit powders for their use as natural colorants of breakfast cereals. The hue of the extruded products ranged with the fruit source: blueberry ($h = 50.22^\circ$), Concord grape ($h = 70.08^\circ$), cranberry ($h = 58.02^\circ$),

and red raspberry ($h = 53.58^\circ$). These products were all darker and more red than the extruded control ($h = 88.39^\circ$).

Moisture Properties

Moisture content and water activity (Table 5.2) were affected by extrusion temperature and level of pomace powder, although the differences were not large. Final moisture contents ranged from 4.27 to 7.41 g/100 g. Snacks with 6 g/100 g DF pomace processed at 130°C had the highest moisture content (7.41 g/100 g) and water activity (0.42) while those with 6 g/100 g DF pomace processed at 120°C had the lowest moisture content (3.34 g/100 g) and water activity (0.13).

Moisture content is important in extrusion as a factor in starch gelatinization within the barrel of the extruder and in the expansion of the product after exiting (Lee et al., 2000). As the moisture content and water activity were not correlated with expansion, differences observed in the extruded snacks were not likely due to extrusion *per se* or the addition of the blueberry pomace powder. Differences were more likely due to inconsistencies during secondary drying in the impingement oven.

Expansion Ratio and Bulk Density

Pomace and temperature were significant factors for expansion ratio and bulk density (Table 5.2). As pomace powder levels increased in the extruded snacks, expansion was reduced and bulk density increased. Overall, the control had the greatest expansion and the lowest bulk density compared to all other treatments. The control processed at 120°C had the greatest expansion (5.43:1) and lowest bulk density (4.0 g/100 ml) compared to all other treatment levels. Bulk density increased for all samples as the amount of pomace added increased. For example, for product extruded at 120°C

bulk density increased from 4.0 g/100 ml for control to 8.5 g/100 ml for snacks with 6 g/100 g DF pomace powder. For each temperature level, extruded snacks containing 6 g/100 g DF pomace powder (8.2-8.5 g/100 ml) were denser than control (4.0-7.0 g/100 ml).

Addition of other fruits and vegetables has also been reported to decrease expansion and increase bulk density. Extruded corn cereals formulated with blueberry concentrate created less expanded products and increased bulk density when compared with control (Chaovanalikit et al., 2003). When dried pumpkin peels (30 g/100 g of dry feed) were added to a corn grit extruded snack, the expansion decreased significantly. It has been suggested that the decrease of expansion is due to the increase of dextrinization and weakening of the extruded puff structure (Norfezah et al., 2012). Siddiq et al. (2008) suggested that the fiber and sugars present in the fruit matrix are responsible for the increase in bulk density of the extruded product. Non-starch carbohydrates present in the pomace fiber may bind water more tightly than the protein and starch in the flour. The network formed by the fiber may also be more resistant to flashing off of water as the product exits the extruder die, thereby reducing expansion and increasing bulk density (Yağci & Göğüs, 2008).

Texture

Results from the penetration and snapping tests are shown in Table 5.3. The maximum force on penetration is an indication of the overall hardness of the product. In general, the addition of pomace increased sample hardness, and samples with 6 g/100 g DF pomace showed the highest maximum force (18.78 – 23.75 N). Snacks made with 6 g/100 g DF pomace powder at 110°C were significantly harder (23.75 N) than the control

snack processed at 120°C (9.86 N) and snacks with 4 g/100 g DF pomace powder processed at 130°C (10.19 N). The barrel temperature of the extruder is typically important in the final hardness of the product, with the increase of temperature decreasing the hardness of the final product. It has been suggested that increasing temperature would decrease the melt viscosity (resistance to flow of the polymeric material), which promotes bubble growth, higher expansion and a softer product (Ding et al., 2006). In this study, temperature was not found to be a significant factor.

Crisp products tend to have a force/distance profile with multiple fractures and therefore relatively large “linear distance”. The control processed at 120°C (41.30 mm) had the smallest linear distance, indicating that it would be the least crisp of all samples. Within each temperature group, samples with 6 g/100 g DF pomace powder had the highest linear distance, suggesting they were crisper than other samples. Samples with 6 g/100 g DF pomace powder and processed at 130°C had the greatest linear distance of all samples (141.96 mm). Others have also found that incorporation of pomace influences the crispness of extruded products. When 17-28 g/100 g of apple pomace was added to an extruded puffed cereal, the product crispness increased as level of pomace rose. With added pomace, the extrudate cell walls were less homogenous and more sensitive to the applied force, resulting in more fractures (Karkle et al., 2012). Interestingly, no significant differences were found in gradient force among the samples. Gradient force is an indication of the resistance of the material to breaking, and related to brittleness. Materials that are brittle tend to be fragile, lending themselves towards fracturing (Smith, 1974). Altan et al. (2008b) found that increasing pomace levels increased the gradient force of barley-tomato extrudates, but decreased product crispness. They suggested that

the added fiber increased the cell thickness, but had smaller cells that were more prone to rupture.

Temperature, pomace, and the interaction term were significant factors for snapping force. Extruded snacks processed at 110°C had the highest snapping force (13.15-19.18 N), while the control had the highest snapping force compared to all the other pomace levels. As with the maximum force, snacks made with 6 g/100 g DF pomace powder at 110°C (18.52 N) took more force to snap than the control at 120°C (8.58 N) and the control processed at 130°C (9.48 N). Snacks made with 2 g/100 g DF pomace powder (10.93-13.15 N) and 4 g/100 g DF pomace powder (10.89-13.42 N) were not different than the control.

Sensory

For sensory analysis, a forced-choice preference ranking test was performed as it is less sensitive to bias (Zavala, 1965). Rank sums ranged from 121 to 135 for samples with different pomace levels (Table 5.4). The critical difference value for 4 treatment groups at $p < 0.05$ was 33.8. Thus, there were no significant differences in preference amongst the samples. Panelists were given the option to leave comments to explain ranking of the extruded snacks, and major differences according to the panelists were attributed to texture and color, but differences did not affect overall preference. Panelists perceived the added color as a positive attribute. Rankings of the extruded snacks depended mostly on texture. Higher rankings were associated with perceived crispness while lower rankings were with associated with increased chewiness. Hardness was both considered a positive and negative attribute, and the extrudate with 6 g/100 g DF pomace was often commented on as too hard. Color preference was most affected by the

inclusion of the anthocyanins naturally found within the blueberry pomace powder. Durge et al. (2013) determined that using anthocyanins as colorants produced a highly acceptable color in rice extrudates, with scores of 6.55-8.00 on a 9-point hedonic scale. This implies that blueberry pomace powder may be added as a natural colorant source and a functional ingredient in extruded snacks and still be liked by consumers.

