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Consumer Acceptance and Phytonutrient Assessment of Cold Hardy, Locally Grown Plums

Amber L. Elwell
University of Maine, amber.elwell@maine.edu

MASTER'S THESIS CONSUMER ACCEPTABILITY OF AND PHYTONUTRIENT ASSESSMENT FOR COLD HARDY, LOCALLY GROWN PLUMS

(*Prunus Salicina & Prunus Domestica)*

By

Amber Lynn Elwell

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Advisory Committee:

Angela Myracle MPH, PhD, Assistant Professor of Food Science & Human Nutrition, Advisor

Renae Moran, PhD, Associate Professor of Pomology, University of Maine

L Brian Perkins, PhD, Research Assistant Food Science and Human Nutrition

CONSUMER ACCEPTABILITY OF AND BIOANALYSIS OF BIOACTIVE COMPOUNDS IN COLD HARDY, LOCALLY GROWN PLUMS

(*Prunus Salicina & Prunus Domestica)*

By: Amber Lynn Elwell

Thesis Advisor: Dr. Angela Myracle

An Abstract of the Thesis Presented

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Prunus salicina, Japanese plums, and *Prunus domestica,* European plums, are naturally rich in fiber, minerals, vitamins and phytonutrients. Consuming plums can increase overall fruit intake and can be incorporated into part of a healthy diet. Through local plum production, harvesting can be completed without the need for cross-country shipping, which can increase overall acceptability and nutritional quality of the fruit. Furthermore, the increase in local production can help to boost Maine's economy and creates a market for new plum product production.

The first objective of this study was to determine consumer acceptance of locally grown plum cultivars at a tree-ripened stage. Sensory testing was conducted in the Sensory Evaluation Center at the University of Maine on plum cultivars harvested at Highmoor Farm in Monmouth, ME. The highest rated cultivar for 'overall' acceptability was the Japanese plum, Oblinya, with an average rating of 7.27±1.42. Toka, Kahinta and Superior also had high 'overall' acceptability scores with average ratings of, 6.98±1.4, 6.97±1.55 and 6.9±1.37, respectively. European varieties Early Italian and Caselton also had high 'overall' acceptability ratings at 6.98±1.46 and

6.76±1.35, respectively. In a similar consumer study, fruit was believed to be accepted among consumers with likeness ratings of >5.0. All cultivars tested in this study outperformed this value.

The second objective of this study was to evaluate phytonutrient content of the plum cultivars. Phytochemical constituents were extracted using 80% acidified methanol for twelve locally grown plum cultivars at a tree-ripened stage. Total monomeric anthocyanin, total phenolics, and free radical scavenging ability was measured on the cultivars. The European plum Caselton had the highest anthocyanin content (1242.83±14.05mg /100 g.) Toka had the greatest antioxidant capacity, demonstrating 50% inhibition of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (1.28±0.14 mg/mL.) Toka also had the highest total phenolic content (1006.04±21.88 mg/ 100g.) Statistically significant differences were found between Japanese and European varieties for total phenolic content and free radical scavenging ability, with Japanese cultivars having higher values in both categories.

Cold-hardy plum cultivars that can be produced in Maine were successfully cultivated and were widely accepted among consumers. Producing plums that are well liked by consumers provides a new agricultural sector for Maine’s farmers, in turn boosting local economies and creating a new market for processed plum products. The evaluation of consumer acceptance and bioanalysis of plum cultivars establishes a deeper understanding of novel, tasty, and healthy plums for producers to incorporate into their production programs. This study fills the gap in knowledge of cold-hardy cultivars, and demonstrates that tree-ripened cultivars Oblinya, Toka, Kahinta, Superior and Early Italian all have favorable marketing potential for tree-fruit farmers in Maine.

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CHAPTER 1

INTRODUCTION

The prevalence of chronic health conditions in the United States is rapidly increasing. According to the Center for Disease Control and Prevention, almost half of all adults in the United States (117 million people) have one or more chronic health conditions (1). Consequences of these conditions include a decreased quality of life and an increased economic burden on individuals, families and healthcare systems. (2). A healthy lifestyle is the most relevant and cost-effective method of combatting chronic disease. Plum cultivars *Prunus domestica* (European plums) and *Prunus salicina* (Japanese plums) have been evaluated for their nutritional benefits. These plums are naturally high fiber, vitamin, mineral, and phytonutrient content. Consumption of plums can be incorporated as part of a healthy diet providing an overall increased fruit intake for Americans.

Research on the health effects of plums shows promising results for their anti-inflammatory and antioxidant characteristics (3). Epidemiological studies have revealed many beneficial effects of the consumption of fruits and vegetables with a high phenolic content. The protective effects of dietary phytochemicals against oxidative stress-related diseases are due to their contribution and maintenance of redox homeostasis in cells. (4,5). Cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction. This may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions (5,6). The benefits of phenolic compounds include the prevention of heart disease, stroke, and certain cancers (5). Polyphenols are known as secondary metabolites of plants that are generally involved in defense against ultraviolet radiation or aggression by pathogens (6). More than 8,000 polyphenolic compounds have been identified in various plant species. Specific phenolic compounds include phenolic acids, flavonoids, and lignans. These phenols have been identified as a significant dietary source of antioxidants (7). There is also a subclass of polyphenols called anthocyanins, which are responsible for the red, purple, and blue hues found in fruits and vegetables. Anthocyanin isolates and anthocyanin rich mixtures of bioflavonoids have been found to provide many benefits to human health. These benefits include protection against DNA cleavage, estrogenic activity, enzyme inhibition and lipid peroxidation (7,8). Therefore, it is possible that increasing the consumption of plums, which are high in polyphenolic compounds, may play a role in helping to decrease the prevalence of chronic diseases such as cardiovascular disease, diabetes, cancer, and arthritis.

Consumption of plums in the US is lower than most other types of fruit, with a per capita yearly consumption average of 0.5 kg (9). Low consumption of plums is in part due to poor quality that occurs with harvest at an early stage of maturity, which is needed to prevent bruising and premature ripening during the shipping duration. When plums are harvested at more advanced stages of maturity, there is a better fruit quality and greater consumer acceptance (10). Local plums can help boost Maine's economy, eliminate the need for cross-country shipping, and contribute to improved consumer acceptability of plums. Local cultivars also prevent degradation of the fruit's nutritional quality by reducing the time between harvest and consumption. If plums are grown closer to their intended market, they can be harvested at later stages of ripeness to increase fruit quality and reduce long-distance shipping practices.

The state of Maine is often referred to as the "Vacationland" of the country. This is in part due to the extensive coastlines, beaches, mountains, foliage in the fall and many other spectacular views. Each summer Maine's population increases from a little over one million to around thirteen million through the tourists that come to visit (11). This period of July through September is known as the peak tourism season which coincides with the ripening of plums.

Maine has an abundant number of apple orchards so the addition of plum orchards would be beneficial for the state. There are many Japanese (*P. salicina)* and European (*P. domestica)* plum varieties that can survive cold temperature growing conditions in the north. The USDA growth zones for Maine include zone 3b to 6a. The minimal range for these temperate zones is -35°F to -5°F during winter months (12). Objective testing of fruit quality and consumer acceptance is needed to provide valuable information. This information may be used by producers and farmers to select the most competitive cultivars that can survive Maine winters. By introducing local plum varieties to consumers, there is room for increased consumption of plums along with an expansion of Maine's local agriculture.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF PLUMS

Plums are a remarkably diverse group of fruit that comprises several different species and sub-groups (13). Plums belong to the *Prunus* genus of plants and are relatives of the peach, nectarine, and almond. All plums are considered “drupes” fruits that have a hard stone pit surrounding their seeds. Two of the significant plum groupings include *Prunus domestica*, the European plum and *Prunus salicina***,** the Japanese plum (14).

2.1.1 JAPANESE PLUMS

Prunus salicina Lindl., commonly known as Japanese plum, originated in the Yangtze River basin and spread across eastern China where it continues to be cultivated several thousand years later (15). Though *Prunus salicina* is native to China, the common name Japanese plum is used since the first imports of this fruit to the United States came from Japan in the late 19th century (15,16). Currently, Japanese plums are the predominant plum produced on an international basis (16). Japanese plums are typically grown in temperate zones, but some cultivars that have become adapted to the subtropics. China is the most significant producer of this type of plum, but Europe and the United States also produce large quantities of Japanese plums (14).

A unique trait of the Japanese plum is the variability in color of the peel and flesh. Peel color can be black, purple, red, green, or yellow. Flesh color can be yellow or red with many shades of both colors, and in some cultivars, the flesh is a combination of yellow and red

(16). The plums that have red flesh are commonly referred to as blood plums, which is unique to the Japanese cultivars. The variety and brightness of the color are what draws many consumers to the Japanese plum, and makes it favorable for eating as whole, fresh fruit (14).

Previous reviews and research on the phytochemical content of plums and health benefits have focused on European plum cultivars (17). However, with the increase in yield and consumption, there is currently growing interest in the nutritional aspects of Japanese plums. Like European plums, Japanese plums are a good source of fiber, vitamin C, and potassium. The major phytochemicals found in Japanese plums are anthocyanins, carotenoids, flavanols, proanthocyanins and hydroxycinnamic acid derivatives (16). The health benefits of these phytochemical compounds may lead to increased interest in the Japanese plum variety.

The vibrant deep purple and red colors found in Japanese blood plums make these cultivars a rich source of anthocyanins. Phytochemicals are predominantly found in concentrated levels within the peel of the plum versus the flesh (14,16). Total monomeric anthocyanin content varies widely between Japanese plum varieties, due to the wide color range for these cultivars. The main anthocyanins in Japanese plums are cyanidin-3-glucoside and cyanidin-3-rutinoside (18). The anthocyanin content of the blood plums, which have dark-flesh and dark-peel, make this fruit a significant source of dietary anthocyanins (16). Japanese blood plums can have total anthocyanin levels that are close to 300 mg per 100 g, exceeding or comparable to berry fruits and are therefore, regarded as some of the most abundant sources of anthocyanins (19). Typically, the red-fleshed Japanese blood plums have a much higher flesh/peel content ratio of anthocyanins (0.1-0.2 mg) compared to the yellow-fleshed varieties (0.003-0.06 mg), respectively (18). The high levels of anthocyanin in the flesh of Japanese blood plums is a novel discovery since the content in most anthocyanin-rich fruits (i.e., berries, grapes, European plums, etc.) are usually concentrated solely in the skin/peel with little to no anthocyanin levels in the flesh (16).

The length of time that Japanese plums remain growing and maturing on the tree has a substantial impact on phytochemical content. Delaying harvest results in a significant increase in total phenolic, anthocyanin and carotenoid contents (19,20). The chemical reactions that occur during maturation releases more bound components that result in increases in the concentration of bioactive phenolics (10). The total phenolic content of the peel and flesh increased 1.5-3.1- and 1.7- 4.1-fold respectively, over forty days (unripe to the mature stage) (21). Total carotenoid content and anthocyanin content of both peel and flesh experienced similar increases during ripening. This is another indication for local plum production as the cultivars would remain on the tree until fully ripened, increasing the content of phytochemicals in the fruit.

2.1.2 EUROPEAN PLUMS

Prunus domestica (European plum) is a common garden plum that is known for its diversity in size, color, and flavors and is one of the most desirable plum species due to excellent taste and versatility of uses (22). The European plum is believed to have been discovered around 2000 years ago, having an origin near the Caspian Sea and was introduced in the United States by pilgrims in the 17th century (3). The high sugar content of European plums promotes consumption by individuals of all age groups (23). European plums are mainly used in processed products such as compotes, mousse, dried, canned, jams and the most significant component, prunes (23).

Prunes, which are the dried version of plums, are comprised mostly of European cultivars and are known for their laxative effect, due to their high fiber content of approximately 6 grams of dietary fiber per 100 g serving (3,24). Prunes are created industrially by drying whole plums with hot air at 85-90ºC for 18 hours (17). Many believe that this process of food preservation started near the Caspian Sea, in the same region where European plums were initially discovered (3). Currently, California is the leading producer of prunes worldwide, with a production of 99% of the nation's plum crop and 70% of plums, worldwide. (25).

Prunes contain significant amounts of sorbitol, quinic acid, chlorogenic acids, vitamin K, boron, copper, and potassium (17). These substances and other compounds present in dried European plums may have beneficial health effects when prunes are regularly consumed. Eating prunes as a regular snack may also help to increase satiety, which in turn would reduce food intake, helping to control obesity, diabetes, and related chronic diseases (3,17,26). Furthermore, though plums are very sweet, prunes do not cause massive rises in postprandial blood glucose and insulin, making this food a safe snack for diabetics (17). The high fiber, fructose and sorbitol content of dried plums are what makes prunes a low glycemic snack (24).

According to a study by Kimura et al., the ingestion of prunes has been found to decrease the LDL cholesterol plasma levels in humans who suffer from hypercholesterolemia, as well as plasma and liver cholesterol concentrations in hyperlipidemic rats (27). Furthermore, prune extract and juice have been found to inhibit low-density lipoprotein (LDL) oxidation by using reversed phase HPLC with diode array detection (24). *In vitro* assays have proven prunes to have the highest antioxidant capacity compared to all other dried fruits. Cardiovascular disease is the leading global cause of death, accounting for more than 17.9 million deaths per year (28). Strokes are the second leading cause of global death behind heart disease. These statistics indicate the importance of maintaining lipid control through a healthy diet.

The vast number of gastrointestinal (GI) disorders in this country such as constipation, colorectal cancer, and diverticulitis has become a public health burden. For example, constipation affects almost 14% of the adult population worldwide, which negatively impact the quality of life for those individuals (26,29). Lifestyle modification is the focus of these issues. Increasing dietary fiber intake is known to have positive effects on stool weight and GI transit times (29). Prunes are considered to have a laxative effect and are traditionally used to relieve constipation (30). This is mostly due to the high fiber content, but other components found in the European plum including the sugar alcohol sorbitol and phenolic compounds may contribute to the laxative quality (29). The phenolic compounds are primarily chlorogenic and neochlorogenic acids (17), which are not readily absorbed in the small intestine, and pass into the colon undigested. Lever et al. found evidence linking consumption of chlorogenic acids in prunes (100g/day) to an increase in stool frequency for subjects who suffer from constipation (29).

