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Factors affecting color and anthocyanin content of 'Michigan Purple' potato tubers during tuber development and storage

presented by

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has been accepted towards fulfillment
of the requirements for the

M.S. degree in Horticulture

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Aug. 12, 2004

Date

FACTORS AFFECTING COLOR AND ANTHOCYANIN CONTENT OF 'MICHIGAN PURPLE' POTATO TUBERS DURING TUBER DEVELOPMENT AND STORAGE

By

Elzette van Rooyen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

2004

ABSTRACT

FACTORS AFFECTING COLOR AND ANTHOCYANIN CONTENT OF 'MICHIGAN PURPLE' POTATO TUBERS DURING TUBER DEVELOPMENT AND STORAGE

By

Elzette van Rooyen

Achieving vivid purple color at harvest and maintaining color during storage are important quality factors for purple colored potatoes if growers wish to receive premium prices. To ensure the marketability of the 'Michigan Purple' potato cultivar, we investigated color stability and anthocyanin concentration of 'Michigan Purple' potato tubers during storage and compared it with other colored potato cultivars. In addition, we investigated color and anthocyanin concentrations during tuber development and tentatively identified and quantified the anthocyanins responsible for the purple color in this cultivar. We also investigated pre-and postharvest tools that could possibly enhance the tuber skin-color at harvest and in storage. Preharvest investigations include the evaluation of soil type, plant spacing, nitrogen fertilizer type and application rate, and 2,4-dichlorophenoxyacetic acid (2,4-D) application rate. Postharvest investigations include the evaluation of storage temperature and ethylene application.

Dedicated to my dear parents,

Wilhelm and Ellena van Rooyen.

ACKNOWLEDGEMENTS

I want to thank my parents, Ellena and Wilhelm van Rooyen, my brother Michael and my sister Francoinette for believing in me and constantly giving me the support that I needed to make it through this challenging time in my life. It wasn't easy, but I made it, thanks to you! I also want to thank my friends in South Africa for their love and constant support.

I would like to express my appreciation to Dr. Deirdre Holcroft for inviting me to study with her here at Michigan State University. Thank you for giving me the opportunity to spread my wings. I know you had to leave, but I'm truly sorry that we couldn't keep working together. Fortunately, this gave me the opportunity to work with a very interesting person, Dr. Randy Beaudry. Randy, thank you for your inputs and support and patiently helping me achieve my goal. I enjoyed working with you and learning more about postharvest in your lab.

I would also like to thank the other members of my committee, Dr. Sieglinde Snapp and Dr. Johan Brink for all their help and inputs. Special thanks to Karen and Johan Brink for being so understanding and supportive. I'm glad you decided to live in East Lansing.

There are many special people that I met working at MSU: Kasey and Nick Wierzba, we went through a lot together, good times and bad times, Mauricio Cañoles and Marlene Ayala, you were my second committee, thanks for all the help and support, I don't think I would have been able to do it without the

two of you, Leah Clearwater, another great person I met here, thanks for the good times, Alejandra and Robert, Jen Dwyer and John Treloar.

Lastly I would like to thank a very special person in my life, Conrad Schutte. Thank you for following me to America and being here everyday for the last year to help me. Thanks for all your patience and love.

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INTRODUCTION

Pigmented potatoes and other colored underground organs are currently receiving more market interest for their aesthetic and health properties, especially in the food and restaurant industry. Colored potatoes currently account for 10% of the $ 3.06 billion production in the United States (USDA, 2003). The potato *(Solanum tuberosum* L) is an important crop worldwide, ranking fourth in importance after rice, wheat, and maize.

Color intensity at harvest and during storage is an essential quality factor for colored potatoes if growers want to receive premium prices. In 2001, a purple variety, 'Michigan Purple' was released by the Michigan State University breeding program (Douches et al., 2001). This is an attractive round potato with white flesh and purple skin color, rather than red, which makes it unusual in the tablestock market. The color has been reported to degrade in storage. Thus, to ensure adequate color, it is important to develop pre- and postharvest strategies to maintain or increase the stability and the concentration of anthocyanin pigments.

The anthocyanin pigments responsible for the flesh and skin color of various novelty potatoes have recently been identified (Fossen and Andersen, 2000; Lewis et al., 1998 a, b; Naito et al., 1998; Rodriguez-Saona et al., 1998). The anthocyanins in 'Michigan Purple' have not been identified or quantified despite the importance of color for successful marketing of this variety.

Currently there is no data available on the extent of color loss in the skin during storage, or on pre- or postharvest factors to maintain or enhance anthocyanins.

This project aims to investigate factors affecting color development and stability during production and storage which may be important for the marketability of this new purple-skinned variety. Specifically, the results from this research will provide the information on changes in color of 'Michigan Purple' during storage that will allow growers and storage operators to predict the storage life of the tubers, relative to visual quality. In addition, the research results provides insights into preharvest recommendations for improving tuber skin-color such as soil type and planting space, the use of growth regulator 2,4-D, and the application of nitrogen fertilizer and manure. Postharvest recommendations will include optimum storage temperature and the possible application of ethylene to improve and/or maintain color during production and storage.

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LITERATURE REVIEW

Introduction

Color is an important aesthetic attribute of most fresh and processed fruit and vegetables. In addition, colored commodities are becoming increasingly popular since the discovery of the health benefits of the pigments that are responsible for the color. Hence, interest in pigmented potatoes and products from pigmented potatoes is growing. These potatoes represent a niche market with potential for the gourmet industry based on color. Fortunately, the presence of color (i.e. anthocyanin pigments) in the tuber skin and/or the tuber flesh makes them of potential benefit to human health. Available evidence suggests that anthocyanins could exhibit multiple biological effects such as anti-inflamatory action, antioxidant-antiradical activity, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy, and prevention of cholesterol-induced atherosclerosis (Clifford, 2000; Espin et al., 2000; Mazza and Miniati, 1993; Wang et al., 1997).

Potato History and Importance

Potatoes are the fourth most important crop in the world after rice, wheat, and maize (Salazar and Busch, 2001). It is believed that the potato originated in South America, specifically in the Andean highlands of Bolivia, Ecuador and Peru and was first domesticated more than 6000 years ago in the area around Lake Titicaca (Burton, 1948). In 1532, the Spanish took the potato to Europe where it adapted very well to the climate and soon became an important crop there. It is

believed that the potato arrived in North America about 50 years later. Native Andean potatoes are still cultivated today and display an extraordinary diversity of taste and texture and come in a fascinating array of shapes and colors.

In the United States today, about 1.3 million acres are planted with potatoes and the industry itself has an estimated value of $3 billion (USDA, 2003). Approximately 37% are utilized as frozen products, 12% as dehydrated products, 12% as chips and shoestrings, 31% as table stock, 6% as seed and 2% for other uses such as starch, flour and canned products (Salazar and Busch, 2001).

In the United States, potatoes are classified by the USDA into three categories: round whites, russets and red-skinned. Potatoes in each category share similar characteristics for example the red-skinned category generally have a low dry matter content and are targeted to the fresh market. Red potatoes comprise approximately 10% of the total potato acreage in the United States today (Andersen et al., 2002). Currently, the most popular red-skinned varieties are 'Red Pontiac' and 'Norland' (Salazar and Busch, 2001).