Even though blueberry pomace decreased expansion and created a firmer extruded snack at 6g/100g DF inclusion, there was still no difference in preference as compared to control. In other studies, extruded snacks formulated with 15-45 g/100g DF *Moringa oleifera* leaf were harder and less expanded, yet were still found to have high overall acceptability (6.13-7.53 on a 9-point hedonic scale) while the control processed with 22 g/100 g moisture content was the least acceptable (4.12) (Liu & Abughoush, 2011).

CONCLUSION

Vacuum belt dried blueberry pomace powder may be a cost effective way to incorporate healthy phytochemicals and fiber into extruded snack products. VBD blueberry pomace powder created darker, blue to purple snacks, and therefore may be used as a natural colorant for extruded snack products. Also, as pomace powder inclusion increased, extruded snacks became less expanded and denser. Snacks containing less than 6 g/100 g percent pomace had similar firmness, crispness, hardness, and snapping force as control. Even though addition of dried blueberry pomace powder affected physical properties, all samples were evenly preferred by consumers.

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Table 5.1: Color of sorghum flour and vacuum belt dried blueberry pomace powder snacks when extruded at three different exit temperatures¹

Exit Temperature (°C)	Pomace (g/100 g dry feed)	L*		C*		h°	
		Mean	SD	Mean	SD	Mean	SD
110	0	75.27 ^a	5.89	15.60 ^a	0.43	85 ^f	0.90
	2	55.19 ^b	0.40	7.38 ^d	0.28	357 ^{bc}	0.31
	4	49.22 ^{bc}	0.89	9.46 ^{bc}	0.39	353 ^{cd}	0.14
	6	25.34 ^d	0.71	7.91 ^{cd}	0.49	346 ^e	1.06
120	0	77.99 ^a	0.68	15.23 ^a	0.58	87 ^f	0.20
	2	54.78 ^b	0.96	7.38 ^d	0.24	361 ^a	0.43
	4	47.27 ^c	0.79	9.96 ^b	0.25	352 ^d	0.42
	6	26.81 ^d	2.94	7.87 ^{cd}	1.47	347 ^e	2.84
130	0	78.21 ^a	3.36	14.87 ^a	1.06	86 ^f	0.27
	2	54.20 ^b	0.83	7.28 ^d	0.29	359 ^{ab}	0.63
	4	47.49 ^c	1.12	9.68 ^{bc}	0.31	353 ^d	0.35
	6	29.18 ^d	1.45	8.39 ^{bcd}	0.73	346 ^e	3.24

¹Means in the same column followed by the same letter are not significantly different (p<0.05).

Table 5.2: Moisture properties, expansion, and bulk density of extruded sorghum snacks with added vacuum dried blueberry pomace powder when extruded at three different exit temperatures.¹

Temperature (°C)	Pomace (g/100 g dry feed)	Moisture Content (g H ₂ O/100g)		Water Activity		Expansion Ratio		Bulk Density (g/100ml)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
110	0	5.70 ^f	0.01	0.25 ^{cd}	0.027	4.88 ^b	0.47	6.5 ^d	0.3
	2	5.92 ^{def}	0.03	0.22 ^{de}	0.003	4.20 ^{cd}	0.32	7.9 ^{abc}	0.8
	4	6.32 ^{bc}	0.05	0.26 ^c	0.004	4.29 ^{cd}	0.16	7.4 ^{abcd}	0.7
	6	6.26 ^{bcd}	0.2	0.33 ^b	0.006	4.22 ^{cd}	0.25	8.2 ^{abc}	0.6
120	0	5.73 ^{ef}	0.17	0.24 ^{cd}	0.02	5.43 ^a	0.43	4.0 ^e	0.6
	2	4.70 ^g	0.07	0.17 ^f	0.007	4.28 ^{cd}	0.41	7.1 ^{bcd}	0.7
	4	6.09 ^{cde}	0.16	0.24 ^{cd}	0.003	4.29 ^{cd}	0.21	7.3 ^{abcd}	0.9
	6	3.34 ⁱ	0.02	0.13 ^g	0.005	4.03 ^d	0.16	8.5 ^a	0.4
130	0	6.50 ^b	0.16	0.23 ^{de}	0.015	4.51 ^c	0.18	7.0 ^{cd}	0.9
	2	4.27 ^h	0.08	0.15 ^f	0.019	3.98 ^d	0.23	7.7 ^{abcd}	0.6
	4	5.70 ^f	0.04	0.21 ^e	0.001	4.21 ^{cd}	0.21	7.0 ^{cd}	0.6
	6	7.41 ^a	0.24	0.42 ^a	0.011	3.49 ^e	0.40	8.3 ^{ab}	0.5

¹Means in the same column followed by the same letter are not significantly different (p<0.05).

Table 5.3. Penetration and snapping force of the extruded sorghum snacks with added vacuum dried blueberry pomace powder when extruded at three different exit temperatures.¹

Temperature (°C)	Pomace (g/100g dry feed)	Max Force (N)		Linear Distance (mm)		Gradient Force (N/s)		Snapping Force (N)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
110	0	16.79 ^{ab}	6.13	83.55 ^{ab}	42.14	8.32 ^a	6.05	19.18 ^a	6.00
	2	17.55 ^{ab}	5.21	67.65 ^{ab}	42.25	13.30 ^a	10.31	13.15 ^{abc}	3.17
	4	15.22 ^{ab}	4.47	98.46 ^{ab}	56.79	5.79 ^a	3.27	13.42 ^{abc}	3.21
	6	23.75 ^a	8.79	132.26 ^{ab}	60.52	10.22 ^a	5.84	18.52 ^{ab}	4.95
120	0	9.86 ^b	5.27	41.30 ^b	11.50	8.30 ^a	8.19	8.58 ^c	2.48
	2	14.11 ^{ab}	5.39	78.10 ^{ab}	49.93	8.96 ^a	8.17	12.88 ^{abc}	3.46
	4	12.72 ^{ab}	4.15	62.92 ^{ab}	44.89	7.72 ^a	6.60	11.85 ^{abc}	2.33
	6	21.74 ^{ab}	13.57	87.38 ^{ab}	48.42	17.30 ^a	11.74	13.20 ^{abc}	2.42
130	0	13.31 ^{ab}	7.76	70.14 ^{ab}	33.42	6.32 ^a	3.76	9.48 ^c	2.05
	2	16.04 ^{ab}	5.77	82.28 ^{ab}	46.36	5.29 ^a	3.94	10.93 ^{bc}	4.09
	4	10.19 ^b	5.68	67.95 ^{ab}	66.38	3.93 ^a	1.85	10.89 ^{bc}	5.83
	6	18.78 ^{ab}	4.15	141.96 ^a	51.97	13.91 ^a	5.80	12.20 ^{abc}	2.42

¹Means followed by the same letter in the same column are not significantly different.