Prunes can also have a positive impact on reducing the risk of osteoporosis. A recent study by Rendina et al. compared the effects of several dried fruits (plums, apple, apricots, grapes, and mangoes) in the restoration of bone in osteopenic mouse models (31). Their results found that prunes, apricots, and raisin diets improved whole body and spine bone mineral density (BMD) compared to the control. The results also show that prunes were the only dried fruit that had an anabolic effect on the trabecular bone in the vertebra that prevents bone loss in the tibia. When compared to the other dried fruits in this study, prunes were the only food that could down-regulate osteoclast differentiation, while up-regulating osteoblast and glutathione activity (31,32). This means prune consumption may aid in slowing bone cell breakdown while increasing the cells that help to build up bones. The authors of this study suggested that compared to other dried fruits, prunes have a unique effect on bone health.

2.1.3 AGRICULTURAL PRACTICES

Plums are known as a climacteric fruit, meaning that they continue to ripen after they are harvested. This leads to commercial plum producers to harvest plums at a pre-mature state to account for the ripening process during transportation and sale (33). Unfortunately, this method of pre-mature harvesting leads to a decreased fruit quality, consumer acceptance, and nutrition content (12). However, if plums were harvested at a fully tree ripened stage, it would result in very short shelf life of only three to four days due to senescence, which is deterioration that comes with age (10,12). Locally plum production would help this dilemma by allowing for mature-harvesting. This practice would eliminate the need for cross-country shipping and transportation.

The continued ripening that occurs in plums, post-harvest is due to the production of ethylene, which is an oxygen-dependent olefin gas and pheromone (34). Ethylene production accelerates the process of senescence, reducing the time plums can be consumed, post-harvest. The conversion of L-methionine into ethylene causes this process. All fruit products undergo the process of senescence, which is deterioration that alters the aroma, color, texture, flavor and nutritional properties causing decreased consumer acceptability (12,35). Many commercial producers reduce the effects of ethylene by using modified atmospheric packaging (MAP) or, more commonly, will cold-store the produce at 0-5ºC in 80-95% humidity (10,36). The cold storing of plums can lead to chilling injury of the fruit, which can lead to a decrease in consumer acceptance. A "mealy" texture and flesh browning are the two major chilling injuries for plums, and the development of flesh browning in plums is always accompanied by flesh transparency, known as gel breakdown (37).

One method to improve plum quality post-harvest is proper temperature management. This would involve keeping cultivars at 0ºC versus 5ºC, during the storage, shipping, and marketing stages and would extend the quality of the plum and reduce the changes of “mealiness” or flesh browning (12,33,37). A second method to improve the quality of plum cultivars is to grow local cold-hardy plums and sell these cultivars at local farm stands and supermarkets during the harvest season in the state of Maine.

2.1.4 CONSUMER ACCEPTABILITY

Growing cold-hardy plums with bountiful yield are essential for sustainability. However, the long-term success of these cultivars depends on consumer acceptability. When consumers are shopping for fruits and vegetables, they look for attributes in the following order; 1) color and appearance, 2) flavor (taste and aroma), 3) texture, and 4) nutritional value (38). The appearance of the product is the most critical quality, and this usually determines whether a product is purchased. Nutritional components of the product are ‘hidden' characteristics that can affect us in ways we cannot see and is becoming more valued by consumers. The commercial growing practices of early harvest in preparation for shipping and storing is having a negative impact on consumer acceptance of plum cultivars and decreasing the nutritional quality of the fruit (13). Other consumer acceptability tests have shown that harvesting plums closer to their ideal tree ripened stage of maturity results in high consumer acceptance (39).

Consumer acceptability of fruits tests many factors including visual and olfactory characteristics, and texture(38). Consumer acceptability tests are the most effective means of assessing product quality. Hedonic scales are widely used in these tests to quantify consumer acceptance with a numeric rating that relates to the “degrees of likeness” (1-dislike extremely to 9-like extremely). Some errors can occur using the hedonic scale to measure consumer acceptance. An example includes ‘the error of central tendency' in which participants avoid choosing options on extreme ends of the spectrum. Another issue is the position order, where the first sample tested can affect the responses for the remaining samples in a particular test (40).

The initial appearance of a product is the most important attribute to consumers (38). Anthocyanins (responsible for red, purple and blue hues), chlorophylls (responsible for green colors), carotenoids (responsible for orange and yellows), and flavonoids (responsible for yellow) of the components that make up the colors and nutritional health benefits of the plum samples (13,38,41). The predominant bioactive that makes up the color characteristic of most plums are anthocyanins (7,19,41). The shape, size, and general make-up of fruit are also essential attributes since consumers look for larger produce that is free from blemishes and uniform in shape (38). Overall, the appearance of a product is the initial determining factor for consumer purchase and consumption.

Other important characteristics of consumer acceptability are flavor and aroma. These two sensations can be affected by many factors including temperature change, pressure, cooking methods, and overall fruit quality. Barret et al. defines flavor as the aroma and the taste of a product, and explains that it can be broken down into five categories; sour, bitter, sweet, salty and umami (broth or meaty sensation) (38). Flavor is the most critical component of consumer acceptability that dictates consumer purchase or consumption of a particular product (38). Volatile compounds released during mastication also play a role in the overall acceptance of a product (38). Research by Crisosto et al., found that the existence of a sweet aroma and flavor caused a higher consumer acceptance, compared to products with an overwhelming sour flavor profile (33,42).

Plums are a unique fruit in that they have very sweet flesh, but a bitter/sour skin. Because of this, it is essential to pay particular attention to the °brix/acid ratios (42). The °brix to acid ratio is the leading predictor of consumer acceptance of plums (13). °Brix is defined as the amount of total soluble solids in a product or in the case of plums the amount of fructose (sugar) present and is expressed as °brix (43). Titratable acidity represents the total acid in a product and in the case of plums is reported as percent malic acid. Another study conducted by Crisosto et al. found that the amount of time fruit is left on a tree to ripen directly impacts the °brix and titratable acidity values (44). The longer that plums are allowed to ripen on a tree, the higher °brix, and lower acidity it will have thus increasing the °brix/acid ratio (44). This study also found significant changes in texture, astringency, and aroma in cultivars that were allowed more extended periods to ripen on a tree, increasing overall consumer acceptability of the fruit (44). This is another reason why locally grown cultivars may have higher consumer acceptance, versus cultivars that are shipped across the country as the local fruit would be fully ripened.

Texture is another important parameter in determining consumer acceptance and can be measured subjectively and objectively (38). The turgor pressure (osmotic pressure found inside a plant cell wall) is a quantifiable measurement and can be obtained using a penetrometer (13,38). Measurement of turgor, or flesh firmness, can help tree farmers determine when fruit is ready for harvest. The average range of flesh firmness that indicates ripeness for plums is 2-3 pounds for immediate sale, and 3-4 pounds if the plums are to be held in brief storage (13). Most plums have a firmness of 2 pounds or less when consumed. As plums ripen, they become more susceptible to rain cracking. The harvest of plums can be scheduled to avoid heavy rains if they are at sufficient maturity (38). Like other attributes, the texture is also affected by stage of ripeness. If a plum is harvested too early, it will have moisture loss and internal breakdown, and if a plum is harvested too late, it will have a "spongy" texture (33). The proper determination for the state of maturity for plum cultivars is an important factor in increasing consumer acceptance.

2.2 NUTRITIONAL BENEFITS OF PLUMS

Through the intake of phytonutrients from plums, consumers may benefit from a reduction in chronic low-grade inflammation. Individuals looking to benefit from these effects are those with diabetes mellitus, obesity and cardiovascular disease where there is an increased level of inflammation (45,46).

2.2.1 TOTAL PHENOLICS

Polyphenols, otherwise known as phenolic compounds, are secondary plant metabolites that act as protectants and provide color (47,48). The attributes of polyphenols create astringent and bitter compounds when they are consumed. Phenolic compounds are believed to be the primary antioxidants in plums, and the predominant polyphenol found in plums is neochlorogenic acid (3-O-caffeoylquinic acid), a type of polyphenol that comes from the subclass of phenylpropanoids (49). Phenolic compounds can be absorbed in the small intestine and have varying bioavailability (47). The microflora found in the colon can act to break phenolic compounds apart. In the liver, these compounds are methylated for elimination (6,47).

When allowed to ripen fully on the tree, the phenolic compounds in plums can increase 1.5-4.7 fold (16). Polyphenols help to inhibit reactive oxygen species by donating a hydrogen molecule to the free radical and prevent dioxygen formation (47,51). This free radical scavenging ability of phenolic compounds can help with diseases such as diabetes mellitus, osteoporosis, obesity and cardiovascular disease by reducing inflammation. With these health conditions, low-grade inflammation and high circulatory blood glucose levels lead to advanced glycation end products and reactive oxygen species (ROS). Polyphenols can help to reduce this chronic, low-grade inflammation by scavenging these ROS (52). The slowing, controlling or reversing of low-grade inflammation is likely to be an important way to prevent, or reduce the severity of, the onset of conditions affecting a person’s health and well-being.

2.2.2 ANTIOXIDANTS

Many studies have proven that dietary antioxidants can reduce oxidative stress and increase overall health through their ability to scavenge free radicals. Free radical production is continuously occurring in cells as part of the standard cellular function, but an excess of free radical production has been found to play a role in many diseases (53). A shared property among all free radicals is the presence of an unpaired electron. This property makes free radicals highly reactive and allowing radicals to freely donate or extract an electron from other molecules, thereby behaving as an oxidant or reductants. Oxygen derivates, specifically superoxide and hydroxyl radicals are the most predominant free radicals that contribute to many disease states. Often, radical formation in the body occurs by several mechanisms that involve endogenous and environmental factors (see figure 1) (53).

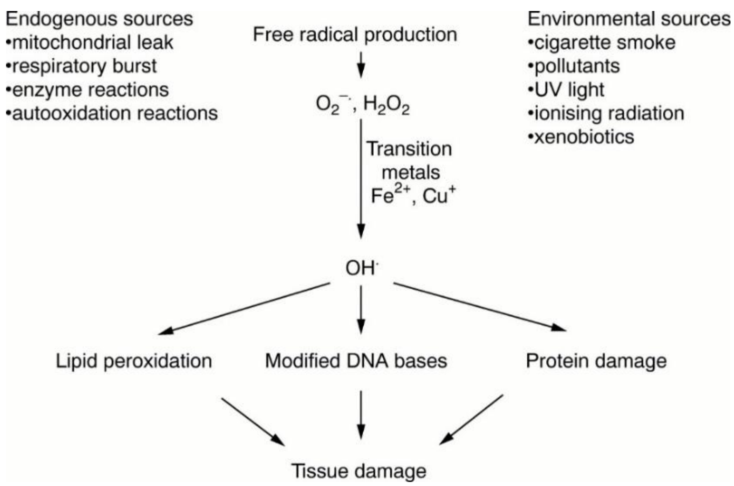


Figure 1: Major sources of free radicals in the body. (53).

Antioxidants are vital in the body as they work to prevent tissue damage caused by free radicals. This is done by stopping the formation of radicals, scavenging them, or promoting their decomposition (3,20,53). Therefore, antioxidants have an important role in the prevention of cardiovascular disease, cancer, diabetes, and other chronic illnesses related to oxidative stress (20,54). Plums are widely known to be rich in antioxidants. The antioxidant or free radical scavenging behavior of plums is more significant than what is found in common fruits such as oranges, strawberries, and apples (54). The antioxidant capacity of plums can be quantified using available quantification methods using DPPH. This information can be used in consumer marketing and can help increase consumer interest in purchasing plums (19).

2.2.3 ANTHOCYANINS

Anthocyanins belong to a subclass of plant phytochemicals known as flavonoids. Anthocyanins are water-soluble pigments that are responsible for the blue, purple and red hues seen in fruits and vegetables. In plants, anthocyanins attract pollinators which promote seed dispersal and protect plants from environmental stressors like ultraviolet radiation and predation (55). Figure 2 shows the chemical structure of an anthocyanin.

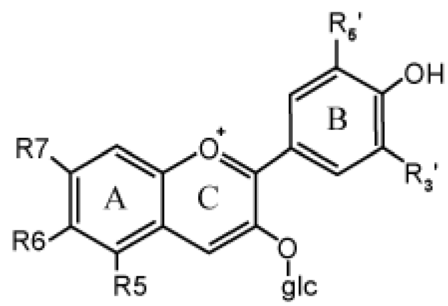


Figure 2: General anthocyanin structure

Source: http://www.micro-ox.com/chem_antho.htm

Anthocyanins are obtained through the diet and are considered non-essential, but are thought to have a positive impact on human health (55). There is research demonstrating the role of anthocyanins in preventing and reducing nutrition-related chronic diseases like cardiovascular disease and diabetes, as a result of their natural antioxidant properties (7,56). In their aglycosylated state, anthocyanins are called anthocyanidins, meaning they lack the presence of a sugar moiety (55). There are six anthocyanidins found universally in plants; cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (57).

The major anthocyanins found in plums are cyanidin 3-rutinoside, cyanidin 3-glucoside, peonidin 3-rutinoside, cyanidin 3-xyloside, and cyanidin 3-galactoside (8,41). These anthocyanins are primarily found in the skin of plums, with the exception of the bright red flesh found in Japanese blood plums (7,19,41). A study by Fanning et al. determined that 33-66% of anthocyanin content in plums are found in the peel, with some studies finding up to 97% in the peel (19). Anthocyanin content is also thought to increase through the ripening process (41). Fanning et al. researched a new plum variety ‘Queen Garnet’, which was developed by the Queensland Government breeding program as a high anthocyanin, high antioxidant plum. The Queen Garnet plum was allowed to ripen on the tree for an additional month, and during that time had an increase in anthocyanin content by 74-98 mg/100 g (19).

Anthocyanin compounds are susceptible to pH levels, with more acidic pH (pH 1-3) leading to mostly flavylium compounds that are the dark red and purple colors and also the most stable (55,57). At more basic or neutral pH levels, there is a loss of pigment or pseudo base formations where anthocyanins become transformed into chalcones (pH 7-8) and are less stable (55,57). Anthocyanins are found to be the most stable at a pH of <2.0 (55).

There have been many *in vitro* studies linking anthocyanin compounds to the antioxidant, anti-inflammatory, and anticarcinogenic properties. There was also a correlation found between the consumption of a diet rich in anthocyanins leading to a decrease in cancer and cardiovascular disease risk (58). However, the research behind the benefits of anthocyanin intake on human health needs further time and investigation. Consumers are currently becoming more health conscious and are seeking foods that provide health benefits. Anthocyanins are one of the most widely investigated polyphenols due to their beneficial health impacts and as a substitute for widely used synthetic food dyes, which can cause toxic effects in humans (59). This shift in consumer values for food products can provide a direction to market products that are rich in anthocyanins, helping to prevent chronic diseases.