In contrast to the past, there is an increase in the amount of colored potatoes available on the market today. Varieties with purple, blue, and red skin and flesh have been released with names such as 'French Fingerling', 'All Blue', 'Chieftain,' 'Norland,' 'Dark Red Norland', and 'Red Pontiac'. These colored potatoes are referred to as 'specialty' potatoes and are intended to appeal to restaurateurs, caterers, and home chefs.

Tuber Quality

Visual appeal, consumer preference, and ability to meet market specifications are criteria of tuber quality. Tuber quality assessment differs depending on the intended use of the tubers; potatoes targeted for the fresh market or for the French fry or chip industries are evaluated differently. In 1991, The United States Department of Agriculture released 'The United States Standards for Grades of Potatoes' in which details regarding potato grades are reported (USDA, 1991). Here follows a summary of some of the factors that are taken into account to determine quality.

Fresh market: Important tuber quality factors are uniformity in size and shape, unmarked skin, flavor, flesh firmness, shallow eyes, and absence of diseases or defects such as internal brown spot (internal rust spot), internal heat necrosis, glycoalkoloids (greening), and mechanical damage.

French fry industry: The most important quality factors are flavor, absence of defects, concentration of reducing sugar (sugars that contain aldehyde groups that can be oxidized to carboxylic acids), and dry matter. Dry matter is measured as specific gravity, which increases as the water content of the potato decreases. The French frying process dehydrates the tuber flesh, thus tubers with low specific gravity (high water content) will result in major water loss and lower fry yield. Specific gravity also affects oil absorption. Tubers with low specific gravity absorb more oil than those with high specific gravity, resulting in greasy fries. The reducing sugar concentration influences the color of the fries. Tubers with a high reducing sugar concentration become dark French fries since the sugars

react with amino acids in the tuber in a non-enzymatic Maillard-type browning reaction.

Chip industry: The best chip potatoes have good flavor, absence of bitterness and off-flavors, low reducing sugars concentration, and high specific gravity. The color of chips depends on the concentration of the reducing sugars in the potato for the same reasons mentioned for French fries; high reducing sugar concentration will result in dark chips. High specific gravity is extremely important in the chipping industry since chip crispness and lack of oiliness greatly depends on it.

Color is a factor that has increased in importance since colored varieties started entering the mainstream market. Although potatoes of different color and shape, apart from some red-skinned cultivars, are not yet readily available in big retail stores, these potatoes can be found in smaller specialty stores. Since the primary selling point of pigmented cultivars is color, good color is a necessity for cultivars such as 'Red Pontiac', 'Norland' and 'All Blue'. Good color development starts in the field, thus growers can make use of certain growing practices to enhance the anthocyanin synthesis in the field.

Preharvest Enhancement of Anthocyanins

In order to sustain quality after harvest, growers have to make correct preharvest management decisions. Nitrogen (N) management is important for obtaining appropriate plant productivity and color. Nitrogen is a critical nutrient for potato plants since it has a great effect on yield and tuber quality. Inadequate

amounts of N will reduce yields, whereas an excess can reduce tuber quality and tuber yield.

Nitrogen fertilizer sources include ammonium sources such as urea, ammonium sulfate, diammonium phosphate, monoammonium phosphate, and ammonium polyphosphate. These sources are used at planting time since the nitrogen is in the ammonium form (NH_4^+) which is less likely to leach into the soil, as is the case with ammonium nitrate or calcium nitrate (NO_3^-) (MSU extension, 2004). Organic matter such as manure can also be used as a source of nitrogen.

Callus and cell suspension culture studies on grapes have shown that the total amounts of nitrogen, as well as the ratio of NO_3- to NH_4^+ strongly affect anthocyanin production and cell growth (Kim and Kim, 2002). To our knowledge, the impact of nitrogen form and application on the development of the anthocyanins in the tuber skin during tuber development and in storage has never been documented.

The second potential preharvest color enhancement strategy is the use of 2, 4-dichlorophenoxyacetic acid (2,4-D). Foliar applications of hormones such as this synthetic auxin have been used commercially to enhance the color of the red potatoes. 2,4-D stimulates the synthesis of ethylene, which is thought to promote the production of anthocyanins in the periderm (Nelson and Bristol, 1975)

The use of 2,4-D as a potential color enhancer became of interest in the 1940s. One of the first reports of auxin as a color enhancer was from that of Fults and Schaal (1948). They observed an improvement in the skin color of red-skinned 'Bliss Triumph' aka 'Red Bliss' in plants fertilized with 20 lbs per acre of

the sodium and ammonium salts and the butyl ester of 2,4-D. Payne et al. (1950) later proved that 2,4-D also improves the color of 'Red McClure' and that the improved skin color did remain stable in storage. The efficiency of 2,4-D as a color enhancer, depends on the application rate and the stage of development of the plant at the time of application (Nylund, 1955).

Postharvest Enhancement of Anthocyanins

Maintaining storage conditions after harvest affect the appearance of the potato including color and anthocyanin concentration. Appropriate temperature and ventilation are extremely important for maintaining tuber quality. Tubers intended for table use are usually stored at 4°C and at high relative humidity (RH). Those intended for processing are stored at higher temperatures such as 10°C and high RH in order to prevent starch converting to sugar, which as previously noted, can cause potato tissue to darken when fried or dehydrated (Sowokinos, 2001).

In addition, storage temperature is also important to maintain good tuber color. Lewis et al. (1999) found that anthocyanins increased in the skin and/or the flesh of 'Arran Victory' (purple skin/white flesh), 'Desiree' (pink skin/ white flesh) and 'Urenika' (purple skin/ purple flesh) tubers over long term storage at 4°C, while tubers stored at 10, 18, and 26 °C did not have an increase in anthocyanins. They argued that reducing sugars act as precursors for anthocyanins and low temperatures stimulate the synthesis of sugars and thus increase the formation of anthocyanins. Sugars can act as precursors to

anthocyanins by either providing the carbon skeletons of the anthocyanins molecule, or through the glycosylation of the anthocyanins. Conversely, Andersen et al. (2002) found that color and anthocyanin concentration decreased in red-skinned 'Norland' tubers after one month of storage at 4 °C. It is possible that this discrepancy in results is due to differences in response of different varieties to temperature.

In apples it has been found that ethylene can increase the rate of anthocyanin synthesis (Faragher and Brohier, 1984). Ethephon, an ethylene generating compound, is commonly applied preharvest as a foliar application to apple trees shortly before harvest to advance the marketing season. It results in an increase in the concentration of anthocyanins, presumably through the increase of the phytohormones chalcone isomerase (CHI) and phenylalanine-ammonialyase (PAL) (Li et al., 2002). To our knowledge, applying ethylene postharvest to increase the periderm color of colored potatoes has not been tested.

Biology and Biosynthesis of Anthocyanins

The term 'anthocyanin' was first used by Marquart (1835) to describe blue, violet, and red pigments derived from plants. Anthocyanins are part of a diverse class of secondary metabolites known as phenolic compounds. Phenolic compounds, synthesized primarily from the shikimic acid pathway, presumably in the chloroplast (Taiz and Zeiger, 1998), accumulate in the vacuole of the plant cell. Phenolic compounds can be divided into two classes based on their

structure: non-flavonoids and flavonoids. Non-flavonoids include phenolic acids and coumarins; flavonoids include anthocyanins, flavonols, flavan-3-ols, flavones, flavanones, flavanonols, condensed tannins, flavan-3-4-diols, chalcones, and isoflavanoids (Figure 1). Anthocyanins, therefore, are synthesized in the cytoplasm but are ultimately localized in the vacuoles (Hrazdina, 1992; Matern et al., 1986; Wagner and Hrazdina, 1984).