Table 5.4: Forced-preference ranking sums for sorghum and vacuum belt dried blueberry pomace snacks extruded at 120°C.¹

	Pomace (g/100 g of dry feed)			
	0	2	4	6
Total Ranked Sum	132	121	135	132

¹Critical value of difference between total ranked sums is 33.8.

CHAPTER 6

VACUUM BELT DRIED BLUEBERRY POMACE POWDER AS A NUTRITIONAL INGREDIENT IN A SORGHUM EXTRUDED SNACK

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ABSTRACT

Vacuum belt dried blueberry pomace powder was added to a sorghum extruded snack as a possible nutritional enhancer. The pomace powder was combined with white sorghum flour at 2, 4, and 6 g/100 g dry feed (DF) and extruded at three exit temperatures 110, 120, or 130°C. The extruded snack was evaluated for total dietary fiber (TDF), total monomeric anthocyanins (TMA), total phenolics content (TPC), and antioxidant activity (AA) by DPPH. Anthocyanidin-glucosides were characterized by HPLC-DAD. TDF was increased in the snack by the addition of pomace powder at 6 g/100 g (7.46-7.49 g/100 g) compared to control (5.67-7.06 g/100 g). Also, pomace increased the TMA of extruded snacks. The snacks made with 6 g/100 g DF extruded at 120°C exit temperature contained the highest TMA (46.35 mg C3G/100 g). Total phenolics also increased with the addition of pomace powder. The control (sorghum only) had the lowest TPC (46.3-49.2 mg GAE/100 g) and samples with 6 g/100 g DF pomace processed at 120°C had the highest TPC (194.9 mgGAE/100 g). Antioxidant activity was correlated with both TMA ($r = 0.867$) and TPC ($r = 0.801$). Snacks that contained at least 4 g/100 g DF or more of pomace powder had at least 50% DPPH inhibition. Fourteen distinct peaks were identified by HPLC that included delphinidin glucosides, cyanidin glucosides, petunidin glucosides, peonidin glucosides, and malvidin glucosides.

INTRODUCTION

Making beneficial diet and food choices has become increasingly important to consumers, particularly as concerns with obesity develop as a worldwide issue for adults and children. In addition to fulfilling daily requirements for macro- and micronutrients,

proper nutritional intake can reduce the risk of chronic diseases, such as type 2 diabetes, cardiovascular disease, and osteoporosis (Anon, 2007). These benefits may derive from traditional nutrients and dietary fiber, as well as other bioactive compounds contained in food ingredients. One area of interest is the development of more nutritious snack options, namely those that deliver greater nutrient density, fiber, antioxidants or other health-promoting phytochemicals. An intriguing possibility, which would promote sustainability and reduce ingredient costs, is to develop products using waste streams from fruit and vegetable processing. In particular, fruit and vegetable pomace, consisting of skins, seeds, and pulp, contains antioxidants and fiber that may be beneficial to health. Such ingredients might be incorporated in a variety of products including beverages, meat and dairy products, baked goods and extruded snacks.

There has been considerable interest in how to best include pomace in extruded products. Researchers have investigated the use of grape, tomato, carrot, onion, guava, and apple pomace as a nutritional ingredient in extruded snack products. In general, research has shown that the inclusion of fruit or vegetable pomace tends to produce less expanded, more dense, and harder products, yet these products are still found acceptable by consumers (Altan et al., 2008a; Altan et al., 2008b; Dar et al., 2012; Hae-Jin & Yang-Kyun, 2000; Tangirala et al., 2012; Karkle et al., 2012).

Researchers have also studied how extrusion changes the dietary fiber or phytochemicals of extruded products that are made with or include pomace. Hwang et al. (1998) found that extrusion of dried apple pomace created a higher yield of pectins that could subsequently be extracted with hot water. This would be important for health implications, as soluble dietary fiber may be helpful in reducing serum cholesterol levels

thereby reducing the risk of cardiovascular disease (Wells & Ershoff, 1961). White et al. (2010) determined that flavonols increased 30-34% in cranberry pomace extruded with corn starch, while ORAC values also increased in some cases. These findings were related to changes in polyphenolic distributions caused by extrusion. Extrusion of cranberry pomace and corn starch resulted in a breakdown of larger procyanidins (with 3-9 degrees of polymerization) producing greater amounts of smaller procyanidins (with 1-2 degrees of polymerization). The authors noted that this might increase the bioavailability of the procyanidins and promote health benefits to be had from the product.

The use of blueberries and blueberry pomace in products is desirable as they contain substantial levels of dietary fiber, vitamin C, vitamin K, manganese and lesser amounts of several micronutrients (Kerr, 2013). The pomace is also a particularly rich source of anthocyanins and other phenolic compounds, and has relatively high antioxidant capacity (Brownmiller et al., 2008). Blueberry products have also been successfully added to extruded snacks. When white cornmeal and spray dried blueberry juice powder (1 g/100 g of dry feed) were extruded together, snacks were produced with acceptable color and higher phenolics (138.5 ppm) and anthocyanin (0.46 mg/100 g) content than cornmeal control, which contained 102.6 ppm phenolics and 0.03 mg/100 g anthocyanins (Camire et al., 2007). Extruded snacks have also been formulated with blueberry concentrate (69° Brix) to incorporate anthocyanins (Camire et al., 2002). Extrusion of freeze-dried blueberry pomace increased the amount of monomer, dimers and trimers of procyanidins, while reducing the anthocyanin content by 53 g/100 g (Khanal et al., 2012; Khanal et al., 2009b). As found with extruded cranberry pomace, the

authors felt this was one way to increase procyanidin components that are more easily absorbed.

The use of fruit and vegetable pomace as an ingredient is best facilitated when it is available in a stable form for transportation and formulation. The creation of pomace powders would meet this goal, but care needs to be taken not to lose or destroy desirable compounds in the pomace during processing. Vacuum belt drying has been shown to be an excellent drying method for heat labile compounds. Vacuum belt drying produced apple pomace powders and muscadine pomace powders with comparable anthocyanin and phenolics content to freeze-dried powder in considerably less time (Yan & Kerr, 2013; Vashisth et al., 2011). Vacuum belt dried sweet potato chips retained up to 94% of the β -carotene content from blanched sweet potatoes, while fried chips retained only 33.1% (Xu & Kerr, 2013). Vacuum belt dried blueberries processed at 90°C had similar total anthocyanin content (9.9-13.9 mg C3G/g DM), total phenolics content (30.2-35.1 mg GAE/g DM) and antioxidant activity (432-451 μ mol TE/g DM) as freeze-dried control (Pallas, 2011).

Vacuum belt drying has produced blueberry pomace powder with good flowability and good color retention with no evidence of browning (Kim, 2012). At this time, no research has been done involving the incorporation of vacuum belt dried blueberry pomace as an ingredient in an extruded snack. The objective of this study was to determine the effects of including vacuum belt dried blueberry pomace on the dietary fiber, anthocyanin content, phenolics content, and total dietary fiber of a sorghum based extruded snack, as well as to characterize individual anthocyanidin-glycosides in the extruded snack.