2.2.4 Vitamin C

Vitamin C is a water-soluble vitamin with antioxidant properties that are essential to life. Vitamin C has multiple roles throughout the body including, but not limited to, scavenging free radicals, enzyme cofactor donating electrons to synthesize collagen, absorption of iron and peptide formation (60). There are two known forms of Vitamin C; the biologically active form L-ascorbic acid (AA) and the oxidized form L-dehydroascorbic acid (DHAA) (61). The most common form of Vitamin C is L-ascorbic acid which is very labile under many environmental conditions such as temperatures above 23ºC and exposed to light, metals, and oxygen (61,62). When two protons are removed from ascorbic acid, it becomes transformed into dehydroascorbic acid. This oxidation can then be reversed by dehydroascorbic reductase which allows for a higher antioxidant capacity (60).

Since humans cannot produce vitamin C, they must consume vitamin C through the diet. The estimated average requirements (EAR) for Vitamin C is approximately 90 mg/day for men and 75 mg/day for women (60). Studies with plums have shown that the vitamin C content per 100 g serving is between 2.5-10.2 mg, with the flesh comprising 85% of this amount and the peel providing 15% (63). Based on this research, a typical 100g serving of plum can offer consumers up to 14% of their daily allowance of vitamin C.

CHAPTER 3

OBJECTIVES

The purpose of this research was to determine the consumer acceptability of locally grown cold hardy plum cultivars. Specific species were *Prunus domestica,* and *Prunus salicina* tested at a tree-ripened stage of maturity. This information will be utilized to help Maine farmers identify favorable varieties to plant. Plum cultivation can help to boost the local economy. A secondary objective of this research was to evaluate the nutritional and phytochemical content of the plum cultivars. This was completed through analysis of anthocyanin, total phenolics content, in addition to measuring antioxidant capacity.

The first objective of this study was to determine which cold hardy cultivars are most accepted by consumers. The consumer testing was held at the University of Maine Sensory Evaluation Center. This testing was International Review Board (IRB) approved, and participants were compensated four dollars for their time. Plum cultivars were grown at Highmoor Farm located in Monmouth, Maine. Consumer acceptability data on specific plums obtained from this research can be shared with local farmers.

The second objective of this study was to evaluate the nutrient and phytochemical content of the plum cultivars. This is an important objective for two main reasons. First, we can use the laboratory analyses in conjunction with sensory evaluations to make a better recommendation on suitable cultivars. Second, by determining total phenolic content, total anthocyanin content, and free radical scavenging capacity, we can identify health benefits related to the plum cultivars.

Highmoor Farm grows a variety of cultivars that can withstand Maine winters. These plums were widely accepted via sensory testing. The introduction of these plum cultivars that are well-liked by consumers could help to provide a new agricultural sector for local Maine farmers. Providing sensory information to the farmers can help them select favorable cultivars for cultivation where the fruit can be readily marketed. Strengthening the agricultural sector by selecting varieties such as Oblinya, Toka, Kahinta, Rosy Gage, and Early Italian, has excellent potential for orchard production.

CHAPTER 4

MATERIAL AND METHODS

4.1 SENSORY EVALUATION

The plums used for this study were harvested from plum trees planted in 2007, as a randomized block design at the University of Maine’s Highmoor Farm in Monmouth, ME. The trees were cultivated using local practices. The cultivars planted included Early Golden, Shiro, Methley, Black Amber, Spring Satan, Queen Rosa and Vanier. Of those varieties, Early Golden, Shiro, and Methley were used for this research. Toka, Kahinta, and Superior were planted in 2006, and Oblinya, Rosy Gage, Castleton, Long John, and Cacack's Best were planted in 2008. All plum trees were alike in vigor and were hand-thinned to a uniform crop load in mid-June.

The sensory testing for the plums was performed in the Sensory Evaluation Center at the University of Maine. The first day of testing occurred on August 16, 2016, and consisted of cultivars Oblinya, Shiro, and Early Italians. The second day of plum testing was on September 7, 2016, and consisted of cultivars Castleton, Rosy Gage, and Superior. The third day of testing occurred on September 15, 2016, and consisted of cultivars Toka, Kahinta, Cacack's Best and Cambridge Gage. The goal was to recruit 100 participants through each testing sessions via the use of flyers, word of mouth, and the University of Maine First Class Computer Conference system announcements. The goal of 100 participants was successful on the second and third testing day. However, there were only 98 participants on the first day of testing. For a copy of the recruitment notices, refer to Appendix B.

The plum samples arrived early morning on the day of the scheduled test (freshly picked within the past 24 hours), were prepared in a negative pressure kitchen, then delivered to participants through a small sliding window at individual stations. The preparation of the plums included washing, drying and slicing the plums fresh for optimal visualization. During the test, the participants were seated at a partitioned desk under lighting that imitated "Northern Daylight 65" for optimal visual effects of the samples. Samples were assigned a three-digit randomized code appointed by SIMS software (Sensory Computer Systems, LLC., Berkley Heights, NJ, USA). This test was approved by the International Review Board (IRB).

Depending on size, each plum was cut into quarters or sixths and pits were removed. The participants who tested the plums were not involved in the study and did not know which cultivars were being evaluated. Each participant received two slices of plums, which were placed in white ceramic dishes labeled with a corresponding three-digit randomized code. Along with plum samples, participants were also provided with a napkin, water (to cleanse palate) and a laminated sheet with plum images printed to scale to help participants answer a sizing preference question. It should be noted that these sheets were not located for the first sensory test, so the data were excluded from results.

Upon arriving at the testing center, volunteers were asked to read the informed consent form before participating in the study and were invited not to participate if they had allergies or a dislike of plums. For a copy of the informed consent forms, refer to Appendix C. All participants received the same test ballot which consisted of demographics, hedonic scale, and Likert scale questions. Demographic and frequency questions preceded sampling. Once participants sampled the plums, they answered five hedonic questions about color, sweetness, skin tartness, texture and overall liking with 1 = Dislike Extremely and 9 = Like Extremely. There was also a section for open-ended comments for each of the plum samples which allowed additional comparisons to be made. The hedonic part of the test was repeated for all samples tested and was presented in the same order. Once sampling and hedonic questions were completed, participants were asked a set of follow-up questions. These questions included a seven-point Likert scale ranging from Strongly Agree to Strongly Disagree, which was used to evaluate the likelihood of purchasing plums. There were also questions about how frequently participants consumed plums, where they would most likely buy plums, and if they would buy locally grown plums over fruit grown elsewhere. Sizing preference was also assessed by using a tangible, to-scale picture reference sheet of plums at three different sizes, 2.5 cm, 3.8 cm and 5 cm in diameter, which is an accurate representation of the size of plums grown in Maine.

4.2 LABORATORY ANALYSIS

For all laboratory analyses, fresh plum samples were juiced to measure ^{0}brix, pH and the rest of the samples placed in a -20ºC freezer to allow testing of titratable acidity. The remaining plums samples were freeze-dried and then held at -80ºC until further use for analysis. The plum samples were kept in storage for up to two months before they were used for phytochemical analyses including total phenolics, antioxidant capacity, and anthocyanin content.

4.2.1 CHEMICAL AND EQUIPMENT SOURCING

Table 1 provides a list of chemicals used for the laboratory analyses in this study. Table 2 provides a list of equipment used for the laboratory analyses in this study.

Table 1. Chemicals used and manufacturer information

Chemical	Manufacturer	Location
0.1 N Sodium hydroxide	Fisher Scientific	Pittsburgh, PA, USA
Folin Ciocalteu reagent	Sigma Aldrich	St. Louis, MO, USA
Sodium bicarbonate	VWR International LLC	Randor, PA, USA
Gallic acid	Sigma Aldrich	St. Louis MO, USA
Potassium chloride	Fisher Scientific	Pittsburgh, PA, USA
Sodium Acetate	Fisher Scientific	Pittsburgh, PA, USA
2,2-diphenyl-1-picrylhydrazyl	Sigma Aldrich	St. Louis, MO, USA
0.3%Trifluoroacetic Acid (TFA)	Fisher Scientific	Pittsburgh, PA, USA
Meta-phosphoric Acid (MPA)	Fisher Scientific	Pittsburgh, PA, USA
Potassium phosphate monobasic	Sigma Aldrich	St. Louis, MO, USA
Phosphoric Acid	Baker	Phillipsburg, NJ, USA
Tris(2-carboxyethyl) Phosphine Hydrochloride	Thermo Scientific	Rockford, IL, USA
Ascorbic Acid Standard	Baker	Phillipsburg, NJ, USA
Dehydroascorbic Acid Standard	Sigma Aldrich	St. Louis, MO, USA
100% Methanol	Fisher Scientific	Pittsburgh, PA, USA

Table 2. Equipment used and manufacturer information

Equipment	**Model Number**	**Manufacturer**	**Location**
Pocket refractometer	PAL-3	ATAGO	Tokyo, Japan
Analytical balance	ALF204	Fisher Scientific	Pittsburgh, PA, USA
pH meter	PB-11	Sartorius AG	Gottingen, Germany
Colorimeter	LabScan XE	Hunter Associates Laboratories, Inc.	Reston, VA, USA
Glass sample cup	04720900	Hunter Associates Laboratories Inc.	Reston, VA, USA
Centrifuge	5424 R	Eppendorf AG	Hamburg, Germany
96-well plates	-	Fisher Scientific	Pittsburgh, PA, USA
Spectrophotometer	ELx808	BioTek Instruments	Winooski, VT, USA
Vacufuge	-	Eppendorf	Hamburg, Germany
Automated Titrator	HHI 84532	Hanna Instruments	Carrollton, TX

4.2.2 SAMPLE PREPARATION

For analyses of Brix, pH, and titratable acidity the plum samples were juiced by blending six pitted plums for fifteen seconds, followed by centrifuging in an Eppendorf Centrifuge 5904R (Hamburg-Eppendorf, Hamburg, Germany) for ten minutes at 4200 RPM at 4ºC using a swing bucket rotor. The relative centrifugal force (RCF) [$(\frac{RPM}{1000})^2$ x *radius* x 1.118] was

3234 RCF. After samples were centrifuged, they were filtered through a Whatman #4 filter paper in a Buchner funnel using an applied vacuum.

Total phenolics, total monomeric anthocyanins, and antioxidant capacity were measured using freeze-dried plum sample extracts that had been stored in -80ºC freezer. A sample representation of ten plums from each cultivar was ground into a powder using a mortar and pestle and extracted to be used in the assays. The extraction method consisted of weighing 3g samples of plum powder and adding 30 mL of 80% acidified methanol. The samples were then extracted using a Polytron homogenizer at top speed for two minutes. During the homogenization process, the samples were kept in ice to avoid heat degradation of the bioactive molecules in the extracts. After homogenization, samples were centrifuged for 20 minutes at 4200 revolutions per minute (rpm), at 4ºC. The supernatant of each sample was collected from centrifuged tubes and placed in a vacufuge to be dried. Once the extraction of the supernatant was evaporated with the vacufuge, the samples were re-suspended in 10 mL of 80% acidified methanol and aliquoted to be used for assays.

4.2.3 COLOR

The CIE L*a*b* objective method for measuring color, a spectrophotometer is used to obtain values for lightness (L*), green to red (a*), and blue to yellow (b*) colors. Values were interpreted in the following way: L*= 0 is darkest or absolute black, L*= 100 is lightest or absolute white. Negative a* values are in the green spectrum, positive a* values are in the red spectrum. Negative b* values are in the blue spectrum, and positive b* values are in the yellow spectrum.

Analysis of color was performed using a colorimeter using Hunter Lab software, version 4.10. The sensor used for plum testing was the LX16824, with an area view of 12.7 mm. The instrument was standardized using black and white tiles. The column position used for the colorimeter was CIELAB, with a brightness of 457 nm and two illuminant observers (D65/10 and F02/10). Samples were prepared for the colorimeter test by slicing one vertical section of the plum parallel to the ‘seam' of the plum carefully to not come in contact with the pit. The slice was immediately placed on a petri dish (Falcon 50 x 9 mm) and covered with a black cylinder that accompanies the colorimeter. After all the setting and configurations are set up, the sample is read and repeated twice for each plum, using opposites sides of each sample. The results were averaged for each L*, a*, b* value.

4.2.4 BRIX

Brix values represent the concentration of soluble solids in a liquid and are used to determine sugar content. ^{o}Brix was measured using filtered juice samples. The analysis of the percent of soluble solids (Brix) in the samples was performed with a digital hand-held "pocket" refractometer that was zeroed between tests using ultra-pure water and blotted with a Kim-tech wipe. Two drops of the sample were transferred onto the lens of the refractometer using a disposable pipette. All samples were analyzed in triplicates, and an average value was calculated for each.

4.2.5 pH

pH is a measurement of the concentration of hydrogen ions in a solution and thereby measures the strength of an acid. Before testing samples, the pH meter was standardized with buffers pH 4.01 and pH 7.00. pH was measured with a pH meter. The samples were analyzed in triplicates with average values calculated for each sample.

4.2.6 TITRATABLE ACIDITY

Titratable acidity is a measurement related to the acidity of a sample and is expressed as a percent of the predominant acid in a solution. The Hanna Titrator was calibrated for pH using the following buffers: pH 4.01, pH 7.01 and pH 8.20. The pump was calibrated using the manufacturers 'Acidity in Fruit Juice Calibration Standard' (HI 84532-55), and the 'Acidity in Fruit Juice High Range Titrant' (HI 84532-51) was used to measure percent malic acid within 3% accuracy. Titratable acidity was recorded as percent malic acid, the predominant acid found in plums. Titratable acidity was measured in triplicate for all samples. Once the samples were juiced, precisely 5mL of the plum sample was added to the titratable acidity cup, 45mL of ultrapure water was also added, and measurements were recorded.

4.2.7 TOTAL PHENOLICS

The measurement of total phenolic content is achieved by measuring the electron transfer between the Folin-Ciocalteu reagent and the phenolic compounds present in a sample, is also known as the gallic acid equivalent method (64). The measurement is represented as the total reducing capacity of the sample. This is a mixture of phosphomolybdate and phosphotungstate which is used in the colorimetric in-vitro assay of phenolic acids.

The samples for the total phenolics assay were diluted (see Table 3 for dilution factors) to fit the linear range of the plate reader (0.2-1.2). In a 96—well plate, 20µL of each sample was mixed with 90µL of Folin-Ciocalteu reagent that was previously distilled with water (9:1 water: sample), and 90µL of sodium bicarbonate (6g/100mL water). The final well volume was 200µL. The plate was left to incubate for 90 minutes at room temperature (in the dark) and then measured in a spectrophotometer at 750 nm. Gen5™ software, version 2.0 (BioTek) was used to collect readings from the spectrophotometer. A standard curve was drawn based on the absorbance of gallic acid (GA) at different concentrations (0, 25, 50, 100, 150, 200 and 250 µg/ml). The standard stock for total phenolics was made by dissolving 0.01g of gallic acid with 80% acidified methanol and brought up to a volume of 10 ml in a volumetric flask. Seven working standards were prepared from this stock solution.