Anthocyanins are the most widespread group of pigmented flavonoids and are responsible for most of the red, pink, purple, and blue colors observed in various plant organs such as the flowers, fruit, roots, stems, leaves, and bracts (Taiz and Zeiger, 1998).

Flavonoids have a basic carbon skeleton containing 15 carbons (C6-C3-C6) with 2 aromatic rings connected by a 3-C bridge (Taiz and Zeiger, 1998) (Figure 2). The carbon skeleton of all flavonoids is derived from acetyl CoA and phenylalanine. The A-ring is formed from three acetate units and phenylalanine gives rise to the B-ring and the 3-C chain of the heterocyclic C-ring (Macheix et al., 1990). The flavonoid biosynthesis pathway starts with phenylalanine which is metabolized to cinnamate by the enzyme phenylalanine ammonia lyase (PAL). Cinnamate in turn forms p-coumarate through the enzyme cinnamate 4-hydroxylase (C4H). p-coumarate eventually forms p-coumaroyl coenzyme A (CoA) through the action of enzyme 4-coumarate: CoA ligase (4CL) (Lewis et al., 1998a) (Figure 3).

The next step is the formation of the yellow C-15 skeleton naringenin chalcone (4,2',4',6' -tetrahydroxychalcone) through the condensation of one

molecule of 4-coumaroyl CoA with three molecules of malonyl CoA. This step is catalyzed by chalcone synthase (CHS) (Koes et al., 1994). The malonyl CoA needed for the reaction is derived through the carboxylation of acetyl CoA. Naringenin chalcone or the isomeric flavanone is the central intermediate in the synthesis of all flavonoids (Macheix et al., 1990). Naringenin chalcone is then isomerized by chalcone isomerase (CSI) to the colorless naringenin (5, 7, 4'-trihydroxyflavanone). Naringenin is converted to dihydrokaempferol (DHK) by flavanone 3-hydroxylase (F3H) or alternatively to the flavones by flavone synthase (FS).

From here, DHK can be hydroxylated by flavonoid 3'hydroxylase (F3'H) to produce dihydroquercetin (DHQ) or by flavonoid 3', 5'hydroxylase (F3'5'H) to form dihydromyricetin (DHM) (Holton and Cornish, 1995). Flavonol synthase (FLS) can catalizes the formation of flavonols kaempherol, quercetin and myricitin from DHK, DHQ and DHM, respectively. In order to form anthocyanins, the enzyme dihydroflavonol reductase (DFR) reduces the dihydroflavonols (DHK, DHQ and DHM) to flavan-3,4-diols (leucoanthocyanidins) (Seigler, 1998). Anthocyanidin synthase (ANS) catalyzes oxidation and dehydration of these compounds to produce anthocyanidins. Anthocyanins are formed by adding a sugar to the aglycone by UDP-glucose-flavonoid-3-glycosyltransferase (3GT). The enzyme, anthocyanidin-3-glucoside rhamnosyltransferase (RT), catalyzes the addition of rhamnosyl to the glucose of the anthocyanin molecule.

Induction of anthocyanin biosynthesis

Photoinduction by wavelengths in the UV, visible and far-red regions is probably the most important environmental factor to play a role in anthocyanin biosynthesis in above-ground plant parts. It has been found that in most systems little or no anthocyanin is formed in darkness (Camm et al., 1993; Dong et al., 1998; Grisebach, 1982; Kakegawa et al., 1987) and that light enhances the rate of synthesis of anthocyanins and flavonoids (Lewis et al., 1998).

The enzymes in the phenylpropanoid pathway (phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H) and 4-coumarate: CoA ligase (4CL) reach maximal activity after 15 hours of irradiation (Hahlbrock et al. 1976). Anthocyanin accumulation is directly associated with phenylalanine ammonia-lyase (PAL) activity in apples (Mazza and Miniati, 1993). Furthermore, PAL activity has only been observed in the red part of apple skin.

Other triggers for anthocyanin biosynthesis are cold stress (Shichijo et al., 1993; Christie et al., 1994; Graham, 1998), osmotic stress induced by sucrose (Cormier et al., 1989; Decendit and Merillon, 1996), glucose (Tholakalabavi et al., 1997) and mannitol (Tholakalabavi et al., 1994), deficiency in nitrogen (Bongue-Bartelsman and Philips, 1995; Do and Cormier, 1991), deficiency in phosphorus (Dedaldechamp et al., 1995; Rajendran et al., 1992; Trull et al., 1997), low pH (Suzuki, 1995), exposure to methyl jasmonate (Franceschi and Grimes, 1991), wounding (Ferreres et al., 1997), pathogen infection (Dixon et al., 1994), and exogenous growth regulators such as auxin and cytokinins (Rajendran et al., 1992; Sakamoto et al., 1994).

The biosynthesis of color in underground organs is not well understood. Lewis et al. (1998a) investigated the factors that regulate the biosynthesis of anthocyanins and flavonoids in colored potato tubers. They hypothesize that the exposure of the aerial parts of the plants to light induces a 'trigger' molecule which is then transported to the tubers to initiate anthocyanin biosynthesis.

Although one could speculate that anthocyanins are dependent on carbohydrates from photosynthesis, plants grown in the dark, supplied with a carbohydrate source (sucrose), produced tubers, but the tubers did not accumulate anthocyanins. Further evidence to support a 'trigger' molecule includes the polar nature of anthocyanin accumulation in tubers of plants exposed to light, seeming to appear at the stem end first and then gradually accumulating across the tuber over time. Further work is required to identify the nature of this 'trigger' molecule.

After growing potato plants in the dark, it was found that some cultivars that usually produce highly pigmented tubers still produced limited color in the tubers, however, less colored cultivars failed to produce any color in the dark. Both types produced significantly higher amounts of phenolics including flavonoids and anthocyanins after exposure to white light.

The authors suggest in the dark the precursor(s) of these compounds are depleted in an earlier part of the pathway such as phenolic acid production and/or that phenolics, flavonoids, and anthocyanins are all controlled by different control mechanisms. That the various components of the phenolic pathway are governed by different control mechanisms is supported by the fact that in the

tubers of 'Desiree', a less intensely colored variety, the anthocyanin pathway is virtually inactive while the phenolic and the flavonoid pathways show some activity, judging by end products.

Lewis et al. (1998a) suggest that light causes the preferable diversion of the flavonoid intermediates (naringenin-chalcone and naringenin) to synthesize anthocyanin whereas in the absence of light, only the biosynthetic pathway of the flavonoid precursors and the branch pathway to catechin (a flavan-3-ol) appear to be active. After exposure of minitubers to light, there appears to be an increase in PAL and other enzymes downstream from PAL. This correlates with the rate of increase in anthocyanin, flavonoid and phenolic biosynthesis. This derepression of the enzymes seems to require the exposure of the leaves to light, and not the tubers.

The role of glutathione-s-transferases in the accumulation of anthocyanins

Glutathione-S-transferases (GSTs) are enzymes responsible for the conjugation of the tri-peptide glutathione (GSH) to hydrophobic, electrophilic, and usually cytotoxic substrates (Marrs, 1996). They can be found in both animal and plant organisms. Plant GSTs were first identified for their ability to detoxify herbicides. It was found that the genes of a specific subclass of GSTs termed type III GSTs, are induced by auxin and ethylene treatments and numerous stresses including oxidative stress, pathogen attack, and heavy-metal toxicity. The GST enzymes also play a role in the normal metabolism of secondary metabolites such as anthocyanins. According to Irzyk et al. (1995), anthocyanin

pigments require GSH conjugation for transport from the cytoplasm into the vacuole. If the anthocyanin pigment remains too long in the cytoplasm, it becomes toxic to the cell and the further production of the anthocyanin is jeopardized.