MATERIALS AND METHODS

Chemicals

Acetone, methanol, and acetonitrile were purchased from Fisher-Scientific (Thermo Fisher Scientific Inc., Waltham, MA). Potassium chloride, sodium acetate, hydrochloric acid, and phosphoric acid were purchased from J. T. Baker (Avantor Performace Materials, Inc., Center Valley, PA). Folin-Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl, total dietary fiber kit, and gallic acid were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO). Dephinidin, cyanidin, and peonidin were bought from Extrasynthese (69726 Genay Cedex, France). Diatase enzyme was purchased from E. C. Kraus (Independence, MO).

Processing

Pomace Production:

Individually quick frozen rabbiteye blueberry (*Vaccinium ashei*) varieties ‘Brightwell’ and ‘Tifblue’ were donated from the Blueberry Research and Demonstration Farm (Alma, Ga) and kept in a -20°C freezer. Berries were removed from the freezer and crushed in a Hobart Model 98 grinder (Hobart Corporation, Troy, OH). After crushing, berry mash was heated in a steam kettle to 40°C. Pectinex Ultra SP-L (Novozymes, Bagsvaerd, Denmark) was added at mash (0.26 ml/kg) and held for 1.5 hr. The mash was then pressed in a pilot scale hydraulic fruit press (Bucher HP 14 Laboratory Press, Bucher Industries AG, Murzlenstrasse 80, CH-8166 Niederweningen) yielding ~70% juice. The resultant pomace was frozen and stored at -20°C.

Vacuum Belt Dried Pomace Powder Production:

As needed, blueberry pomace was removed from the -20°C freezer and allowed to thaw for 24 h at 4°C. The pomace was dried using a pilot scale Bucher Dryband (Bucher Unipektin AG, Switzerland) continuous vacuum belt dryer (Figure 6.1). Pomace was placed directly onto a 22 cm Teflon belt that passed over 3 convection heating plates (zones 1, 2, and 3). In addition, a 22.9 cm radiation plate was set 4.7 cm above zones 1, 2, and 3. All conduction heating zones and the radiation plate were set at the same temperature during processing. Samples were dried at 90°C and a vacuum of 2.7-3.3 kPa for 60 min. Dried pomace was ground in a pilot scale food processor (R10DVV, Robot Coupe U.S.A., Inc., Jackson, MS) for 1 min.

Extrusion

Harvest pearl white sorghum flour was donated from ADM (ADM Milling Co., Plainview, TX). Dried blueberry pomace powder was added to the sorghum at 0 (control), 2, 4, and 6 g/100 g pomace powder:sorghum flour dry feed (DF). The sorghum and blueberry pomace snack was extruded in a pilot scale 25:1 twin screw extruder (MPF30, APV Baker, Inc., Staffordshire, England). The screw profile configuration was: (3) 1.5D twin lead feed screws, (1) 1D twin lead feed screw, (5) 30° forward peddles, (3) 1.5D twin lead feed screws, (2) 60° forward peddles, (3) 60° backward peddles, (3) 60° forward peddles, (3) 1.5D twin lead feed screws, (7) 60° forward peddles, (5) 30° forward peddles, and (1) 1D single lead discharge screw. The extrusion die included a 2mm diameter hole. Calibration of the solids feeder and water input was done before processing. Formulations were transported via a screw pump solids feeder and mixed with water to a final moisture content of 17.5 g H₂O/100 g. Exit temperatures were set at

110, 120, or 130°C. After extrusion, samples were dried in an impingement oven at 90°C for 10 min. Samples were packaged and stored at -20°C prior to chemical analysis.

Chemical Analysis

Total Dietary Fiber

Total dietary fiber (TDF) was measured by AOAC Official Method 985.29 (Anon, 2005). In duplicate, 1 g of ground, dried sample was combined with 50 ml 0.08M phosphate buffer. To the solution, 0.1 ml of heat stable alpha-amylase was added and the mixture was incubated at 95-100°C in a water bath for 30 min. During heating, samples were shaken every 5 min by hand. Samples were then removed and allowed to cool to room temperature (25°C). After cooling, 10 ml of 0.275 M NaOH solution was added followed by 5 mg of protease. The sample was placed in a 60°C water bath and allowed to incubate for 30 min with constant agitation. Samples were then removed from the water bath and allowed to cool to room temperature (25°C). After cooling, 10 ml of 0.325 M HCl solution was added followed by 0.3 ml amyloglucosidase. The samples were incubated in a 60°C water bath with constant agitation for 30 min. After removal, 280 ml of preheated 98% ethanol was added, and the material was allowed to precipitate for 60 min. After precipitation, samples were filtered through a fritted crucible (coarse, 40-60 µm) containing 0.5 g Celite. Samples were rinsed 3 times with 20 ml of 78% ethanol (v/v), 2 times with 10 ml of 95% ethanol (v/v) and 2 times with 10 ml of acetone. Rinsed samples were dried in a vacuum oven at 70°C for 24 hr. Protein was determined by the total Kjeldahl nitrogen method with a conversion factor of 6.25, and ash was determined by the burn method. To determine any background contributions to mass, a blank

containing no sample was also analyzed. TDF in g/100 g was calculated using Equation (Eqn) 1:

$$\frac{W_r - P - A - B}{W_0} * 100 \quad \text{Eqn. 1}$$

where

W_r = weight of residue

P = protein of residue

A = ash of residue

B = contributions from blank

W_0 = initial weight of sample

Extract Preparation

Three and a half grams of sample were ground in a mortar and pestle and placed in a 50 ml sterile plastic tube with 35 ml of DI water. Diatase, an enzyme cocktail composed of α -amylase and β -amylase, was added to the tube at a concentration of 1.3 μ l/ml to break down the starches of the extruded sample and to improve extraction of phytochemicals. The sterile tubes were capped and placed in a water bath at 60°C with constant agitation for 1 h. After the enzymatic reaction was completed, tubes were placed in a -20°C freezer for storage.

Tubes were removed from the freezer and allowed to thaw for 12 h at 4°C. After thawing, the samples were combined with 35 ml of acidified methanol (0.01% HCl) and allowed to reflux for 60 min. After refluxing, samples were cooled to room temperature and centrifuged (Centrifuge Model 228, Fisher-Scientific, Thermo Fisher Scientific Inc., Waltham, MA) at 3400 rpm for 3 min. The supernatant liquid was decanted off, and the remaining precipitate was re-suspended in 35 ml of 70% acidified methanolic solution

(v/v). The re-suspended sample was refluxed for 60 min and centrifuged. Refluxing in acidified methanol and centrifugation was done two more times. All supernatant fractions were pooled together and evaporated under vacuum to remove the methanol. The remaining extract was brought up to 50 ml with DI water and stored in amber vials. Extractions were done in triplicate.