Total phenolics were calculated using a standard curve (regression curve) drawn using Excel software with concentrations (X), and absorbances (Y) of the working standards and a regression equation was obtained (expressed as Y= a +bX). The total phenolics concentrations of each plum sample were calculated using the regression equation, [x=(y-a)/b] with (y) being the test sample's absorbance. The actual content of total phenolics can then be calculated with the prior concentrations, weights of plum samples, and dilution factors. Total phenolics were tested in triplicate on the plate using three separate extractions. From these separate extractions, an average was calculated along with the standard deviation. The calculations for total phenolics is as follows:

A. Gallic Acid Standard Curve (slope *m* and y-intercept *b*), Sample concentration (x) = Linear Regression

B. (sample concentration * dilution factor) * re-suspension volume / sample weight (g) / 1000 * 100 = Total Phenolic Content per 100 mg freeze-dried

Table 3. Dilution factors used for determining total phenolics.

Sample	**Dilution (Parts Water: Parts Sample)**
Methley	15:1
Rosy Gage	7:1
Castleton	7:1
Early Italian	15:1
Cambridge Gage	15:1
Toka	31:1
Kahinta	31:1
Oblinya	15:1
Cacack's Best	15:1
Shiro	15:1
Superior	31:1

4.2.8 TOTAL MONOMERIC ANTHOCYANINS

Total monomeric anthocyanins are unique in their structure, and subsequently, their color is sensitive to change in pH. At a pH of 1.0, anthocyanins are in their oxonium form and are colored. At a pH of 4.5, anthocyanins are in their hemiketal form and are colorless. This change in color at known pH values allows for the use of a spectrophotometer in determining anthocyanin content of a solution where the difference in absorbance of a pigment is proportional to its concentration(56).

Total anthocyanin content of samples was determined using a pH differential method, as previously described by Lee et al., with modifications. Two buffers were prepared to begin the total anthocyanin assay. The first buffer was a pH of 1.0 and was created with potassium chloride (0.025M). In a beaker, 0.4650g of KCL was dissolved in ultra-pure water and brought up to a volume of 240mL in a beaker. The pH of this solution was measured, and then the pH value was adjusted to 1.0 (±0.05) with concentrated HCl. The solution was transferred to a graduated cylinder and Quantum statis (Qs) 250 mL with ultra-pure water. The second buffer was made to a pH of 4.5 with sodium acetate (0.4M). To make this buffer, 13.6075g of $CH_3COONa3H_2O$ was added to a beaker and dissolved in ultra-pure water to 225 mL. The pH was then measured and adjusted to 4.5 (±0.05) with HCl (3M). The solution was then transferred to a graduated cylinder and Qs 250 mL with ultra-pure water.

The sample preparations began by determination of dilution factors for each cultivar. The samples were diluted with a pH 1.0 buffer until the absorbance at 520 nm was within the linear range (0.2-2.0) of the spectrophotometer (see table 4 for dilution factors). In a 96-well plate, 20µL of the sample was dispensed into a microplate in triplicate and diluted with 180µL of buffer (pH 1.0 or pH 4.5). The plate was left to incubate for 30 minutes before being measured. A spectrophotometer read absorbance at 515 and 690 nm. Gen5 software, version 2.0 (BioTek) was used to collect readings from the spectrophotometer. Anthocyanin content was reported as cyanidin-3-glucoside equivalents, which is the predominant anthocyanin in plums. Each sample was prepared in triplicate, and the average was calculated along with standard deviations. The equation used to calculate total monomeric anthocyanins is as follows:

$(A*MW*DF*10^3)E*0.59$; where:

A= (A515nm-A690nm) pH 1.0-(A515nm-A690nm) pH 4.5

MW (molecular weight)= 449.2g/mol for cyanidin-3-glucoside

DF= dilution factor

0.59=path length in cm (for 200µL liquid in each well)

E= 26900 molar extinction coefficient, in L*mol---1*cm---1, for cyanidin---3---glucose

10^3= factor for conversion from g to mg

Table 4. Dilution factors used for determining total anthocyanins

Sample	**Dilution (Parts Water: Parts Sample)**
Methley	7:1
Early Golden	Undiluted
Rosy Gage	Undiluted
Castleton	7:1
Early Italian	3:1
Cambridge Gage	Undiluted
Toka	3:1
Kahinta	1:1
Oblinya	7:1
Cacack's Best	7:1
Shiro	Undiluted
Superior	Undiluted

4.2.9 ANTIOXIDANT CAPACITY

This method for assessing antioxidants uses DPPH, which is a standard abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark purple colored crystalline powder composed of stable free radical molecules. These free radicals transform from a deep purple to a pale-yellow color when neutralized by antioxidants in a sample. This color change causes a decrease in light absorption at 515 nm when the mixture is read by a spectrophotometer, which allows the determination of percent inhibition of free radicals, or free radical scavenging capacity of a sample (65).

The DPPH working stock solution ($3x10^{-4}$M) was prepared by dissolving 0.0118 g of DPPH in 100mL of 100% methanol. The original plum extracts were all diluted into five different concentrations with absorbance ranging from 0.2 to 2.0 after reacting with the DPPH solution. The plate preparation consisted of a mixture of 150µL 80% acidified methanol and 150µL of DPPH as control with 100% methanol used as a blank. Once the proper dilutions for each plum sample were found, 150µL of the diluted sample was dispensed into each well, with 150µL of DPPH solution. Each sample was dispensed in triplicate, and the average of the readings was recorded along with the standard deviation. Once the samples and the DPPH were mixed, the plate was left to incubate at room temperature for 30 minutes in the dark and then read at 515nm using the spectrophotometer. The calculations for total antioxidants are as follows:

A. Weight of plum (g)/ suspension volume/ dilution factor

B. Concentration / Inhibition = Regression Line

C. (50 – y-intercept) / slope = IC_{50}

4.2.10 FLESH FIRMNESS

Dr. Renae Moran evaluated flesh firmness pressure during harvest on five-ten fruit of each cultivar on tree-ripened plums. Flesh firmness was measured with a Fruit Hardness Tester model 803 penetrometer with an 8-mm tip (General Specialty Tools and Instruments, Secaucus NJ). The plums were each tested on two sides, and to do so the plunger of the penetrometer was pushed into a freshly cut surface until it penetrated the marked line on the instrument to measure flesh firmness. To assure accuracy, the meter was zeroed in between all readings.

4.3 STATISTCAL ANALYSES

Data were analyzed using the GLM procedure of SAS version 9.1 (SAS Institute, Cary, NC) with the LSMEANS statement for means separation between homogenous subsets. Excel (Microsoft Excel, Redmond, WA, USA) was used to calculate means, standard deviations, the coefficient of variance, total anthocyanins, total phenolics and DPPH scavenging abilities. Results were considered statistically significant at ($P \leq 0.05$) for all testing.

CHAPTER 5

RESULTS

5.1 SENSORY EVALUATION

5.1.1 PANELIST DEMOGRAPHICS

Participants for all the sensory tests completed the same ballot using tablets provided by the Sensory Evaluation Center. Each test took an average of fifteen minutes to complete. Out of all participants 61.4% were females, and 38.6% were male. The largest age group was represented by the 18-24-year-old population at 45%, and the lowest represented group was those over the age of 60 with only 5.7%. Most participants (56.4%) consumed plums one to four times per year and 6.7% of participants responded that they never consume plums. When asked to respond to the statement, "I often think about the health benefits of foods before I choose to eat them," 76% of participants agreed with this statement. Of the total participants, 83.5% stated that they were very likely or somewhat likely to purchase plums grown locally in Maine, over a plum grown outside of the state. Additionally, for the second and third test, participants were provided index cards with real-size images of plums measuring 1", 1.5" and 2" in diameter. This was not able to be measured in the first test as the index cards were not able to be located. The size preference question revealed that, 54.5% of consumers preferred a 2" plum compared to 45.5% who would choose a plum that was 1.5" or 1" in diameter plum.

Table 5. Demographic characterization of the 3-session consumer panel (n=98, 100 and 100).

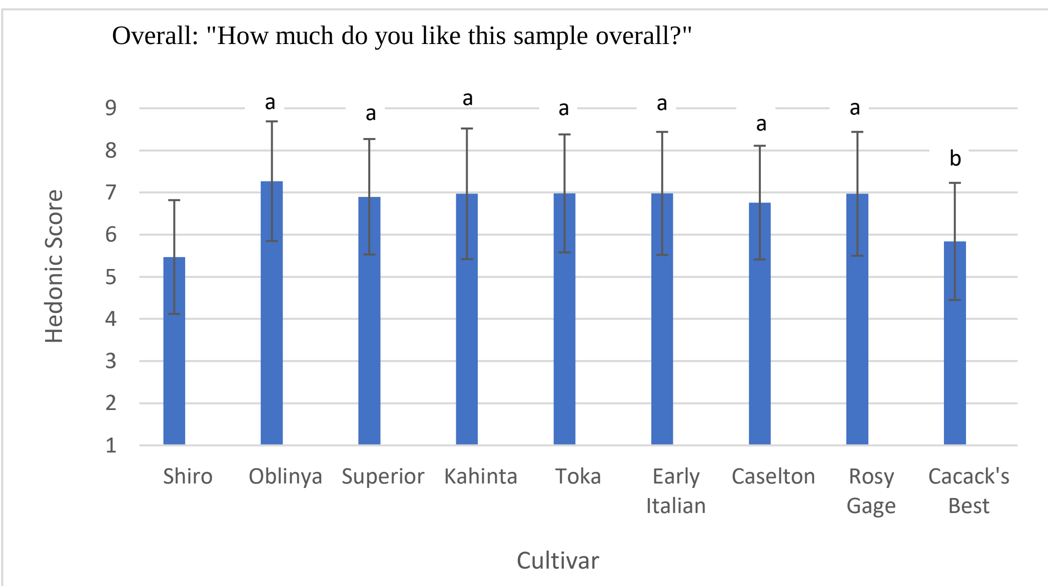
Variable	Response category	Percentage of total
Gender	Male	38.6
	Female	61.4
	Prefer not to say	0.0
Age in years	18 – 24	45.0
	25 – 38	35.2
	39 – 59	14.4
	60+	5.7
Frequency of plum consumption	Never	6.7
	1 – 4 times per year	56.4
	1 – 2 times per month	25.8
	1 – 2 times per week	9.7
	3 or more times per week	1.6
Preference for plum size (diameter)	1”	2.5
	1.5”	43.0
	2”	54.5
Response to "I often think about the health benefits of foods before I choose to eat them."		
	Agree strongly	43.6
	Agree somewhat	32.5
	Agree slightly	18.4
	Neither agree or disagree.	2.3
	Disagree slightly	1.0
	Disagree somewhat	1.6
	Disagree strongly	.06
How likely would you be to purchase a plum grown locally in Maine, over a plum grown outside of the state?		
	Very likely	51.3
	Somewhat likely	32.2
	Neither likely or unlikely	15.4
	Somewhat unlikely	.06
	Very unlikely	.03

5.2.1 CULTIVAR ACCEPTABILIITY

5.2.1.1 OVERALL ACCEPTABILITY RATINGS

The highest ratings for "Overall" acceptability were seen in Oblinya with a rating of 7.27 using the 9-point hedonic scale. This rating was not statistically different from Kahinta, Toka, Early Italian, and Rosy Gage, Superior and Castleton that had average ratings of 6.97, 6.98, 6.98, and 6.97, 6.9 and 6.76 respectively. Shiro and Cacack's Best were the lowest scoring cultivars with ratings 5.47 and 5.84 respectively. Overall acceptability of plum cultivar ratings by participants were all found to be over 5.0, with the cultivar Oblinya being the only plum rated above 7.0. Overall acceptability for cultivars is depicted in figure 3.

Figure 3 Overall Hedonic Rating Average

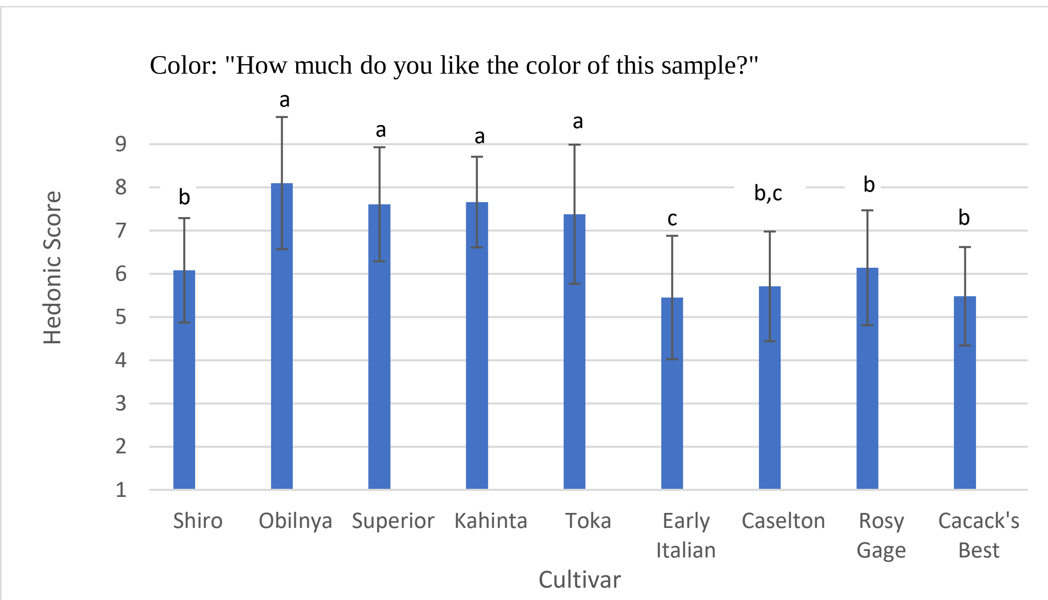


Average overall acceptability scores using hedonic ratings shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.2.1.2 COLOR RATINGS

The color was rated highest for Oblinya with an average rating of 8.10 but was not statistically different from Kahinta, Superior and Toka at 7.66, 7.61 and 7.38 respectively. Rosy Gage, Shiro, and Castleton had no statistical difference with ratings of 6.14, 6.08, and 5.71 respectively. Early Italian was rated the lowest at 5.45 but was not statistically different from Cacack's Best that was rated 5.48. (Figure 4).