Thus, if 2,4-D or ethylene are applied to the plants, it will cause the upregulation of GST genes and the synthesis of GST enzymes. These enzymes will conjugate glutathione to the anthocyanin precursors in the cytoplasm and will eventually allow for it to be transported into the vacuole.

Primary structure of anthocyanins

Anthocyanidins

Anthocyanidins are the structural pigment part (i.e. chromophore) of an anthocyanin molecule. It is also called the aglycone as opposed to anthocyanins which are called the glycone. Macheix et al., (1990) define anthocyanindins as the hydroxylated and methoxylated derivatives of phenyl-2-benzopyrilium. Today there are 15 known anthocyanidins, although only six (pelargonidin (Pg), cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt), and malvidin (Mv)) are widespread and contribute to pigmentation of plants (Mazza and Miniati, 1993). These common six anthocyanidins differ in the number of 3' and 5' hydroxy and methoxy groups on the B ring (Macheix et al., 1990) (Figure 4).

Three anthocyanidins, Pg, Cy and Dp, provide the whole range of flower color from pink, orange and scarlet to mauve, violet and blue, either singly or as mixtures (Goodwin, 1965). Cyanidin is the most commonly found anthocyanidin

in fruits (90%). It is followed in decreasing order by Dp (35%), Pn (30%), Pg (20%) and Pt and Mv (15%).

Anthocyanins

A relatively unstable anthocyanidin becomes a more stable anthocyanin when a sugar is added to the aglycone. Anthocyanins can be modified by glycosylation, methylation, and acylation (Koes et al., 1994). An anthocyanidin may be glycosylated and acylated by different sugars and acids, therefore there are 15-20 times more anthocyanins than anthocyanidins. The most common sugars to bind to anthocyanidins are glucose, galactose, rhamnose, and arabinose. Glycosylation at position 3 on the C-ring results in monoglycosides. The most common substitutes for monoglycosides are glucose, arabinose and galactose (Mazza and Miniati, 1993). For further variation these sugars can also bind in combinations in the form of di- and trisaccharides, or the sugar residues can be acylated by c-coumaric, caffeic, ferulic, sinapic, p-hydroxybenzoic, malonic, oxalic, malic, succinic or acetic acids (Macheix et al., 1990).

Color change through structure change

Hydroxylation, methylation, and glycosylation

Increasing the number of hydroxyl groups on the B-ring causes the visible absorption maximum of the anthocyanidin to shift to longer wavelengths, causing a color change from orange to blue (bathochromic shift) and the destabilization of the molecule (Hrazdina et al., 1970). Methylation of the free hydroxyl groups on the B-ring (Mazza and Miniati, 1993) and the addition of a sugar to the

anthocyanidin molecule (Goodwin, 1965) both cause a perceived reddening in color, also referred to as a hypsochromic shift. In addition, methylation of the B-ring will stabilize the molecule (Figure 4).

Stability

Stability in vivo

pH and copigmentation are the primary factors affecting stability and color of the anthocyanin pigment. Shifts in pH can alter pigment structure (Mazza and Miniati, 1993). Four basic pH-dependant forms exist (Figure 5). At pH $\leq$2, the anthocyanins exist only in the orange to red flavylium cation (AH^+). Raising the pH to pH 4-5 causes a fast acid-base reaction. The hydroxyl groups at position 4' and 7'deprotonate to form the blue quinonoidal base (Macheix et al., 1990). The first deprotonation reaction occurs at about pH 4 (A) and the second at pH 7 (A^-).

While this reaction is occurring, a slower pseudo-acid-base reaction also takes place. The water molecules act as nucleophiles, attacking the flavylium cation at positions 2 and/or 4 and forming the colorless hemiacetal also known as pseudobase or carbinol (B). This reaction accounts for the color loss of the pigment. The red flavylium can be restored with acidification, but the colorless carbinol can open the C-ring to form a yellow or colorless unstable chalcone (C) which can undergo irreversible degradation (Brouillard et al., 1997).

Copigmentation is a color stabilization mechanism that prevents or reduces nucleophilic addition of water to the flavium cation. It occurs from pH 1 to neutrality. A copigment has no color of its own, but when added to a solution

containing anthocyanins it will stabilize, enhance, and modify color (Mazza and Miniati, 1993). Addition of copigments can cause a shift in the λ_{max} towards a bluer color (bathochromic shift) and increase color intensity (hyperchromic shift).

There are two kinds of copigmentation: intramolecular and intermolecular. Intramolecular copigmentation usually refers to the covalent binding of aliphatic and/or aromatic organic acids to the sugars on the anthocyanin molecule, whilst intermolecular copigmentation occurs when other compounds binds non-covalently to the anthocyanin molecule. Compounds that act as copigments include polyphenols, flavonoids, nucleic acids, and amino acids. The type and the concentration of the anthocyanin and the co-pigment, as well as the pH and the temperature of the medium can influence co-pigmentation (Mazza and Miniati, 1993).

Stability during processing

Enzymatic degradation

According to Macheix et al. (1990) there are several enzyme systems involved in anthocyanin degradation: ß-glycosidase (also called anthocyanases), peroxidases and polyphenol oxidases (PPO). ß–glycosidase hydrolyzes the anthocyanin to the anthocyanidin (aglycone) and glycoside, followed by the decomposition of the anthocyanidin (Huang, 1955). Peroxidase is found to enhance the degradation of the aglycone.

High temperatures

High temperatures are often used to prevent PPO activity in fruit and thus protecting the anthocyanins from degradation, e.g. blanching fruit prior to processing or preservation. Conversely, thermal degradation of anthocyanins has been studied for strawberry (Markakis et al., 1957), black raspberry (Daravingas and Cain, 1968), raspberry (Tanchev, 1972), Concord grape (Calvi and Francis, 1978), and sour cherry (Cemeroğlu et al, 1994). Raising temperatures can accelerate color loss in fruit juices. However, a short time/high temperature process is recommended for retention of pigments in red fruit juices since anthocyanin loss seems negligible for treatments exposed to 100 °C for less than 12 min (Macheix et al., 1990).

Light

Light is needed for the synthesis of anthocyanins, however light can also accelerate the degradation of anthocyanins. For this reason, fruit juices are generally stored in the dark. Acylated forms of anthocyanins are the most light stable. Copigmentation with polyhydroxylated flavonoids, isoflavonoids, and aurone sulfonates can make anthocyanins light-stable (Macheix et al., 1990).

Analytical Techniques for Anthocyanin Identification and/or Quantification

There are several techniques for isolating and identifying anthocyanins. Here follows three techniques, see review on analysis techniques by Kong et al. (2003) for more.

High performance liquid chromatography-HPLC

Since the 1980's the use of HPLC in identifying anthocyanins has become very popular. The concept of HPLC is based on the separation of a mixture of components by passage through a chromatographic column. The mobile phase, which contains the mixture of components, passes through the stationary phase which is a column filled with solid particles. The components of the solution migrate according to the non-covalent interactions of the compound with the column. The chemical interactions of the mobile phase and sample with the column determine the rate of migration and separation of components contained in the sample (Fallon et al., 1987).