Total Monomeric Anthocyanins

Total monomeric anthocyanins (TMA) were determined by the pH differential method as described by Giusti and Wrolstad (2001). One milliliter of extract was combined with 4 ml potassium chloride buffer, and one milliliter of extract was combined with 4 ml of sodium acetate buffer and allowed to equilibrate for 15 min. Solutions were measured with a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA) at wavelengths of 520 nm and 700 nm. All measurements were done in triplicate. Concentrations were reported as cyanidin-3-glucoside (C3G) as determined by Eqn. 2:

$$TMA = \frac{(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}}{(\epsilon \times 1)} \times MW \times DF \times 100 \quad \text{Eqn. 2}$$

where

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$$

MW = molecular weight of C3G

DF = dilution factor

ϵ = molar absorptivity coefficient of C3G

Total Phenolic Content

Total phenolics content (TPC) was determined colorimetrically by the Folin-Ciocalteu method as described by Amarowicz and others (2004). One milliliter of extract was diluted with 9 ml of DI water. Two milliliters of the diluted sample was combined with 6.5 ml of DI water. After mixing, 0.5 ml aliquot of Folin-Ciocalteu phenol's reagent and 1 ml of saturated sodium carbonate solution were added and the contents were vortexed for 15 s. The test tubes were then left to rest at room temperature (25°C) for 40 min. Afterwards, absorbance readings were taken at 750 nm using a Spectronic Genesys 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA). Gallic acid was used to prepare a standard curve. Readings were done in triplicate and results reported as gallic acid equivalents (mg GAE/100g).

Antioxidant Activity

Antioxidant activity (AA) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Su & Chien (2007). To determine the free-radical scavenging potential, 0.05 ml of extract was added to 5 ml of DPPH solution (0.025 g/L) and allowed to react in the dark for 30 min at ambient temperature (~20°C). Absorbance was read at 515 nm. Antioxidant activity was calculated as percentage of DPPH discoloration against control (methanol) using Eqn. 3.

$$\% \text{ DPPH Inhibition} = 100 \times \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \quad \text{Eqn. (3)}$$

HPLC Separation of Anthocyanins

Reverse phase analyses of anthocyanins was done with an HP 1090 Series II HPLC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a UV diode array

detector. A Waters Spherisorb (Waters Corporation, Milford, MA) 5 μm ODS (2) (250 X 4.6 mm) column was used for separation of polyphenols as described by Durst and Wrolstad (2001). Extracts were filtered through a 0.45 μm Phenex nylon filter (Phenomenex, Torrance, CA) before injection into the HPLC. The solvent system used a linear gradient of 94% solvent A and 6% solvent B to 75% solvent A and 25% solvent B in 55 min, where solvent A was 4% phosphoric acid and solvent B was 100% acetonitrile. Eluted peaks were detected at 520 nm for anthocyanins. Anthocyanins were identified with standards by elution time and spectral analysis. Anthocyanins were quantified using delphinidin as an external standard and reported as $\mu\text{g del}/100\text{ g}$.

Statistical Analysis

Total dietary fiber was done in duplicate. Extractions, TMA, TPC, and HPLC were done in triplicate. Analysis of variance was performed using JMP (SAS Institute Inc., Cary, NC). Tukey's post hoc testing was used to determine different means.

RESULTS AND DISCUSSION

TDF, TMA, TPC and AA

Measured values of total dietary fiber (TDF), total monomeric anthocyanins (TMA), total phenolics content (TPC), and antioxidant activity (AA) are shown in Table 6.1. Levels of TDF ranged from 5.67 to 7.73 g/100 g dry weight (DW) and temperature, pomace and the interaction term were significant factors. In contrast, TMA, TPC, and AA were dependent on pomace level only.

For dietary fiber, the control processed at 130°C has the lowest TDF (5.67 g TDF/100 g product) and the snack with 6 g pomace/100 g DF blueberry pomace

processed at 120°C had the largest TDF (7.73 g TDF/100 g product). The TDF content for the control (5.67-7.06 g TDF/100 g product) is in agreement with other reported values for sorghum flour (6.6-11.45 g TDF/100 g product) (USDA, 2011; Picolli da Silva & de Lourdes Santorio Ciocca, 2005). According to the USDA Nutrient Database, there is 2.4 g TDF/100 g fresh weight in whole blueberries (USDA, 2011). Branning et al. (2009) reported much higher values of total dietary fiber (40.8 TDF g/100 g pomace) for blueberry pomace, with lignin being the predominate fiber (14.4 g/100 g pomace). Lignin is a common insoluble fiber found in the plant cell wall material. Pectin is the major soluble fiber associated with blueberries, but is reduced during ripening (Proctor & Peng, 1989). Also, some of the pectin present in the whole blueberries may have been broken down during the juice processing as pectinase was added to increase juice yield. Likely, most fiber measured was insoluble lignin. Even though blueberry pomace has been reported to contain a higher amount of dietary fiber there was not sufficient dried pomace powder added to the extruded snack to substantially change the fiber levels.

The source of pomace and other flours or starchs used during extrusion will dictate the amount of pomace needed to increase fiber levels.. When added at 5 g/100 g or more, by-products of cauliflower processing increased fiber content of expanded cereal snacks to 7.6-11.6 g TDF/100 g, as compared to 5.6 g TDF /100 g of the control (Stojceska et al., 2008). Addition of 2.5-7.5 g/100 g mango peel powder to macaroni increased soluble dietary fiber from 3.59 g/100 g to 4.46-5.63 g/100 g, and insoluble dietary fiber from 8.58 g/100 g to 9.34-12.2 g/100 g (Ajila et al., 2010).

Inclusion of pomace powder was the only significant factor for total monomeric anthocyanins. For control, no anthocyanins were detected, which is expected as white

sorghum does not contain these compounds. Overall, as the level of added dried pomace powder increased, so did the anthocyanin content of the extruded material. Samples with 2, 4 or 6 g/100 g DF contained 8.56-9.8, 18.71-28.12, and 33.43-37.83 mg C3G/100 g, respectively. Extrusion temperature had some effect on TMA as the sorghum snack with 6 g/100 g DF pomace powder processed at 120°C contained the highest level of anthocyanins (46.35 mg C3G/100 g). These values are lower than previously observed for blueberry pomace and white sorghum extruded material. Khanal et al., (2009a) produced extruded snacks with 102.3-175.7 mg/100 g anthocyanins. Differences were likely to the higher level of blueberry incorporation (30 g/100 g) and higher feed moisture (45 g/100 g) used in their study. While extrusion had little effect on TMA of the extruded snack, extrusion can cause changes in polyphenolic compounds. When blueberry concentrate was used as a colorant for breakfast cereals extruded from white corn, it was found that while an acceptable color was attained, there was 42% reduction in anthocyanin content (Camire et al., 2002).