Figure 4 Color Hedonic Rating Average



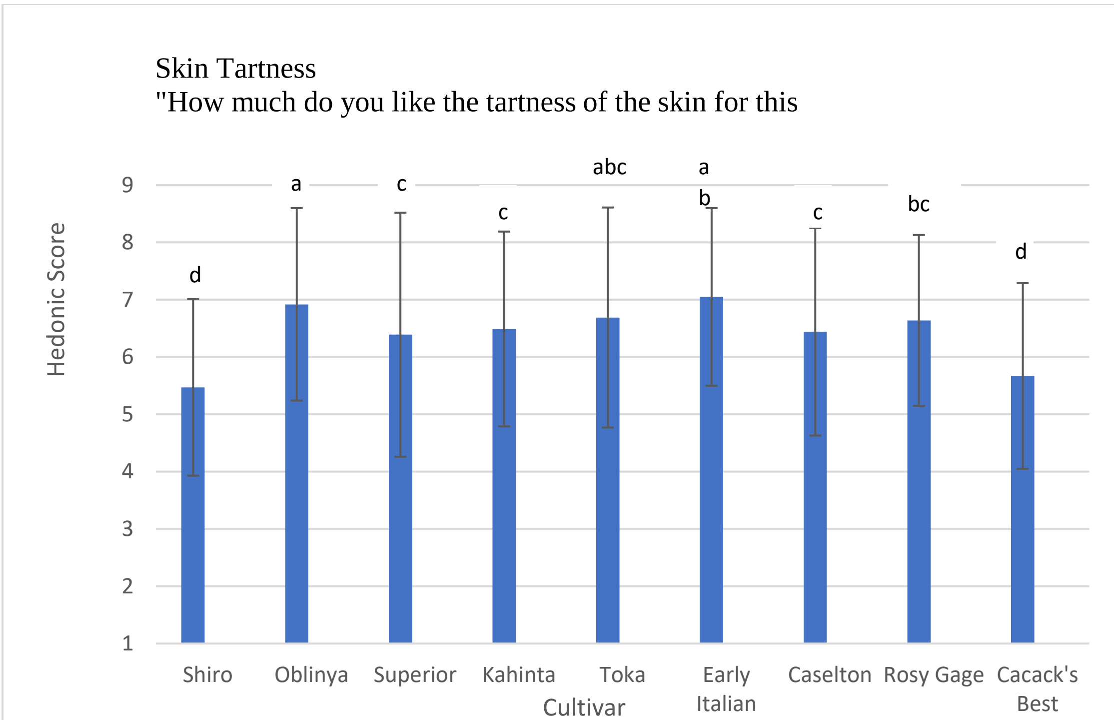
Average color scores using hedonic ratings shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.2.1.3 SKIN-TARTNESS RATINGS

Early Italian had the highest rating for skin tartness at 7.05, and this cultivar was the only plum rated >7.0 in this category. Early Italian was not rated statistically higher than Oblinya, Toka, or Rosy Gage which had average skin-tartness ratings of 6.92, 6.69, and 6.64

respectively. Kahinta, Castleton, and Superior were rated lower than Early Italian, but were not statistically different from each other with average ratings of 6.49, 6.44 and 6.39 respectively. Cacack's Best was rated lowest among all the cultivars with a rating of 5.47 but not statistically different from Shiro 5.67 (Figure 5).

Figure 5 Skin-Tartness Hedonic Rating Average

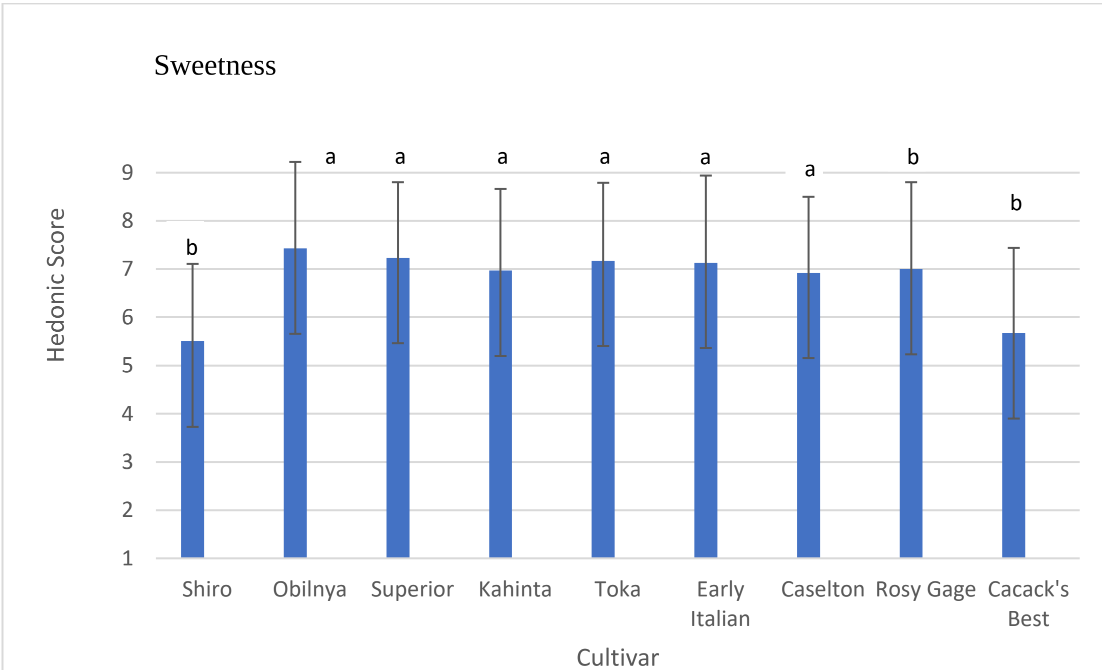


Average skin tartness scores using hedonic ratings shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey’s HSD.

5.2.1.4 FLESH SWEETNESS RATINGS

Oblinya was rated highest for flesh sweetness with an average score of 7.43. However, this was not statistically different from Superior, Toka, Early Italian, Kahinta or Castleton whose average ratings were 7.23, 7.17, 7.13, 6.97 and 6.92 respectively. Cacack's Best was rated the lowest with an average score of 5.5 but was not statistically different from Shiro and Rosy Gage with ratings of 5.67 and 7.0 respectively. Average flesh sweetness ratings are depicted in figure 6.

Figure 6. Flesh Sweetness Hedonic Rating Average.

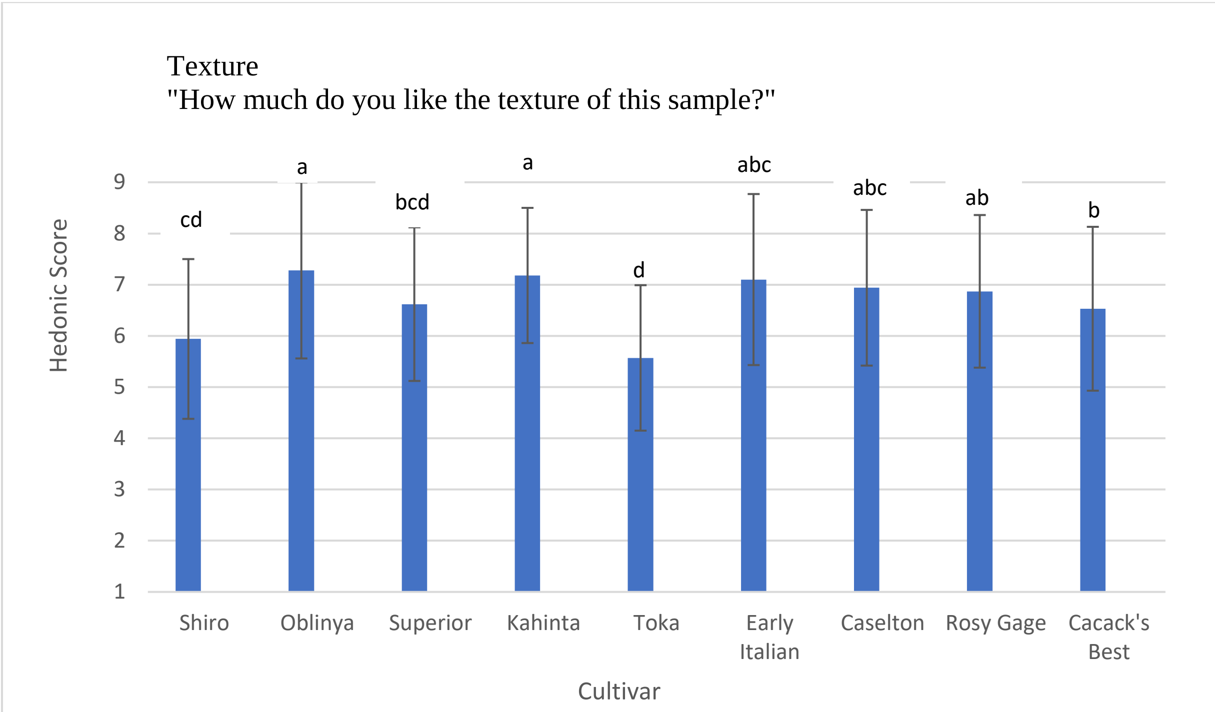


Average flesh sweetness scores using hedonic ratings shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.2.1.5 TEXTURE RATINGS

Oblinya was rated the highest for texture with an average score of 7.28. This was not significantly different from Kahinta, Early Italian, Castleton, or Rosy Gage who had scores of 7.18, 7.1, 6.94, 6.87 respectively. Toka had the statistically lowest average rating 5.57±1.42 but was not significantly different from Superior, Cacack's Best, and Shiro with rating averages of 6.62 6.53 and 5.94 respectively. Average texture ratings are depicted in figure 7.

Figure 7. Average Texture Hedonic Rating Average,



Average ~~flesh sweetness~~texture rating scores using hedonic ratings shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.3 LABORATORY ANALYSES

5.3.1 BRIX, TITRATABLE ACIDITY, BRIX/ACID RATIO, pH, AND FLESH FIRMNESS

Long John had the highest ^{o}Brix with 25%, with the lowest ^{o}Brix found in Shiro at 6.7%. The lowest pH was in Superior at 2.75; the highest pH was in Cambridge Gage at 3.8. Flesh firmness was greatest in Superior and Caselton at 5.0 lbf and 5.0 lbf respectively. Flesh firmness was greatest in Superior and Castleton at 5.0 lbf and 5.0 lbf respectively.The lowest flesh firmness was found in Shiro at 0.9. Average values with standard deviations for ^{o}Brix, pH, TA and flesh firmness are depicted in table 6.

Table 6: Soluble solids concentration, titratable acidity, Brix/acid ratio, pH and flesh firmness of plum samples, shown with the standard error of the mean.

Cultivar	Soluble Solids (%)	Titratable Acidity (%)	SSC: MA	Juice pH	Firmness (lbf)
Methley	11.7 ± 0.01 m[y]	1.34 ± 0.05 g	8.66	3.26 ± 0.04 e	--
Early Golden	10.6 ± 0.01 n	1.98 ± 0.01 bc	5.33	3.04 ± 0.01 h	--
Rosy Gage	19.5 ± 0.01 e	1.67 ± 0.1 e	11.65	3.21 ± 0.01 f	3.3 ± 0.4 bc
Castleton	17.3 ± 0.04 h	1.23 ± 0.03 h	14.02	2.93 ± 0.01 i	5.0 ± 0.5 a
Early Italian	22.1 ± 0.01 c	1.87 ± 0.01 d	11.79	3.72 ± 0.01 bc	--
Cambridge Gage	18.1 ± 0.01 g	1.19 ± 0.01 hi	15.12	3.80 ± 0.01 a	--
Toka	19.8 ± 0.01 d	1.6 ± 0.01 f	12.34	3.41 ± 0.01 d	1.8 ± 0.6 cd
Kahinta	14.8 ± 0.01 k	2.03 ± 0.02 ab	7.26	3.14 ± 0.01 g	3.8 ± 0.7 ab
Oblinya	13.6 ± 0.08 l	1.95 ± 0.04 c	6.96	3.05 ± 0.01 h	2.2 ± 0.1 c
Cacack's Best	15.3 ± 0.01 j	1.0 ± 0.01 j	15.24	3.71 ± 0.01 c	--
Shiro	6.7 ± 0.01 o	1.3 ± 0.01 g	5.12	3.15 ± 0.01 g	0.9 ± 0.1 d
Superior	15.7 ± 0.01 i	2.08 ± 0.01 a	7.55	2.75 ± 0.01 j	5.0 ± 0.6 a
Long John	25 ± 0.01 a	0.87 ± 0.01 k	28.7	3.40 ± 0.01 d	--
Gras Ameloriat	23.4 ± 0.01 b	0.72 ± 0.01 l	32.5	3.74 ± 0.01 b	--
Valor	19.1 ± 0.01 f	1.14 ± 0.01 i	16.7	3.34 ± 0.01 d	--

Y Mean values with standard deviations are depicted. Means single-degree of freedom t-tests detected separation. Means followed by the same letter within cultivar or type are not significant. Data for flesh firmness was not able to be assessed for every cultivar thus leaving missing data points.

5.3.2 COLOR

The color of each plum sample was measured with the CIE L*a*b objective method within 24 hours of the samples arriving on campus. A spectrophotometer was used to measure color by obtaining values for lightness. For the L* value, L*=0 is darkest, or absolute black, and L*=100 is lightest or absolute white. Negative a* values are in the green color spectrum and positive a* values are part of the red color spectrum. Negative b* values are in the blue spectrum, and positive b* values are in the yellow spectrum.

The highest L* value was found for the skin of Shiro, which was a pale yellow color. Shiro also had the largest negative value as it has a light green-yellow color. Transparent Gage also had a large negative number as it is also light green in color. The highest a* value was recorded for the skin of the Japanese plum Kahinta, which is a rich, vibrant red color. The flesh for most plums are yellow in color so many of these flesh readings have positive b* values. The skin for Cacack's Best and Caselton were the only negative b* values as they have a rich purple-blue skin color. The lowest L* value was found for the skin of Oblinya which has a dark red color. Average L*a*b*, objective measurement of color values are depicted in table 7.

Table 7: Color values of plum samples shown with the standard error of the mean.