Spectrophotometry

Spectrophtometry is used to measure the extent to which solutes absorb light at specified wavelengths in the region of 200-800 nanometers. This technique has important applications: the amount of light absorbed at a specified wavelength can produce information on the concentration of a solute; a reaction rate can be determined by plotting absorbed light as a function of time; a plot of absorbed light at different wavelengths (absorption spectrum) can give information on the chromophore (the part of the molecule that absorb light), and can help in the identification of the compound.

NMR

Nuclear magnetic resonance (NMR) is a phenomenon that occurs when the nuclei of certain atoms with gyromagnetic properties such as hydrogen are immersed in a static magnetic field and, when exposed to a second oscillating magnetic field, will tend to align itself with that field by spinning at a rate that is

proportional to the strength of the applied field. If the sample is radiated at the precessional frequency, absorption of energy to the excited state takes place. When the sample returns to the normal state, energy will be released and this will be measured. The energy is directly related to the number and kind of nuclei in the molecule. A NMR spectrum is constituted from a plot of frequency, relative to a standard, versus intensity (Mussinan, 1993). This technique is used for structure determination and peak identification of anthocyanin molecules (Mazza and Miniati, 1993).

Anthocyanin Functions in Plants

From an ecological point of view, the ability of anthocyanins to add color to plants is probably their most significant function. Color in plants contributes to pollination, fertilization, and seed dispersal by animals. Anthocyanins can also act as a light screen against damaging UV radiation (reviewed by Steyn et al., 2001) and photoinhibition in leaves, a role sometimes ascribed to kaempferol (Buchanan et al., 2000). Anthocyanins are also associated with resistance to pathogens in *Brassica* species, sunflowers, pea seedlings and maize (Mazza and Miniati, 1993; Buchanan et al, 2000). The functions of anthocyanins in underground organs is less clear, however, betalains, the pigments in red beets, have been associated with the indication of nutrient stress and pathogen resistance (Mabry, 1980; Piattelli, 1981; Stafford, 1994), and improved viral defense (Sosnova, 1970).

Anthocyanins in Underground Organs

Anthocyanin accumulation in underground tissues can result in the formation of red, purple and blue organs for potatoes, onions, red radishes, sweet potatoes, yams, and garlic. Colored underground organs are now receiving more market interest for their aesthetic and health properties, especially in the gourmet industry. Consequently a considerable amount of research has gone into identifying the anthocyanins in these commodities, as well as trying to improve the color and stability of these compounds.

Potato

In potatoes, the anthocyanins are found in the periderm and/or the peripheral cortex of the tuber, depending on the cultivar (Burton, 1989). The periderm consists of the phellem, phellogen and the phelloderm (Lulai and Freeman, 2001) and the peripheral cortex is the starchy center. According to Howard et al., (1970) tetraploid *Solanum tuberosum* subsp. *tuberosum* have single anthocyanin pigments rather than mixtures as found in *Solanum tuberosum* subsp. *andigena* and cultivated diploid cultivars. Anthocyanins tend to degrade during storage; however Lewis et al. (1999) found anthocyanin concentration to increase during storage at 4 °C. Anthocyanins have been found to decrease during tuber growth (Hung et al. 1997).

The first chemical identification of anthocyanins in a potato was done by Chmielewska in 1935 on 'Negresse' (also known as 'Congo'), which is a cultivar with purple-black skin and flesh. The anthocyanin identified were negretein, a

malvidin derivative: Mv-3-(p-coumaroyl-rutinoside), and a 3-monoglucoside of the then unknown petunidin. The petunidin-3-monoglucoside molecule was later identified as petanin. Andersen et al. (1991) identified the complete structure of petanin as petunidin 3-*O*-[6-*O*-(3-*O*-*E*-*p*-coumaroyl-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside]-5-O-*β*-D-glucopyranoside. In addition, Fossen et al. (2000) identified the novel 3-*O*-[6-(4-ferulyl-*O*-*α* -rhamnopyranosyl)-*β*-glucopyranoside]-5-O-*β*-glucopyranosides of petunidin and malvidin in this cultivar.

The isolation and characterization of ten anthocyanins from diploid and tetraploid potato species led to the discovery of p-coumaroyl-3-rutinoside-5-glucoside derivatives of all six common anthocyanidins, plus the 3-rutinoside derivatives of Pg, Cy, Dp, and Pt in potato (Harborne, 1960). Harborne (1960) identified the anthocyanins in red *Solanum phureja* as the rhamnosylglucosides of cyanidin and pelargonidin, and in the red *Solanum tuberosum*, the rhamnosylglucosides of peonidin and pelargonidin. According to Fossen et al. (2000) and Ishii et al. (1996) the major anthocyanin in red-fleshed tubers is pelargonidin 3-*O*-[6-*O*-(3-*O*-*E*-*p*-coumaroyl-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside]-5-O-*β*-D-glucopyranoside) also known as pelanin. Naito et al. (1998) isolated pelanin and pelargonidin 3-*O*-[6-*O*-(4-*O*-*E*-feruloyl-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside]-5-O-*β*-D-glucopyranoside from red tubers of an anthocyanin-rich tetraploid.

Lewis et al. (1998b) identified the anthocyanins in the dark purple cultivar 'Urenika' and the medium purple cultivar 'Arran Victory' as petunidin-3-(p-coumaroyl-rutinoside)-5-glucoside and malvidin-3-(p-coumaroyl-rutinoside)-5-

glucoside. The petunidin derivative was found in similar concentrations in both cultivars and the malvidin derivative at a higher concentration in the darker 'Urenika'.

Hung et al. (1997) determined that more than 90% of the anthocyanidins in the periderm of 'Norland' are pelargonidin and peonidin. Lewis et al. (1998b) identified the anthocyanins in the red cultivars 'Desiree' and 'Redflesh' as pelargonidin 3-(*p*-coumaroyl-rutinoside)-5-glucoside and peonidin 3-(*p*-coumaroyl-rutinoside)-5-glucoside. Sachse (1973) also identified pelargonidin 3-(*p*-coumaroyl-rutinoside)-5-glucoside and peonidin feruloyl-3-rutinoside-5-glucoside in varieties 'Urgenta' and 'Desiree'. Lewis et al. (1998c) analyzed eight wild *Solanum* species and found lower anthocyanin levels than in colored *Solanum tuberosum* as tubers of these wild species were mostly white, with some showing slight purple color around the eyes.

Onions

The onion plant (*Allium cepa* L.) produces white, yellow or purple bulbs, depending on its genetic makeup. All the anthocyanidins except malvidin have been found in onions. Anthocyanins can be found in crystalline form in the epidermal cells (Mazza and Miniati, 1993). Early work done by Robinson and Robinson (1932) identified a cyanidin 3-pentoseglycoside. Fouassin (1956) identified three cyanidin derivatives, including mono- and diglucosidic derivatives.

Fossen et al. (1996) analysed three red onions and other *Allium spp.* and found them to contain several or all of the following anthocyanins: 3-(6'-malonyl-

3'glucosylglucoside), 3-(3', 6'-dimalonylglucoside), 3-(6'-malonylglucoside), 3-(3'-malonylglucoside), 3-(3'-glucosylglucoside) and 3-glucoside of cyanidin. They also found trace amounts of two pelargonidin derivatives and the 3, 5-diglucosides of cyanidin, and peonidin were reported for the first time in red onion. Gennaro et al. (2002) reported that cyanidin derivatives constitute more than 50% of total anthocyanins in 'Tropea' red onions (*Allium cepa* L.) and in addition detected delphinidin and petunidin derivatives for the first time in onions.