Pomace powder level was also the only significant factor for total phenolics content (Table 6.1). The sorghum-only control contained 46.3-49.2 mg GAE/100 g TPC. Depending on the variety, sorghum may have different levels of phenolics, anthocyanins, and procyanidins. White sorghum, in particular, has only phenolic compounds (Awika & Rooney, 2004). Consistent with TPC measured for the control sorghum extruded snack, Kamath et al., (2004) found that white sorghum flour contained 46.1-76.3 mg phenolics/100 g. The major phenolic acids in sorghum were found to include *p*-coumaric and vanillic acids (Guenzi & McCalla, 1966). As with the TMA, TPC increased with increasing pomace powder level. Extruded snacks with 6 g/100 g DF pomace powder

contained the highest level of phenolics (194.9 mg GAE/100 g). For samples with 6 g/100 g DF pomace powder processed at 130°C, TPC was lower than for those processed at 110°C and 120°C. Processing at the higher temperatures may have destroyed some phenolics during extrusion. Total phenolics were reported to be reduced by 24-46% during extrusion of oat cereals (Viscidi et al., 2004).

Pomace level and its interaction with extrusion temperature were significant factors for antioxidant activity (Table 6.1). Antioxidant activity (AA), as measured by the percent inhibition of the DPPH radical, increased with the level of pomace powder. Samples with more than 4 g/100 g DF of pomace powder generally had greater than 50% DPPH inhibition (49.75-65%). AA was correlated with both TMA ($r=0.867$) and TPC ($r=0.801$), implying that both anthocyanins and phenolics contributed to the antioxidant activity for the extruded snack with pomace powder, while the phenolics in the sorghum contributed to the antioxidant activity in the control. Blueberry polyphenolics have been reported to have high antioxidant activities, displaying 59.9-87.5% DPPH inhibition (Su & Silva, 2006). While extrusion temperature had some interactive influence, no clear trends were seen as to the direct effect of temperature on AA. In products made from barley-fruit and vegetable by-products, high-temperature extrusion reduced antioxidant activity as temperatures over 80°C destroyed the antioxidant properties (Altan et al., 2009).

HPLC

Fourteen distinct peaks were identified by HPLC-DAD, 520 nm (Figure 6.2). The elution order was as follows: delphinidin-3-galactoside, delphinidin-3-glucoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-glucoside, petunidin-3-

galactoside, cyanidin-3-arabinoside, petunidin-3-glucoside, peonidin-3-galactoside, petunidin-3-arabinoside, peonidin-3-glucoside, malvidin-3-galactoside, malvidin-3-glucoside, and malvidin-3-arabinoside. This elution order is consistent with those seen in literature (Lee et al., 2002; Lohachoompol et al., 2008) for blueberry anthocyanins. The anthocyanidin-glucosides were quantified using the standard addition method with delphinidin (Table 6.2). Consistent with the total monomeric anthocyanins, no anthocyanins were detected within the control sorghum extruded snack. For each anthocyanidin-glycoside, the level of pomace powder was the only significant factor. For all extruded snacks, malvidin-glycosides were the most abundant with malvidin-galactoside being the anthocyanin with the highest content (2.24-9.14 $\mu\text{g}/100\text{ g}$). Rabbiteye 'Tifblue' berries have been associated with 14 different anthocyanidin-glycosides with malvidin-3-galactoside accounting for 20.3% of the total anthocyanin content (Prior et al., 2001). One concern is the degradation of anthocyanins due to high heat in the extruder. The rate of degradation of various anthocyanidin-glycosides has been reported to be similar, and the degradation rate constant of the compounds was found to be 3 times higher at 125°C than at 100°C when bilberry extract was exposed to dry heat (Yue & Xu, 2008). White and others (2010) hypothesized that during extrusion starch may be protective of the anthocyanins as degradation was not as great as expected in corn starch extruded snacks with cranberry pomace.

CONCLUSIONS

Adding blueberry pomace may be an option for increasing the nutrient-density of extruded snacks, and vacuum belt drying is a convenient means of creating pomace

powders with good physical and chemical quality. The snack TDF increased with the addition of blueberry pomace greater than 2 g/100 g. Also, as the amount of added pomace powder increased, so did the anthocyanin and phenolics content, and 6 g/100 g pomace powder processed at 120°C contained the highest anthocyanin and phenolics content. Sorghum provided some phenolics as observed in the control extruded snack. Antioxidant activity was correlated with both anthocyanins and phenolics, and extruded snacks with 4 g/100 g or more pomace powder had 50% or greater DPPH inhibition. Extruded snacks contained 14 distinct anthocyanidin-glucosides with malvidin-glucosides having the highest concentration.

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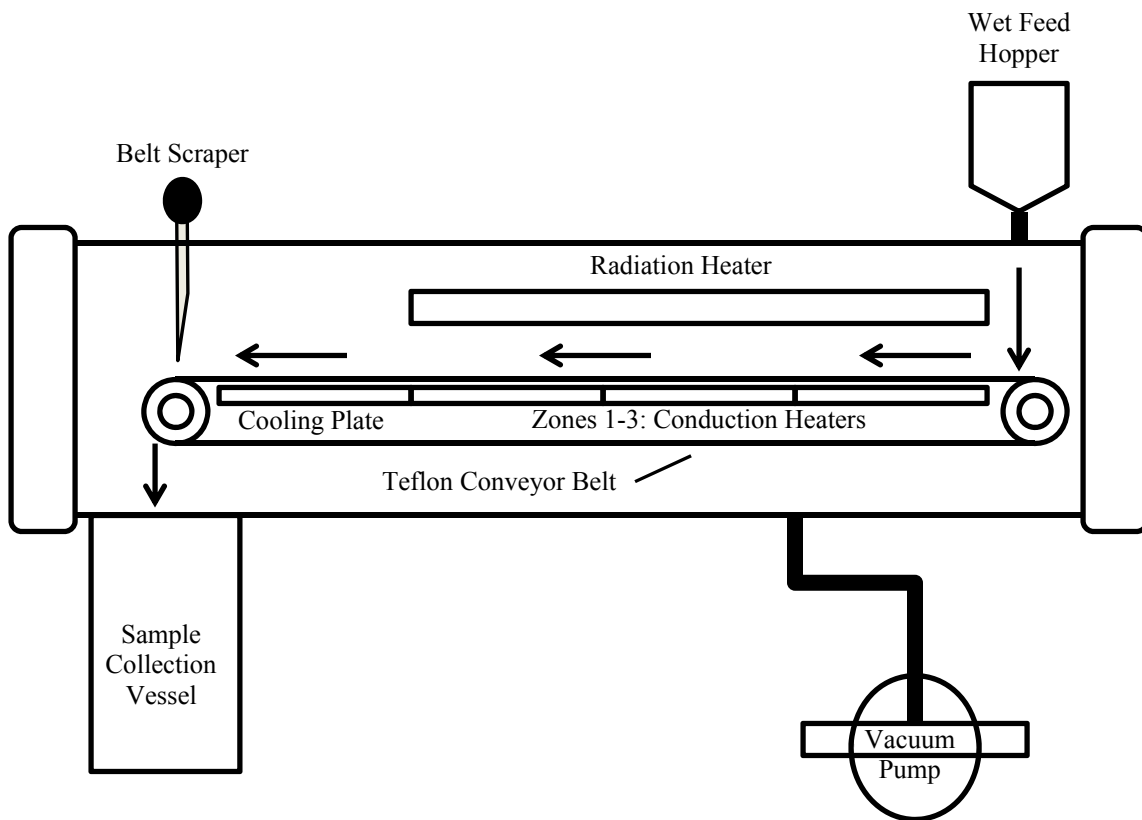