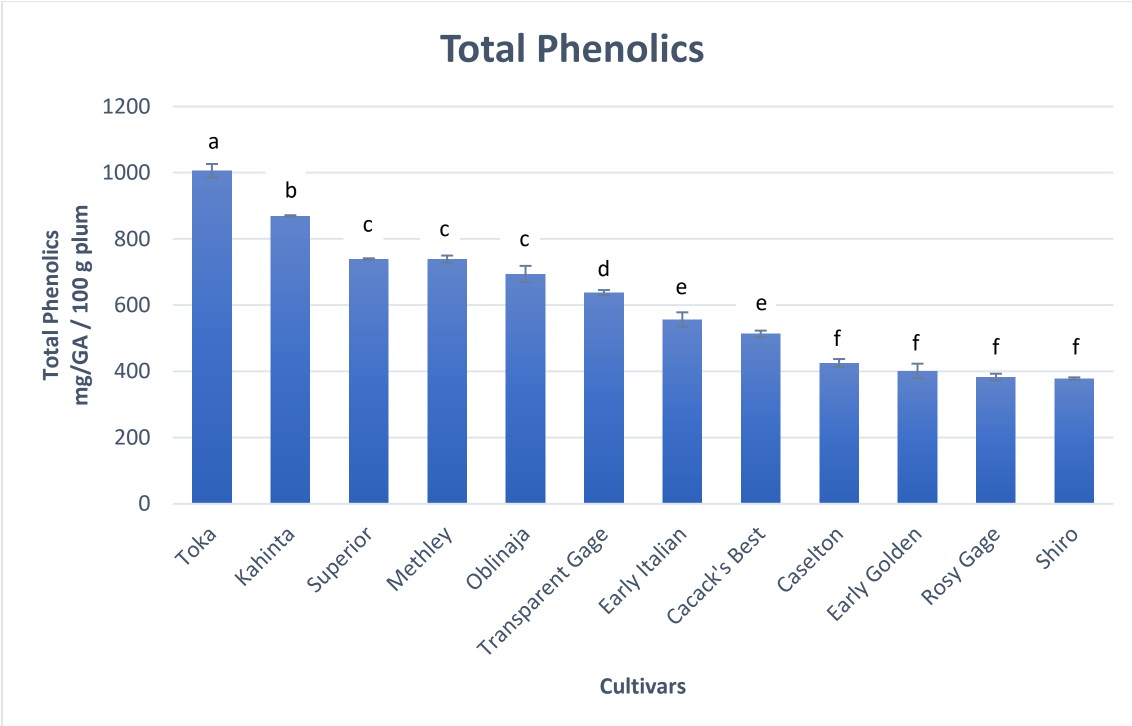
Cultivar	**L***	**a***	**b***
Shiro Skin	59.63±2.58	-2.28±0.42	32.41±4.22
Shiro Flesh	36.53±2.53	1.02±0.78	28.87±1.5
Oblinya Skin	21.73±6.47	6.72±2.72	0.11±2.77
Oblinya Flesh	37.74±1.56	17.78±1.75	29.06±3.2
Early Italian Skin	26.04±1.55	6.04±2.59	0.27±1.57
Early Italian Flesh	33.62±5.42	0.82±1.13	21.68±5.26
Kahinta Skin	28.02±1.73	24.61±4.36	8.92±3.87
Kahinta Flesh	37.82±2.58	14.7±1.86	37.01±1.21
Cacack's Best Skin	25.05±2.83	0.35±0.43	-3.36±2.16

Cacack's Best Flesh	44.66±3.08	1.98±1.01	37.17±1.28
Cambridge Gage Skin	36.71±1.98	0.82±1.1	40.36±2.65
Cambridge Gage Flesh	48.03±3.19	-0.9±2.69	32.27±2.65
Toka Skin	26.21±1.54	14.54±3.6	6.25±2.12
Toka Flesh	36.09±2.27	3.91±0.73	35.73±2.37
Superior Skin	30.61±4.3	19.8±1.8	15.1±5.9
Superior Flesh	37.69 ± 0.17	10.52±0.23	39.02±0.98
Rosy Gage Skin	47.62±3.25	18.93±0.36	30.71±3.31
Rosy Gage Flesh	34.41±3.72	5.11±1.18	33.37±2.47
Castleton Skin	26.46±2.89	1.38±1.04	-5.11±2.0
Castleton Flesh	26.65±4.86	4.05±1.60	24.42±6.07

5.3.3 TOTAL PHENOLICS

The total phenolic content was measured for each plum sample to assess polyphenol content of each cultivar. Toka had the highest concentration of total phenolics (1006.04 mg GA/ 100 g plum ± 21.88), and Kahinta had the second highest concentration (868.87 mg GA/100 g plum ± 2.71) but was statistically significantly lower than Toka. The lowest concentration of total phenolics was found in Shiro (377.61 mg GA/ 100 g plum ± 2.48) but was not statistically significantly different from Castleton (425.22 mg GA/ 100 g plum ±9.9), Early Golden (400.79 mg GA/ 100 g plum ±20.33) and Rosy Gage (382.54 mg GA/ 100 g plum ±10.26). Measurements obtained for total phenolic content are shown in Figure 8.

Figure 8. Total phenolics of plum cultivars.



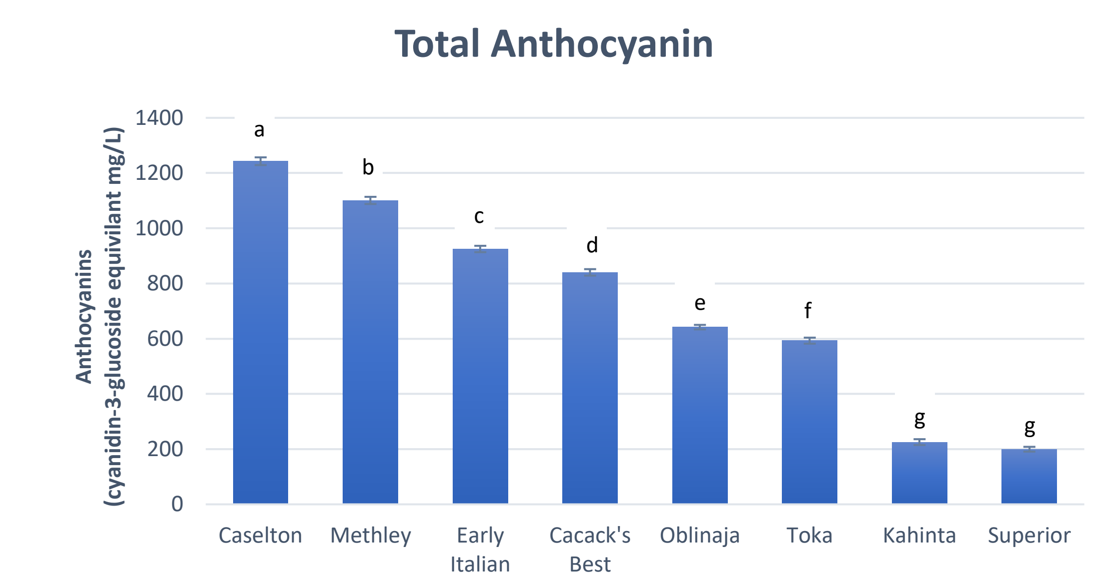
Average phenolic content represented at mg/100 g of freeze-dried plum shown with standard error of the mean. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.3.4 TOTAL MONOMERIC ANTHOCYANINS

Total anthocyanins were measured in each plum cultivar. Anthocyanins are based on red, purple, and blues hues found in fruits and vegetables. Several cultivars of the yellow variety were measured for anthocyanin content but were not included in the results since they had virtually no anthocyanin content.

Of the samples measured, Castleton had the highest concentration of anthocyanins (1242.83 mg/100 g plum ± 14.05), and Methley had the second highest concentration (1100.82 mg/ 100 g plum ± 12.98) and was statistically lower than Caselton. Out of the cultivars tested, Superior had the lowest number of total anthocyanins (199.57 mg/100 g plum ± 8.65) but was not statistically different from Kahinta (228.92±10.71). The cultivars Early Golden, Cambridge Gage, Cacack's Best, Rosy Gage and Shiro were measured for anthocyanin content, but excluded from the graph due to their yellow hues. Data obtained for total anthocyanin content are shown in Figure 9.

Figure 9. Total anthocyanin of plum cultivars, shown with the standard error of the mean.

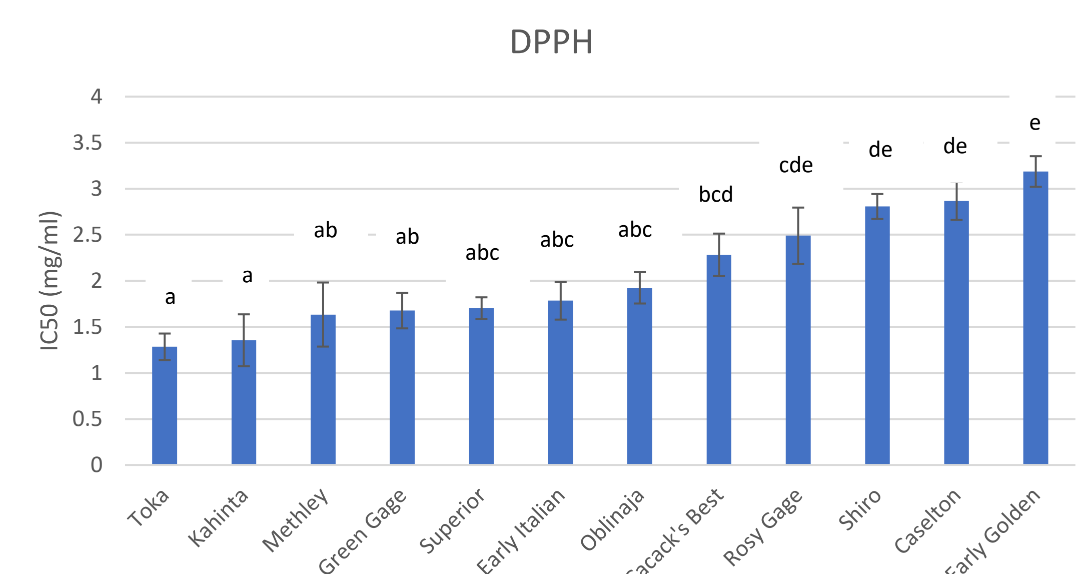


Average total monomeric anthocyanin content represented at mg/100 g of freeze dried plum. Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at $P \leq 0.05$ by Tukey's HSD.

5.3.5 FREE RADICAL SCAVENGING CAPACITY

Free radical scavenging capacity, measured as the concentration needed to scavenge 50% of the free radical compounds, was variable among cultivars. Toka and Kahinta had the most effective DPPH scavenging activity with a 50% inhibition (IC50) of DPPH ($3x10^{-4}$M) of 1.28 mg/ml ± 0.14 and 1.35 mg/ml ± .28 respectively. Early Golden had the least effective DPPH scavenging ability with an IC50 of 3.19±0.16.

Figure 10. Average IC50 DDPH Scavenging Abilities of Plum Cultivars



Average IC50 was calculated using linear regression. IC50 for Gallic Acid is used as an equivalent at (5.68µg/mL). Homogenous subsets identified with like letters, those not sharing like-letters are considered statistically significantly different at *P*≤ 0.05 by Tukey's HSD.

CHAPTER 6

DISCUSSION

6.1 INTERPRETATION OF CONSUMER ACCEPTABILITY

Consumer acceptability is a method of assessing consumer response of a product prior to that product being in the market. This is crucial for determining the success of that specific product. When consumers are choosing fresh fruit, they are considering several factors including the color, appearance, flavor, texture and nutritional value. Consumer acceptability is an important process in determining the success of an item (38). Some large-scale consumer testing studies used in stores, as conducted by Crisosto et al., deem fruit to be accepted by consumers as long as the likeness rating is >5.0. (33,37,42). To eliminate ambiguity in this research, the acceptance level was set at a level ≥ 7. This indicates that a consumer likes the sample moderately or above. If a cutoff of >5.0 is utilized for plums, cultivars that are only slightly liked by consumers could be introduced to market. If a plum that is neither liked nor disliked (score of 5), or only liked slightly (score of 6) enters the market, the fruit may not be purchased or consumed. For this research, Shiro and Cacack's Best received the lowest scores (5.47, 5.84 respectively) for overall likeability. These two varieties serve as an example of why the level is set at >7, to ensure that the most acceptable plums are identified. This is evidenced from results that Shiro has bland flavor and lacks color and Cacack's Best has a bitter aftertaste. All other plum cultivars were ranked favorable among consumers in overall likeability. There is not a set standard for evaluation of fresh fruit, therefore there needs to be additional research to develop such a tool for consumer acceptance of fresh produce (40).

Color is one of the most salient visual cues for consumers. Product color could be considered the most important sensory attribute since a person eats with their eyes (66). The color of a product is what draws consumers in and influences whether they will purchase and/or eat that item. In this research color was one of the highest rated aspects of the sensory evaluation. The brighter colors were better received by consumers. When presenting samples to participants, the plums were cut fresh to prevent oxidative browning. This is important as the oxidation of plums results in a brown color and development of a mealy texture making the fruit unappealing to consumers (33).

In this research, the color was rated significantly higher for Japanese plums than the European plums. Japanese plums have a rich vibrant colors like pink, red, yellow and orange. Many of the European varieties have deep purple skin color and yellow flesh. The reason for this difference in color ratings may be due to Japanese plums having variability in color of the peel and flesh. The variety and brightness of the color are what typically draws many consumers to the Japanese plums, making them favorable for eating as a whole fresh fruit (10,15,16). The varieties of cultivars tested provide many different combinations of skin and flesh colors for both types of plums.

Introducing both Japanese and European varieties to a market that has minimal diversity of plums can help farmers expand their orchards. This research demonstrates that skin and flesh color are important attributes to consumer acceptability, but they should not be the only indicator of which type of plums consumers will purchase. Nutrition content, texture, flavor and overall quality are also important indicators of consumer acceptance.

An important aspect of product acceptance and consumer preference is texture. Texture is also one of the most influential drivers of food aversion (67). An example of this can be seen with the plum cultivar Shiro, which has a soft, watery texture and therefore was not as well-liked among consumers. Toka received a poor rating for texture but was highly rated in all other categories. This may be that due to the fact that it had a soft, spongy texture. Oblinya, Kahinta, and Superior were consistently rated highest for all attributes tested by consumers, including texture and overall acceptability. These plums have attributes of balanced sweet to tart flavors and superior color which lead to their high ratings. Plum producers interested in planting new cold-hardy varieties need to account for all attributes of plums and understand that texture plays an essential role in acceptability. Consumers generally prefer a firm flesh for plum cultivars and this is achieved by harvesting the plums at their ideal state of maturity, which is the tree-ripened stage. Optimal harvest time is important to avoid moisture loss or excessive moisture and gel breakdown, which can lead to alterations of the texture and flavor of the plum.