Red Radish.

Radishes occur in a variety of color such as white, purple, yellow, and black. However, it is the red radishes that receive the most attention as a natural alternative to artificial colorants. Giusti et al. (1998) identified the major pigments in 27 red radish cultivars to be pelargonidin 3-sophoroside-5-glucoside, mono- or di-acylated with cinnamic and malonic acids. Harborne et al. (1958) found pelargonidin 3-sophoroside-5-glucoside in red radish to be acylated with p-coumaric and ferrulic acid, referred to as Raphanusin A and B respectively. In purple radishes, the anthocyanin composition is the same as in red radish; however the aglycone moiety pelargonidin is replaced by cyanidin.

Sweet potato and yams

Sweetpotatoes come in an array of colors. The anthocyanins of the purple sweetpotato (*Ipomoea batatas* L.) cultivar 'Ayamurasaki' were identified as 3-(6, 6'-caffeylferulylsophoroside)-5-glucoside of cyanidin and peonidin (Yoshimoto et

al., 2001). In addition, Terahara et al. (2000) identified another anthocyanin in this cultivar as cyanidin 3-O-(2-O-(6-O-(E)-p-coumaroyl-β-D-glucopyranosyl)-β-D-glucopyranoside)-5-O-β-D-glucopyranoside.

Odake et al. (1992) identified the chemical structures of two major anthocyanins in 'Yamagawamurasaki' as 3-caffeylferulysophoroside-5-glucoside of cyanidin and peonidin. Goda et al. (1997) isolated two new acylated anthocyanins from the same purple cultivar and through spectroscopic analysis identified these compounds as 3-O-(6-O-trans-caffeyl-2-O- β-glucopyranosyl- β-glucopyranoside)-5-O- β-glucoside of cyanidin and peonidin.

The popular edible yam (*Dioscorea alata* L.) usually has white tubers, however, some varieties contain purple pigment. According to Shoyama et al. (1990) the following anthocyanins have been isolated in *D. alata*: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-diglucoside, cyanidin 3-*O*-rhamnoside, cyanidin 3-*O*-gentiobioside and cyanidin 3-*O*-glucoside acylated with ferulic acid. In addition, Shoyama et al. (1990) isolated cyanidin and peonidin 3-*O*-gentiobioside.

Garlic

Pigmentation of garlic (*Allium sativum* L.) is due to the presence of carotenoids, chlorophylls and anthocyanins, the latter being localized in the inner scales of leaves of the bulb (Mazza and Miniati, 1993).The three major anthocyanins are cyanidin 3-glucoside, cyanidin 3-glucoside monoacylated, and cyanidin 3-glucoside diacylated with an aliphatic acid. Fossen and Andersen (1996) found that acidified methanolic extract of inner scale leaves of garlic

contain mostly anthocyanins with aliphatic acylation. These are the rare 3', 6'-dimalonylglucoside (13%) and 3'-malonylglucoside (3%) of cyanidin, in addition to cyanidin 3-(6'-malonylglucoside) (71%) and cyanidin 3-glucoside (12%).

Research Hypotheses and Objectives

Demand for plant products with greater health and aesthetic appeal suggests the development of pre- and postharvest intervention strategies to improve the development and stability of anthocyanin pigments in novelty potatoes, such as 'Michigan Purple'. With this in mind we wanted to investigate the development of anthocyanins during tuber development, as well as in storage and compare this with other colored potatoes. We hypothesized that the preharvest factors that may impact stress responses such as soil type and planting space, rate of nitrogen application, and application of the synthetic auxin 2,4-D, will affect the synthesis and stability of anthocyanins. In addition, we hypothesized that storage temperature regime will impact anthocyanin stability, and that postharvest ethylene application through initiation of stress responses, will result in increased biosynthesis of anthocyanins in the skins of 'Michigan Purple' potatoes.

Objectives

1. Quantify changes in color of 'Michigan Purple' potatoes during storage and compare these to changes in other colored potatoes: 'Dakota Rose', 'Chieftain', 'Norland' and 'California Red'.
2. Quantify the changes in purple color during tuber development in 'Michigan Purple' potatoes
3. Investigate and compare color of 'Michigan Purple tubers grown in muck and mineral soils, and investigate the effect of different planting spaces in mineral soils.
4. Investigate and compare color of 'Michigan Purple' tubers grown under different nitrogen fertilization rates.
5. Investigate and compare tuber color of 'Michigan Purple' plants sprayed with 3 different 2, 4-D rates.
6. Investigate the effect of postharvest temperature manipulation on the synthesis of anthocyanins in tubers of 'Michigan Purple' and red-skin cultivars.
7. Investigate the change in color of 'Michigan Purple' tubers exposed to postharvest ethylene.

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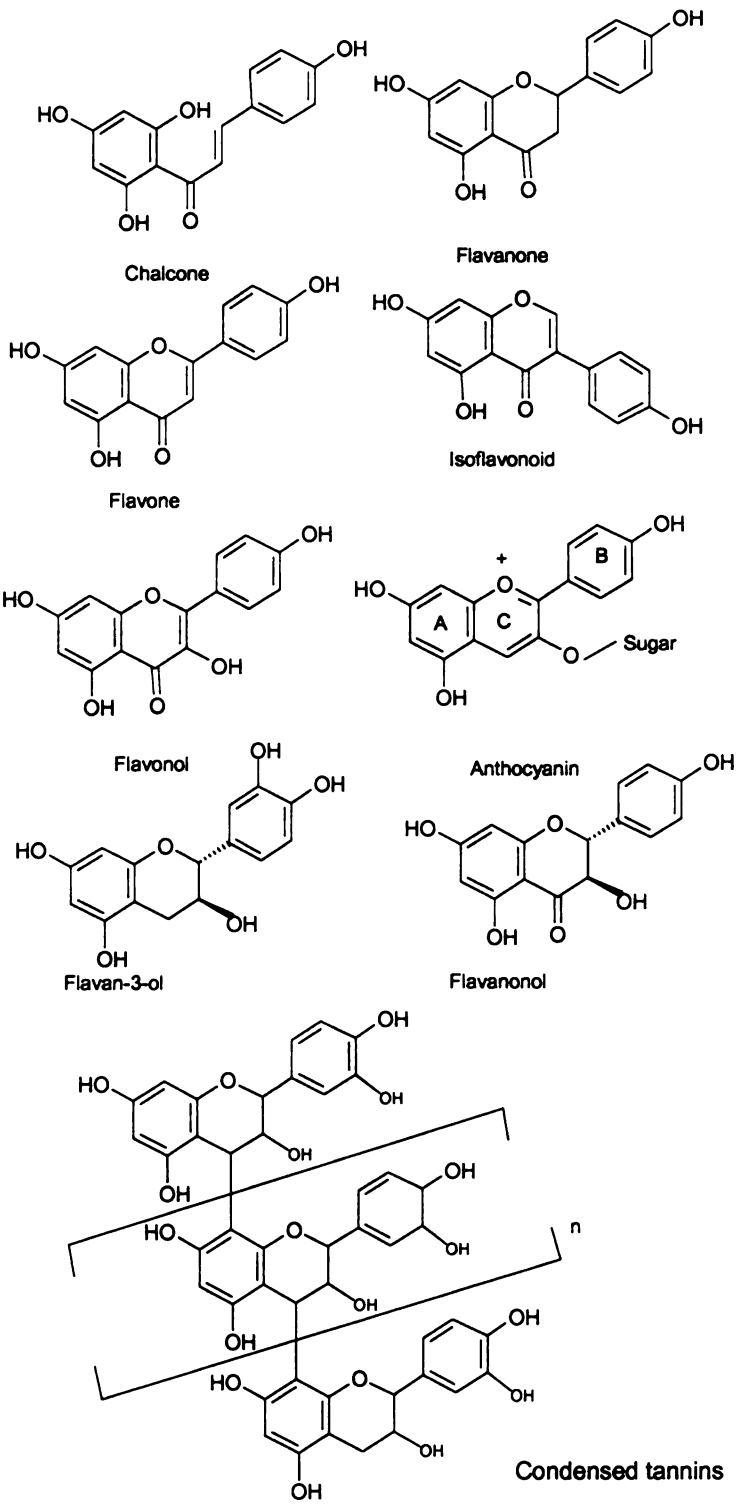