Figure 6.1: Schematic of a continuous vacuum belt dryer. A vacuum belt dryer is a chamber that contains a belt that conveys material over multiple heating zones and a cooling zone. Radiation type heating is also used to increase drying rate.

Table 6.1: Total dietary fiber (TDF), total monomeric anthocyanins (TMA), total phenolics (TPC) and antioxidant activity (AA) of extruded sorghum snacks with vacuum belt dried blueberry pomace powder extruded at three different exit temperatures.¹

Pomace (g/100g)	Temperature (°C)	TDF (g/100g)		TMA (mg C3G/100 g)		TPC (mg GAE/100 g)		AA (% Inhibition)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	110	6.02 ^{de}	0.22	n.d.		46.3 ^e	1.3	27.38 ^e	7.03
	120	7.06 ^{abcde}	0.02	n.d.		48.7 ^e	17	27.36 ^e	3.03
	130	5.67 ^e	0.49	n.d.		49.2 ^e	4.2	24.18 ^e	2.72
2	110	6.26 ^{bcde}	0.01	9.80 ^e	2.91	89.5 ^d	2.1	37.47 ^{de}	9.19
	120	7.23 ^{abcd}	0.13	8.56 ^e	2.34	90.4 ^d	2.4	41.81 ^{cd}	7.12
	130	6.08 ^{cde}	0.15	9.39 ^e	1.75	88.9 ^d	14	46.65 ^{cd}	6.76
4	110	7.53 ^{ab}	0.36	18.71 ^d	4.30	125.6 ^c	9.6	51.87 ^{bc}	10.30
	120	6.93 ^{abcde}	0.25	27.38 ^c	4.98	122.6 ^{cd}	7.0	63.58 ^{ab}	10.05
	130	7.22 ^{abcd}	0.52	28.12 ^c	1.90	123.2 ^{cd}	13	49.74 ^{bc}	5.67
6	110	7.49 ^{abc}	0.76	37.83 ^b	3.22	165.2 ^{ab}	11	59.99 ^{ab}	8.24
	120	7.73 ^a	0.35	46.35 ^a	2.98	194.9 ^a	13	65.00 ^a	8.79
	130	7.46 ^{abc}	0.36	33.43 ^b	3.56	144.4 ^{bc}	56	64.34 ^a	7.40

¹Means in the same column followed by the same letter are not significantly different (p<0.05), and n.d. is none detected.

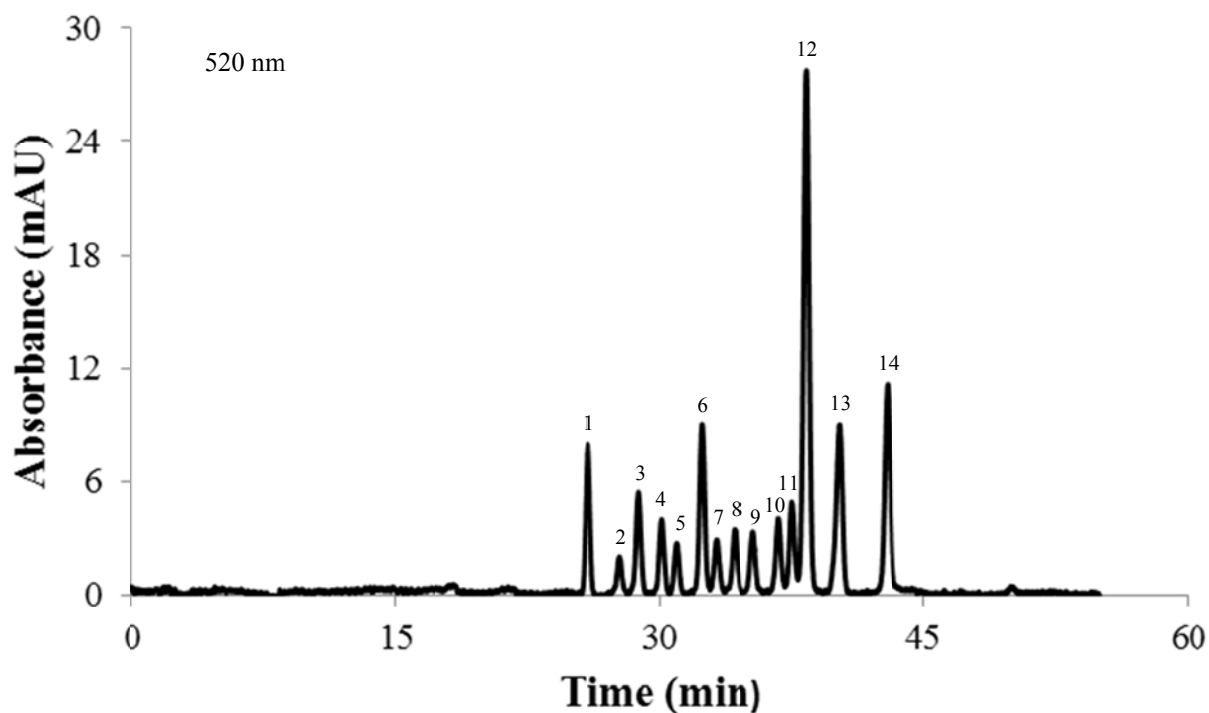


Figure 6.2: HPLC chromatogram of sorghum and vacuum belt dried blueberry pomace extruded snack. The elution order was (1) dephinidin-3-galactoside, (2) delphinidin-3-glucoside, (3) cyanidin-3-galactoside, (4) delphinidin-3-arabinoside, (5) cyanidin-3-glucoside, (6) petunidin-3-glucoside, (7) cyanidin-3-arabinoside, (8) petunidin-3-glucoside, (9) peonidin-3-galactoside, (10) petunidin-3-arabinoside, (11) peonidin-3-glucoside, (12) malvidin-3-galactoside, (13) malvidin-3-glucoside, and (14) malvidin-3-arabinoside.