Plums are a unique fruit that have a sweet flesh with a tart skin profile. The term 'taste' refers to the perception of sweet, sour, bitter and salty, which are detected by the gustatory receptors found primarily in the oral cavity on the tongue (66). The sweetness of a product can be indirectly measured using ^{0}brix which is soluble solids concentration (SSC), and the tartness of a product is indirectly measured through titratable acidity (TA). SSC and TA are important measures that growers use to assess potential consumer acceptance (68). A study performed by Crisosto et al. found that the greatest consumer acceptance for peaches was found when the ^{0}brix was $\geq$10%. It has also been determined that a higher SSC: TA ratio has been associated with greater consumer acceptability (13,68). The results of this study varied slightly from the literature regarding sweetness and tartness ratings. In this study, Shiro, which had the lowest SSC: TA ratio of 5.12, was rated lowest among other cultivars.

However, Cacack's Best, which was also rated poorly, had one of the highest SSC: TA ratios at 15.24. Cacack's Best may have also been poorly rated due to its bitter after taste that is released during mastication (22,38). The plum cultivars Kahinta, Oblinya and Superior, were all rated highly by consumers in all categories and had SSC: TA ratios of 7.26, 6.96 and 7.55, respectively. This may indicate that consumers typically prefer balanced sweetness and tartness when consuming plums.

Plum producers and consumers need to consider all attributes in a plum. Choosing a cultivar based solely on a single characteristic like SSC: TA ratios may result in reduced consumer acceptance as color, appearance, texture, mouthfeel and nutritional value may be just as important as taste and flavor. A negative attribute, such as texture, can directly impact the acceptability of a fruit. Therefore, choosing a cultivar based on a single characteristic may not be sufficient to determine the most favorable fruit. This highlights the importance of understanding which plum attributes attract consumers.

6.2 MARKETING CAPACITY OF PLUMS

In consumer testing, 94% of consumers agree with the statement "I often think about the health benefits of the foods before I choose to eat them." Fifty-seven percent of consumers reported infrequent consumptions of plums, *despite* knowing that the plums provide significant health benefits. This correlates with USDA data, that indicates plum consumption is lower than most other types of fruit with a per capita yearly consumption of 0.5 kg (9). Several factors support this information: 1) there is a lack of diversity of plums in the grocery store, 2) local plums are not predominant in the market, 3) plums do not have a good storage capacity. Introducing plum varieties provides an opportunity to diversify the local market and increase overall plum consumption(69).

6.2.1 INTRODUCING COLD-HARDY VARIETIES TO MARKET

Recently, consumers are focused in the health benefits of food and are becoming more interested in locally sourced products (70). Community supported agriculture (CSA), farm stands, farmer's markets and supermarket chains that are increasing offerings of local foods to meet consumer demands. In this study, 84% of consumer reported that they were likely to purchase a plum grown in the state of Maine, over a plum produced outside of the state. The increase in Maine's population during the summer months provides further opportunity to sell locally grown plums, since the harvest coincides with the peak tourism, July through September. This trend for buying local is also affected by the perception that the local food may have higher quality and less of an environmental impact (69,70). It has been found that plums shipped across country may have inferior qualities compared to local varieties. With the increased tendency to buy local, orchard owners have a chance to expand their orchards to grow more varieties of cold-hardy, consumer-tested plum cultivars.

Plums are climacteric fruit which means they continue to ripen after harvest, which makes stage of ripeness an important attribute for plum sales and consumption. Commercial harvesting practices involve cross-country shipping of under-ripened fruit to account for continued maturation during postharvest(33). These under-ripe harvesting practices decrease consumer acceptability and overall palatability due to chilling injury and gel-breakdown resulting in the browning of the flesh and a mealy texture making the fruit unappealing to consumers(10,33). There is also limited diversity of plums in the market as only a few cultivars can withstand this early harvesting and long-distance shipping practices. Local plum production would eliminate the need for prolonged shipping and storage, and consumers would benefit from the increased quality, variety, and availability of cultivars. By selling local plums, it would allow the fruit to be harvested closer to their ideal state of maturity, increasing overall consumer acceptance.

6.2.2 UTILIZATION OF PLUMS

Increasing local plum production needs to coincide with the development of value added products that utilize plums after the harvest season is over. Value added products could allow consumers to obtain the health benefits of plums year round. An example of a new market may be the beverage industry (71). Research has shown that there are negative impacts related to consumption of sugary soft drinks. Consumers have started to turn their focus to nutritious, healthy beverage choices. Fanning et al. reported various methods of extracting and clarifying juice to retain the nutritional properties (3,16,71). Anthocyanins are heat and light sensitive pigments and can be easily destroyed during the processing of fruits and vegetables (8,72). A study by Kim and Padilla-Zakour found that processing two different cultivars of plums resulted in only 28% retention of total anthocyanins after juice processing (73). Juicing of plums may be carried out cold, but thermal processing has been shown to increase juicing yield (16). An alternative to expanding yield of juice, involves treating the cold plums with pectinase enzyme(16,71). The use of the pectinase enzyme increases juice yield, clarity, soluble solids, titratable acidity, the content of total anthocyanins and total phenolics (71). This study was testing the effects of pectinase enzyme on juicing found that juice from several cultivars may be needed to provide the best balance between acidity and sweetness.

More recently there has been advanced processing technologies, like the high-pressure technique, which preserve the fresh flavors and colors of plums(16). Microwave hydro diffusion is a new technology that can be utilized in place of thermal treatment for processing fruit. This processing method is a combination of microwaves and hydro diffusion of essential oils from the inside to the exterior of fruit and uses gravity to collect and separate the juices (74). This method was tested on European plum varieties, and although the phytochemical content was left intact, the juice yield was significantly lower than that obtained by thermal treatment and treatment with enzymes(16). Therefore the best method for extracting plum juices is using cold juicing with the use of the pectinase enzyme.

European plum cultivars are used mostly as a processed plum, found in compotes, mousse, dried, canned, jams and most commonly, prunes (16,30,57). Japanese plums are typically consumed as whole, fresh fruit. This creates a need to market and utilize Japanese cultivars in the development of value added products. The health benefits of anthocyanins and the intense red color of Japanese blood plum juices and extracts provides a potential for their use as a natural red colorant in food products and food blends (16). These plums can be used as a source of natural colorant as they have a similar hue to the synthetic colorant erythrosine (Red 3), which has harmful effects when consumed (75). The stability of plum anthocyanins is also greater than what is seen in similar fruits such as red grape colorants (8,41). Since anthocyanin compounds are pH sensitive and are the most stable at an acidic pH, the properties of Japanese blood plum anthocyanins make them a good candidate for use as a natural red colorant in lower-pH products to preserve the anthocyanin content (16).

As plum production is encouraged, there needs to be new venues for utilization of the fresh fruit after harvest season is over. There is a need for new value added products such as plum juice, wines and colorants. Implementation of processing techniques such as juicing and baking may also provide a valuable means for using these plum crops. This would ensure that the crop is well utilized and that waste is minimized, leading to an increase in profit for tree farmers.

6.3 COMPARISON OF JAPANESE AND EUROPEAN CULTIVARS

Japanese *(Prunus salicina)* plums are cultivated mainly for fresh fruit market while *Prunus domestica* (European) plums are used for the production of prunes (76). Both species of plums have differences in shape, size, taste, appearance and nutritional profiles. Each cultivar type is high in fiber, vitamin C and potassium which have overall beneficial health effects for consumers (16) including protection against immune system deficiencies, cardiovascular disease and eye disease (60). However, there are several significant differences between the two types of cultivars that could influence consumer choice and utilization that will be further discussed in detail.

6.3.1 JAPANESE CULTIVARS

Consumers are drawn in by the bright colors and the balanced sweet to tart profile found in Japanese plums (14,16). Color is correlated with many health benefits since it indicates the type of phytochemical. The vibrant deep purple, blue and red colors found in Japanese blood plums are a good source of anthocyanins at 1100 mg/100 g. The high levels of anthocyanins in the flesh of Japanese blood plums differs from other types as most often anthocyanins are found in the skin of a fruit (16). The only blood plum used in this research, Methley, had the highest content of anthocyanins due to its red flesh and skin. During the 2016 growing season there was a lack of Methley plums so the sample was not used in the consumer acceptability study. Smeriglio et al. found that blueberries have an average anthocyanin content of 430 mg/100 g (58). This makes Japanese blood plums one of the most abundant sources of anthocyanins(16). Anthocyanin content varies significantly for Japanese plums due to the full range of colors observed with these cultivars as the skin and flesh colors for these plums range from a pale yellow to a deep maroon (14).

Consumers rated Oblinya, Toka, Superior and Kahinta, all Japanese plums the highest for overall likeability in this study. This may be due to their bright, vibrant and variety of color that is seen with this type of plum. The high consumer rating of these cultivars may also be caused by the balanced sweet to tart ratio that is seen with these plums. Japanese plums also had significantly higher amounts of total phenolics and better free radical scavenging capacity than seen in the European cultivars.

6.3.2 EUROPEN CULTIVARS

The European plum varieties used in this research had little variation of color, consisting of mostly purple skin with yellow flesh. European plums also had higher anthocyanin content as Castleton, Early Italian and Cacack's Best all had higher levels of anthocyanins compared to Japanese plums. A study conducted by Fanning et al. found that most of the anthocyanin content (approximately 66%) of anthocyanin-rich fruits are often highly concentrated in the skin/peel(16). Therefore, the reason for the higher levels seen in the European varieties may be due to the deep purple color of the skin.

European plums have a higher SSC concentration of 18.5% average. The higher sugar content of European plums sets them apart from Japanese varieties and allows this type of cultivar to be mainly used in processed products such as compotes, jams, mousse and prunes (23). Prunes, which are typically made from drying European varieties may have many beneficial health effects when regularly consumed. Eating prunes as a regular snack help to increase satiety which can help in reducing food intake, rates of obesity and other related chronic diseases including diabetes and cardiovascular disease (24,77). A study conducted by Chang et al. found that the ingestion of prunes can aid in lowering LDL plasma cholesterol levels in patients who suffer from hypercholesterolemia (27,32). In addition, since plums are have higher levels of antioxidants than most other types of fruit, prunes will also have a higher antioxidant capacity, making them a preventative measure against chronic diseases like heart disease and cancer (27).. Prunes and prune juice are high in fiber so they aid in digestion and help to prevent constipation (24). Prune products are found to be high in potassium, vitamin C, and iron. Prunes also have a low glycemic index making them a safe snack for diabetics as they don’t rapidly raise blood sugar levels. Since prunes have a low glycemic index and may health benefit they can be used as a sugar substitute in desserts. Substituting prunes for sugar still adds desirable texture to baked goods. The consumption of prunes as part of a well-balanced diet may help in the prevention of obesity and other related chronic diseases

6.4 BIOACTIVE CONSTITUENTS AND IMPACT ON DIEASE RISK

Fruit is one of the most important food groups in our diet as it has high concentrations of fiber, vitamins, minerals, phytochemicals and especially, antioxidants (78). Pem et al.

discussed the association of low intakes of fruits (and vegetables) with increased risk of chronic diseases such as cardiovascular disease, blood pressure, hypercholesterolemia, osteoporosis, many cancers, COPD and mental health (78). The health benefits associated with fruits are due to the high phenolic content, their anthocyanin compounds and antioxidant capacity (79). There has been an increased interest in the crucial role of antioxidant compounds in fruit which has been shown help to reduce the risk of chronic diseases by protecting the body against free-radical damage.

6.4.1 THE HEALTH BENEFITS OF PHYTONUTRIENT CONSUMPTION

Free radical production is continuously occurring in the body, due to the production of oxygen radicals from normal metabolism (52). Overtime, free radicals can cause cellular damage which is correlated with oxidative stress (53). This process can lead to chronic diseases such as cancer, cardiovascular disease and diabetes. Antioxidants work to prevent tissue damage caused by free radicals, by scavenging and promoting decomposition of the radicals (5,23,53).

Plums are a good source of phenolic compounds and contain several essential phytonutrients including flavonoids and phenolic acids which have strong antioxidant capacities (80). Plums have a relatively high concentration of antioxidant components compared to grapes, pineapples, raspberries and pomegranates (5,32). In this study, the Japanese plum cultivar Toka had the highest total phenolic content and antioxidant capacity among all other cultivars. Japanese varieties Oblinya, Kahinta, and Superior also had high levels of total phenolics. All of the plums used in this study were grown at Highmoor Farm is Monmouth, Maine and therefore had similar growing conditions and were all harvested at the tree-ripened stage. The exception to this was the supermarket plum Early Italian. Since growing conditions of the plums was similar, the variations of phytonutrient content were significant and likely based on the type of cultivar. Fanning et al. found that due to their rich, vibrant color, Japanese varieties tended to have higher total phenolics and free radical scavenging abilities over the European counterparts(16) and therefore have a higher antioxidant capacity.

Anthocyanins are abundant phenolic compounds in nature and are responsible for the red, blue and purple colors seen in plums (41,56). In this study, the European plum Caselton, with its dark purple skin, was found to have the highest levels of total monomeric anthocyanin among all other cultivars. Other European plums, including Early Italian and Cacack's, also had high levels of anthocyanins, as well as the Japanese blood plum, Methley. Research published by Usenik et al. did not find European varieties to have as much anthocyanin content as seen in our study(13,41). The European plums in this study had two to three times higher levels of anthocyanins compared to what was seen in the study by Usenik et al (41). When comparing the research on anthocyanin content between Japanese and European plums, the European varieties are typically found to have higher anthocyanin content(8,41,55). This trend was supported in this research as all the European plums exhibited higher anthocyanin levels than the Japanese varieties, with the exception of Methley. Many *in vitro* studies and a few animal experiments have demonstrated an extensive range of biological functions of anthocyanins including antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities (16). There is an inverse correlation between the consumption of a diet high in anthocyanin compounds and the incidence of certain types of cancer, cardiovascular disease, metabolic syndrome and other degenerative diseases by reducing oxidative damage (72,81).

Cardiovascular disorders are the most significant causes of death worldwide and are a class of diseases linked to platelet aggregation, hypertension and high plasma concentration of LDL cholesterol (28). Dietary antioxidants and anthocyanins have a potential role in preventing these conditions (8,28,82). A study conducted by Hassellund et al. found that purified anthocyanin supplements help to alleviate metabolic risk factors and markers of inflammation in patients with prehypertension (46). The increase in bioavailability of anthocyanins also resulted in a rise in high-density lipoprotein (HDL) cholesterol. This indicates that individuals who consume high levels of plums rich in anthocyanins may reduce their risk of developing risk factors that can lead to cardiovascular issues.