Figure 1. The different types of flavonoids and their structures.

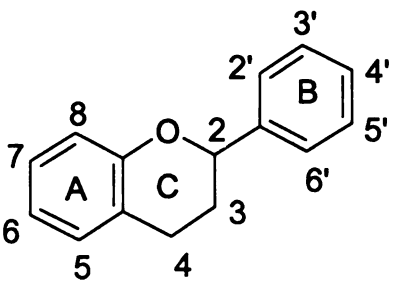
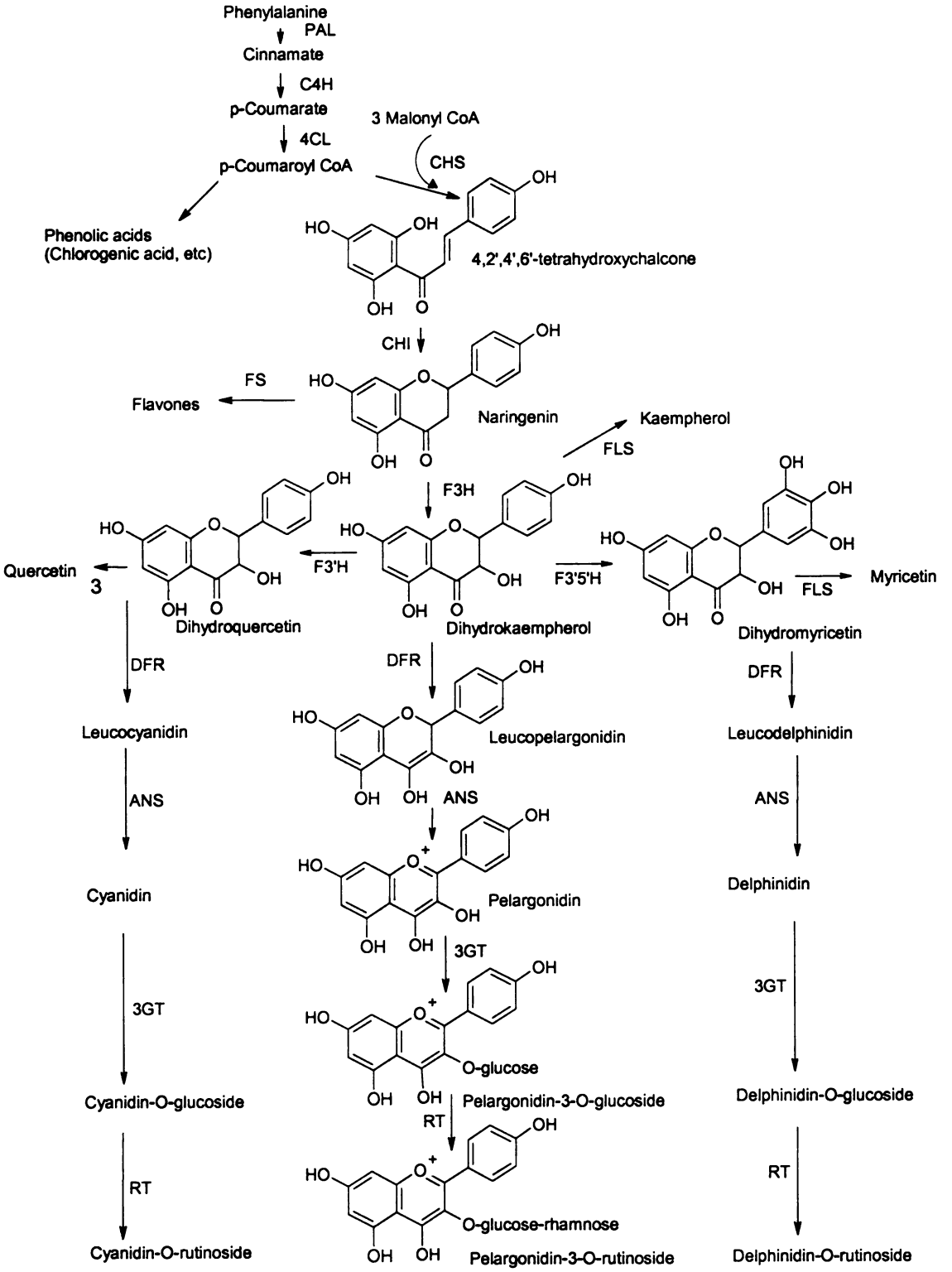


Figure 2. The basic structure of all flavonoids. The A-ring is formed from 3 acetate units (via the malonic acid pathway) and the B-ring with the 3-carbon bridge is constructed from phenylalanine via the shikimic acid pathway.

Figure 3. The phenylpropanoid pathway leading to the synthesis of anthocyanins. The enzymes involved in the reactions are: PAL: phenylalanine ammonialyase; C4H: cinnamate-4-hydroxylase; 4CL: 4-coumaryl-CoA ligase; CHS: chalcone synthase; CHI: chalcone-flavanone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; F3'5'H: flavonoid 3',5'-hydroxylase; DFR: dihydroflavonol reductase; ANS: anthocyanidin synthase; GT: UDPG-flavonoid-3-glucosyltransferase; RT: anthocyanidin-3-glucoside rhamnosyltransferase; FS: flavone synthase and FLS: flavonol synthase.