Table 6.2: Anthocyanins ($\mu\text{g del}/100 \text{ g}$) as determined by HPLC-DAD in a white sorghum and vacuum dried blueberry pomace powder extruded snacks extruded at three exit temperatures.^{1,2}

Anthocyanin	110°C				120°C				130°C			
	0	2	4	6	0	2	4	6	0	2	4	6
Delphinidin-3-galactoside	n.d. ³	0.70 ^d	1.19 ^{cd}	2.23 ^{ab}	n.d.	0.72 ^d	1.66 ^{bc}	2.71 ^a	n.d.	0.81 ^d	1.54 ^{bc}	1.98 ^b
Delphinidin-3-glucoside	n.d.	0.42 ^a	0.52 ^a	0.79 ^a	n.d.	0.44 ^a	0.65 ^a	0.93 ^a	n.d.	0.66 ^a	0.78 ^a	0.78 ^a
Cyanidin-3-galactoside	n.d.	0.62 ^d	1.07 ^{cd}	1.83 ^{ab}	n.d.	0.65 ^d	1.31 ^{bcd}	2.08 ^a	n.d.	0.71 ^d	1.27 ^{bcd}	1.48 ^{abc}
Delphinidin-3-arabinoside	n.d.	0.51 ^c	0.75 ^{bc}	1.46 ^{ab}	n.d.	0.55 ^{bc}	0.91 ^{abc}	1.60 ^a	n.d.	0.57 ^c	0.99 ^{abc}	1.23 ^{abc}
Cyanidin-3-glucoside	n.d.	0.42 ^c	0.79 ^{bc}	1.05 ^{abc}	n.d.	0.42 ^c	0.67 ^{bc}	1.40 ^a	n.d.	0.63 ^{bc}	0.85 ^{abc}	1.10 ^{abc}
Petunidin-3-galactoside	n.d.	0.84 ^e	1.44 ^{de}	2.69 ^{ab}	n.d.	0.84 ^e	1.90 ^{bcd}	2.99 ^a	n.d.	0.97 ^e	1.75 ^{cd}	2.49 ^{abc}
Cyanidin-3-arabinoside	n.d.	0.46 ^c	0.76 ^{abc}	1.29 ^a	n.d.	0.44 ^c	0.89 ^{abc}	1.20 ^a	n.d.	0.57 ^{bc}	0.83 ^{abc}	1.18 ^{ab}
Petunidin-3-glucoside	n.d.	0.44 ^c	0.81 ^{bc}	1.40 ^{ab}	n.d.	0.56 ^c	0.93 ^{abc}	1.47 ^a	n.d.	0.60 ^c	1.13 ^{abc}	1.31 ^{ab}
Peonidin-3-galactoside	n.d.	0.51 ^c	0.83 ^{bc}	1.31 ^{ab}	n.d.	0.59 ^c	1.01 ^{abc}	1.51 ^a	n.d.	0.63 ^c	0.96 ^{abc}	1.38 ^{ab}
Petunidin-3-arabinoside	n.d.	0.49 ^d	0.78 ^{bcd}	1.53 ^{ab}	n.d.	0.55 ^d	0.97 ^{abcd}	1.55 ^a	n.d.	0.63 ^{cd}	1.03 ^{abcd}	1.33 ^{abc}
Peonidin-3-glucoside	n.d.	0.49 ^d	1.01 ^{cd}	1.78 ^{ab}	n.d.	0.55 ^d	1.27 ^{bc}	1.93 ^a	n.d.	0.68 ^{cd}	1.17 ^{bcd}	1.76 ^{ab}
Malvidin-3-galactoside	n.d.	2.35 ^e	4.28 ^d	7.98 ^{ab}	n.d.	2.24 ^e	5.53 ^{cd}	9.14 ^a	n.d.	2.26 ^e	5.36 ^d	7.28 ^{cd}
Malvidin-3-glucoside	n.d.	1.05 ^{bc}	1.95 ^{bc}	3.35 ^a	n.d.	1.02 ^c	1.97 ^{bc}	3.63 ^a	n.d.	1.04 ^c	2.19 ^b	3.24 ^a
Malvidin-3-arabinoside	n.d.	1.14 ^e	1.90 ^{de}	3.59 ^{ab}	n.d.	1.09 ^e	2.41 ^{cd}	4.18 ^a	n.d.	1.30 ^e	2.33 ^{cd}	3.06 ^{bc}

¹Means followed by same letter in the same row are not significantly different ($p < 0.05$).

²Absorbance measured at 520 nm. Vacuum dried pomace powder added to extruded snack at 0 (control), 2, 4, and 6 g/100g dry feed.

³None detected (n.d.)

CHAPTER 7

CONCLUSION

Blueberries are a source of dietary fiber and polyphenols. As this work has shown, it is important to account for effects different processes have on these compounds. Wine, dried pomace powder, and extruded sorghum blueberry pomace snacks products were examined.

Chapter three shows that while blueberry wine contains elevated levels of anthocyanins and phenolics, short term storage can cause drastic changes in some of these compounds. Specifically, monomeric anthocyanins are almost completely lost, implying they were either destroyed or polymerized. Further bench-top testing confirmed that a significant fraction of the blueberry wine anthocyanins were polymerizing and forming stable pigments. While polymerization allows for the stabilization of color within the wine, larger molecular polymers may not be as bioavailable. Further work should focus on determining the degree of polymerization of these stable pigments, as well as considering *in vitro* cell adsorption tests to expound upon changes in bioavailability during storage.

In chapter four, a novel method to create dried blueberry pomace was examined. Vacuum belt drying was able to create a suitable nutritional ingredient for formulated products. Vacuum belt dried blueberry pomace was shown to maintain substantial levels of anthocyanins and phenolics. However, vacuum belt drying is a complex system involving combinations of feed rate, belt speed, and temperature settings. Even though

this paper provided support that vacuum belt drying is an option to produce nutritional pomace powders, the process still needs to be optimized. Therefore, future investigations should focus on improving drying rates while being cognizant of bioactives by determining the optimal combination of feed methods, temperature sequences, and residence times. Also work is needed to fully elaborate the various polyphenols present in the pomace, including phenolic acids and procyanidins. Finally, extended storage studies, manipulating both temperature and humidity, would be beneficial to understand the stability of the powder.

Chapters five and six verified that vacuum dried pomace powder was suitable as a nutraceutical ingredient in extruded products. While addition of pomace powder resulted in a less expanded, denser and harder product it also provided a source of natural color, increased the polyphenolic profile as well as the dietary fiber of the extruded snack, and was equally preferred by consumers when incorporated at levels of six percent and lower.

Future studies should focus on formulation in order to optimize physical attributes of the snacks. This would require sensory testing, including acceptability testing in order to create a more finished product. Additionally, since anthocyanins show lowered stability as pigments when compared with artificial colors, shelf stability of the extruded products should be investigated.