By consuming plums which are rich in phytonutrients, individuals can benefit from a reduction in chronic-low grade inflammation, which can in turn help to prevent diseases such as diabetes mellitus, obesity, and cardiovascular disease (28,52,83,84). When advising patients with these medical conditions, it should be suggested that they increase their fresh fruit intake, specifically plums, which are known to have higher antioxidant capacity over fruit such as apples, strawberries, and oranges (32). The consumption of plums can help to reduce secondary complications of chronic diseases such as diabetes. Diabetic patients have an increased risk of developing neuropathy, retinopathy, and nephropathy. Phenolic acids in the plums help to reduce blood sugar by inhibiting the activity of carbohydrase enzymes. Reduction of hyperglycemia through the diet is essential in preventing these unfavorable conditions (83). Furthermore, the natural fiber content in plums helps to reduce the glycemic index of the fruit, which is yet another method in lowering post-prandial blood glucose spikes (85).

Data from Aune et al. shows that an increased intake of fruits and vegetables to meet the World Health Organization goal of 400g/day can have an inverse association with chronic health-related diseases including cardiovascular disease, cancer, diabetes, and obesity (28). Plums may have an important role in glycemic control and the prevention of chronic diseases if they are incorporated into a healthy and balanced diet. The health benefits of plums should be marketed towards consumers to help increase consumption. This would attract the interest of consumers who are looking for foods that provide health benefits and allow consumers to make more mindful decisions and choose plum cultivars based on their taste, appearance and nutritional properties.

6.4.2 THE NEED FOR INCREASED FRUIT CONSUMPTION

According to the United States Department of Agriculture, National Nutrient Database for Standard Reference, a 100 g serving of plums contains 87% moisture, 46 kcals, 1.4g of fiber, 157 mg of potassium and 9.5 mg of vitamin C (86). Plums are a tasty, small, nutritious snack that are low in calories but high in fiber, vitamins and minerals and phytonutrients contributing to a healthy diet. In this country, the average consumer does not meet the recommended intake of daily fiber. The incorporation of plums into a healthy diet can provide 6% of daily fiber requirements in a single serving, which can help to lower postprandial blood glucose and lower the risk of developing cardiovascular disease (12,46,60).

Fiber is an important component of a well-rounded diet and helps to keep people full and has been shown to reduce intestinal passage rates by forming stool bulk. The forming of stool bulk causes a more gradual nutrient absorption and preventing constipation (24,77,78). Furthermore, the high fiber content of fruit may play a role in calcium absorption, which reduces the 'acid load' of the diet, enhancing bone formation resulting in greater bone strength (87). Fruit consumption, especially plums which have high levels of antioxidants and polyphenols, have positive influences on multiple chronic diseases. Despite these beneficial properties, actual fruit consumption of Americans is far lower than the recommended level of 1.5-2.0 cups per day, which equates to three to four whole plums (88).

Interestingly, the phytochemicals that are found in fruits have been found to act as anti-obesity agents through their role in suppressing growth of adipose tissue (78). Consumption of a diet rich in fruits and vegetables with a variety of phytochemicals have resulted in significant reductions in markers of oxidative cellular damage to DNA and lipid profiles in overweight individuals (89). Also, fruits are very low in energy due to their high water and fiber content, so can be consumed in relatively large amounts to increase satiety and maintain a healthy weight (78). Maintaining a healthy weight and consuming adequate amounts of fruits and vegetables has a crucial in combatting obesity, diabetes and other related chronic diseases.

CHAPTER 7

OVERALL CONCLUSIONS

There is now abundant amounts of evidence linking poor dietary choices with nutrition related chronic diseases, creating a burden for our health care systems in this country. In recent times there has been an increased focus on phytonutrients including antioxidants, phenolic acids, and monomeric anthocyanins. By consuming foods that are high in phytonutrient content, consumers can benefit from a reduction in chronic low-grade inflammation. Individuals who are suffering from diabetes mellitus, obesity, cardiovascular disease and cancer would benefit from this reduction in chronic low grade inflammation in the body (4,12,47,88). Consumers are increasingly interested in choosing foods that fit into a healthy diet. In fact, most participants of this research stated that they often think about the health benefits of food before they choose to eat them. Reducing the risk of these diseases can help to decrease the burden being placed on our health care system and can increase the overall health of our population.

The data collected from this research can be used to market locally grown plum cultivars by understanding consumer preference for plums (14). Farmers can utilize the information obtained on the bioactive constituents from this research to help make sound decisions on which plum cultivars to grow. This research is unique in that compares European and Japanese plum varieties to determine which cultivars would be valuable additions to crops. The local plums have an overall higher quality fruit and provide a new, profitable agricultural sector for farmers, and will help to boost Maine’s economy. The importance of growing and harvesting local plums is that the fruit will have superior taste and flavor that will be appeasing to the consumers that will be purchasing these products.

The cold hardiness and consumer acceptability of local plums found from this research can help fill the gap in knowledge on how to choose varieties based on consumer preference. Tree-ripened cultivars Oblinya, Toka, Kahinta, Superior, Castleton and Early Italian all have favorable marketing potential for tree-fruit farmers in Maine. By evaluating consumer acceptance and bioanalysis of the plum cultivars, there is a substantial understanding of tasty and healthy plums for producers. Furthermore, participants of this study indicated that they were more likely to purchase locally-sourced cultivars, which will have a positive influence on Maine's economy.

CHAPTER 8

STUDY LIMITATIONS

The State of Maine suffered a drought for the summer of 2016, which made it difficult to collect enough plums for the consumer acceptability testing and the bioanalysis for all varieties. Some varieties were only able to undergo bioanalysis testing as there were not enough to test with consumers. With the decreased number of plum cultivars for testing, there was no way to test freshly picked plums versus cold stored plums with our participants. Also, due to improper storage conditions of the plum varieties, measurement of ascorbic acid and dehydroascorbic acid was not able to be performed, as vitamin C is extremely labile and the amounts left in the 2016 plums were virtually undetectable.

CHAPTER 9

FUTURE DIRECTIONS

A follow-up study on additional cultivars should be conducted for future growing seasons. The cultivars Cambridge Gage, Rosy Gage, and Early Golden were not able to be sensory tested and were only able to undergo bioanalysis testing, so these cultivars may be tested in the future. There should also be research conducted on consumer acceptance of fresh picked and cold stored varieties to see if consumer preference and phytonutrient content changes based on storage conditions. In addition to assessing additional varieties, there is a need for the development of new value-added plum products. Examples of this type of market could include juicing, colorants, and non-thermal processing. This would allow consumers to obtain the health benefits of plums after the harvest season is over.

There should also be further research conducted on enzyme activity of cultivars including α-glucosidase inhibitory effects of plums to understand how consuming plums can affect a person with diabetes mellitus. Determination of AA and DHAA in plums can also allow for a more precise assessment of AA content in plum cultivars as vitamin C is a predominant vitamin in plums and has not been assessed in local plums yet. Furthermore, marketing of cold-hardy locally grown plums should be conducted to determine if consumers will purchase these plums compared to plums grown elsewhere. This could be performed by selling plums at farm stands, farmer's markets, and local grocery stores. Assessing storage parameters, additional plum varieties, vitamin C content, and sales of plums can provide valuable information to both consumers and growers.

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APPENDIX A: RECRUITMENT NOTICE FOR PLUM TASTE TESTING

Plum Tasting Recruitment Notice

If you are at least 18 years old and like eating fruit, please help University of Maine researchers evaluate plums.

Testing will take about 20 minutes to complete. Participants will be paid $4 for tasting the plums and completing a survey.

Testing will be held on: Wednesday September 14th between the hours 1:00-4:00 pm and on Thursday September 15th between 1:00-3:00 pm; the testing will take place at the sensory evaluation lab in the back of Hitchner Hall (Room 158A and 158B).

Please call (207) 390-0368 or email amber.elwell@maine.edu for more information.

If you do not like eating tree fruit, or have an allergy to plums, please do not participate.

Tasting will take place on multiple days. If you choose to participate in this research, please do so only once.

To participate in this study please fill out doodle pool with your availability. http://doodle.com/poll/ttvf9daar65xggi7

APPENDIX B: INFORMED CONSENT FOR PLUM TASTE TESTING

You are invited to take part in a research project by Amber Elwell, a M.S. student in Food Science and Human Nutrition, under the direction of Assistant Professor, Dr. Angela Myracle, of the School of Food Science and Agriculture at The University of Maine. The purpose of the research is to determine acceptance of plums that are hearty enough to withstand our local climate. You must be at least 18 years old to take part in this project. If you do not like plums, or have an allergy to plums, please do not participate.

What will you be asked to do?

If you choose to take part in this study, you will be asked to answer a few questions about yourself like your age and gender. You will be served two plum samples. For each sample, you will be asked to rate that particular sample.

Risks

The risks involved in participating in this study are small, and are not expected to be more than those associated with normal eating. The test may take about 20 minutes to complete.

Benefits

You may enjoy eating the various cultivars of plums. Completing the test may help to further grow Maine's agricultural opportunities.

Compensation

Upon completion of the study, you will receive $4 cash. No compensation will be provided if you decide not to complete the study.

Confidentiality

Your name will not be on any files that contain your answers to our questions. Data will be kept in a locked office on password-protected computer. All data will be destroyed by April 1, 2016.

Voluntary

Taking part in this study is voluntary. You may skip questions that you do not wish to answer. If you chose to take part in this study, you may stop at any time. You will only receive compensation if you complete the tasting.

Contact Information

If you have any questions about this study, please contact Amber Elwell at (207) 390-0368 or amber.elwell@maine.edu; or Dr. Angela Myracle at (207) 581-1617 or angela.myracle@maine.edu. If you have any questions about your rights as a research participant, please contact Gayle Jones, Assistant to the University of Maine's Protection of Human Subjects Review Board, at (207) 581-1498 or via e-mail at Gayle.Jones@umit.maine.edu.

APPENDIX C: QUESTIONNAIRE FOR PLUM TASTE TESTING

Attribute 1: Instruction box

Thank you for taking the time to participate in our research.

Attribute 2: choose one

Please indicate your gender:

- Male
- Female
- Prefer not to say

Attribute 3: check one

Please indicate your age range:

- 18-24
- 25-31
- 32-38
- 39-45
- 46-52
- 53-59
- 60+
- Prefer not to say

Attribute 4: check one

Please indicate the highest level of education that you have completed:

- High school
- Some college
- Associate's degree
- Bachelor's degree
- Graduate degree
- Other

Attribute 5: yes or no scale

Do you eat plums?

- Yes
- No

If the answer is no, why not?

Attribute 6: check one

About how often do you eat plums?

- Never
- 1-4 times per year
- 1-2 times per month
- 1-2 times per week
- 3+ times per week

Attribute 7: likert scale

Please tell us how much you agree with this statement: "I often think about the health benefits of foods before I choose to eat them".

- Disagree strongly
- Disagree somewhat
- Disagree slightly
- Neither disagree nor agree
- Agree slightly
- Agree somewhat
- Agree strongly

Attribute 8: Instruction Box

Please evaluate the samples in the order indicated on your screen, and verify that the three-digit code on each of the plum slices match the code on the computer as you rate each sample.
Use the scales to indicate your attitude by choosing the answer that best describes your feelings about the food.
Please take a sip of water before tasting each sample.

Attribute 9: hedonic scale

How much do you like the color of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Attribute 10: hedonic scale

How much do you like the tartness of the skin for this sample?

- Dislike extremely
- Dislike very much

- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Attribute 11: hedonic scale

How much do you like the sweetness of the flesh for this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Attribute 12: hedonic scale

How much do you like the texture of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Attribute 13: hedonic scale

How much do you like this sample overall?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much

- Like extremely

Attribute 14: open-ended comment

Is there anything else you would like to tell us about this sample? If you refer to other samples in this test, please use the sample's three-digit code.

Attribute 15: scale

How likely would you be to purchase a plum grown locally in Maine, over a plum grown outside of the state?

- Very unlikely
- Somewhat unlikely
- Neither likely or unlikely
- Somewhat likely
- Very likely

Attribute 16: select all that apply

Where would you be likely to buy a locally grown plum?

- Supermarket
- Farm stand
- Farmer's market
- Orchard
- Other (please specify)

Attribute 17: check one

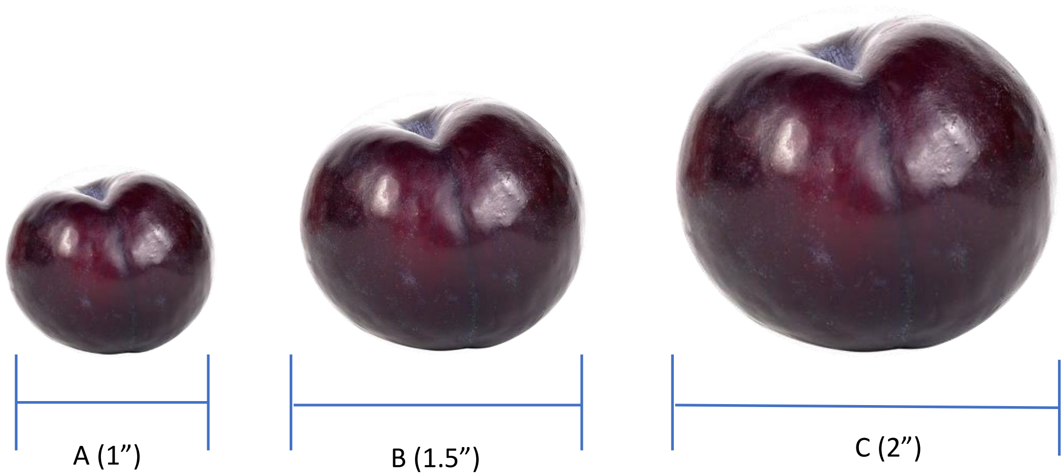
What do you most often utilize plums for?

- Baking
- Salads
- Smoothies
- Jams/jellies
- As fresh fruit
- Other (please specify)

Attribute 18: check one

Please refer to the laminated sheet at your station.

Which of the following plum sizes would you most likely purchase?



Attribute 19: Instruction Box

Thank you very much for your time and opinions. Please raise the window slightly to let the staff know that you are done, and do not forget to pick up your incentive.

BIOGRAPHY OF THE AUTHOR

Amber L. Elwell was born in Rockport, Maine on September 27, 1993. She was raised in Spruce Head, Maine and graduated from Georges Valley High School in 2011. She attended the University of Maine and graduated in 2016 with a Bachelor's of Science degree in Food Science and Human Nutrition. Amber L. Elwell is a candidate for the Master of Science degree in Food Science and Human Nutrition from the University of Maine in August 2018.