Phenylalanine
PAL
Cinnamate
C4H
p-Coumarate
4CL
p-Coumaroyl CoA
3 Malonyl CoA
CHS
Phenolic acids
(Chlorogenic acid, etc)
4,2',4',6'-tetrahydroxychalcone
CHI
FS
Flavones
Naringenin
Kaempherol
FLS
F3H
F3'H
F3'5'H
Quercetin
3
Dihydroquercetin
Dihydrokaempherol
Dihydromyricetin
Myricetin
FLS
DFR
DFR
DFR
Leucocyanidin
Leucopelargonidin
Leucodelphinidin
ANS
ANS
ANS
Cyanidin
Pelargonidin
Delphinidin
3GT
3GT
3GT
O-glucose
Cyanidin-O-glucoside
Pelargonidin-3-O-glucoside
Delphinidin-O-glucoside
RT
RT
RT
O-glucose-rhamnose
Cyanidin-O-rutinoside
Pelargonidin-3-O-rutinoside
Delphinidin-O-rutinoside

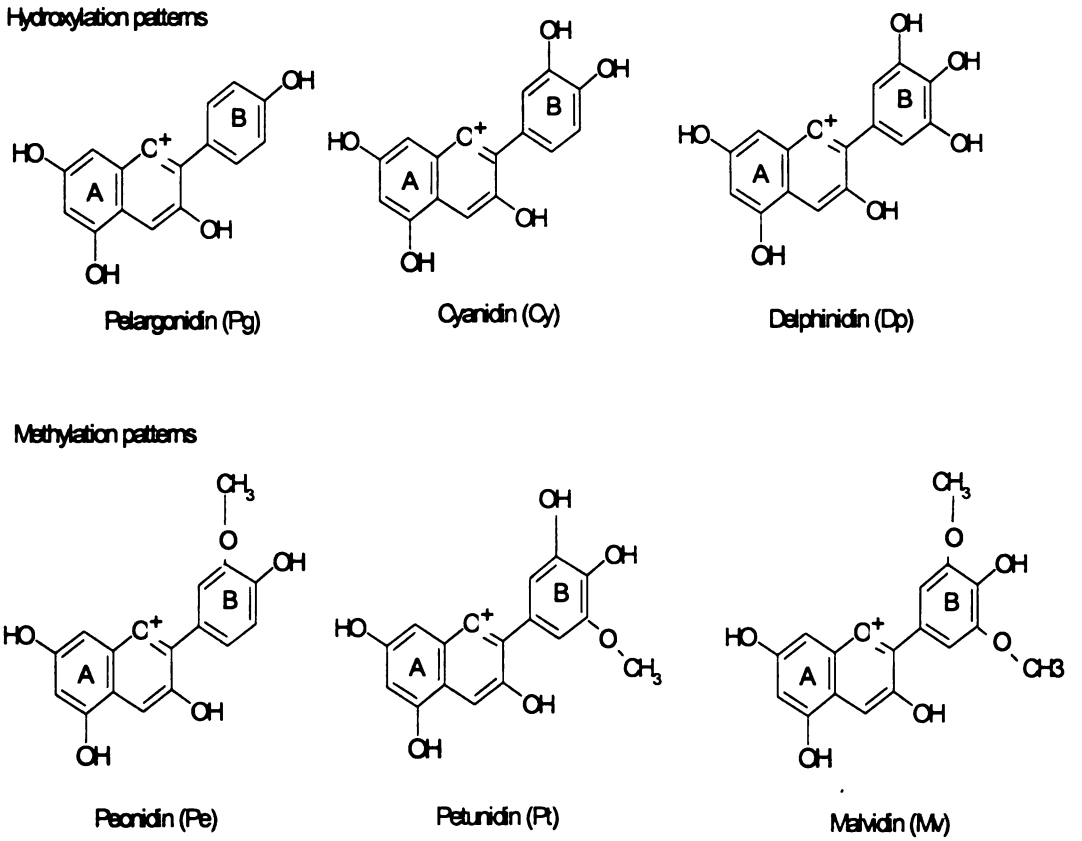


Figure 4. The structures of the six most common anthocyanidins, differing in hydroxylation and methylation positions.

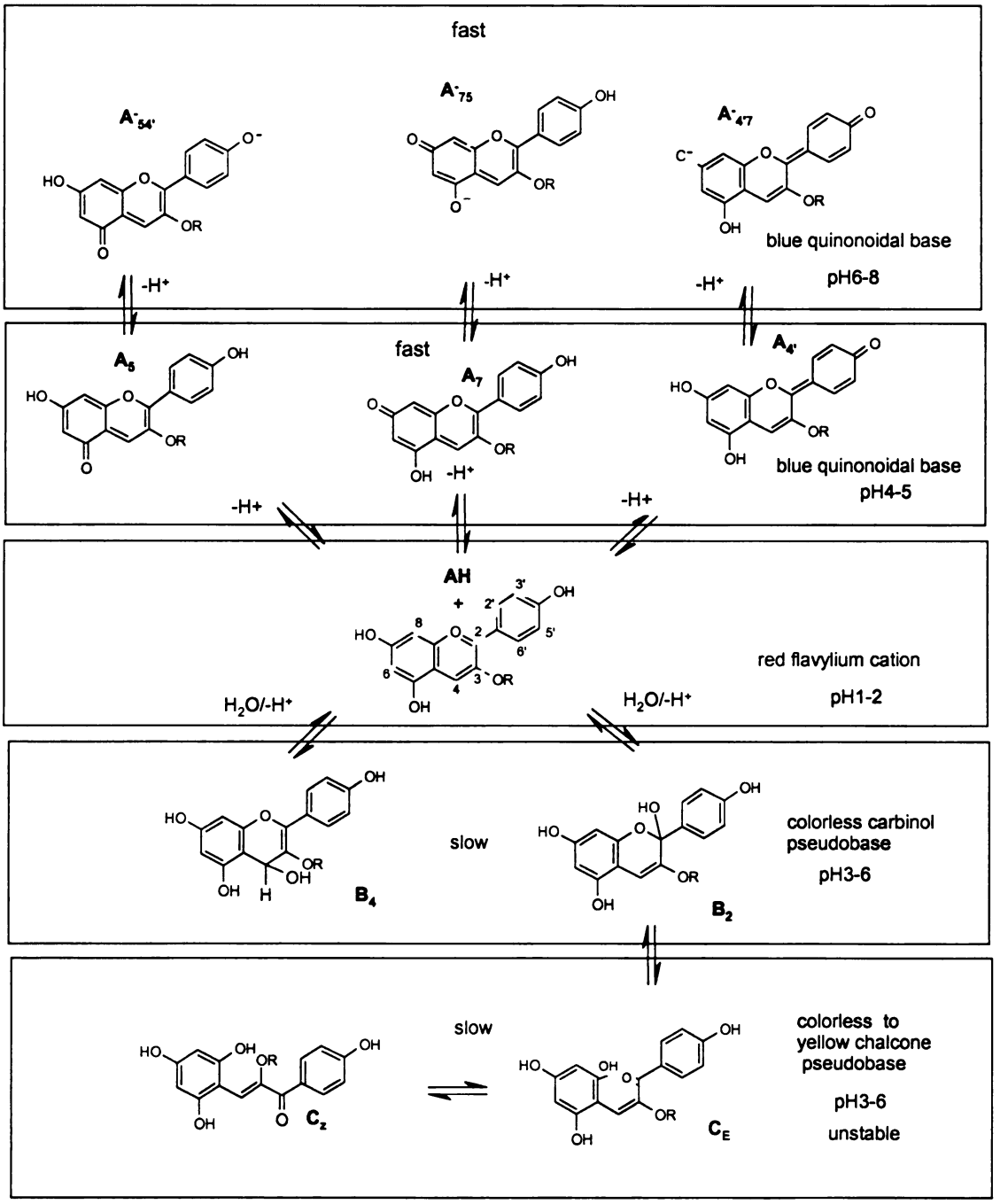


Figure 5. The structural transformation of anthocyanins in aqueous solutions (adapted from Brouillard, 1983).

CHAPTER I

CHANGE IN TUBER SKIN-COLOR OF 'MICHIGAN PURPLE' AND OTHER COLORED POTATO CULTIVARS DURING DEVELOPMENT AND STORAGE

INTRODUCTION

Colored potatoes currently comprise 10 % of the total acreage in the United States (USDA, 2003). The skin color of colored potatoes is due to the presence of anthocyanins in the periderm (Burton, 1989). If the color of red potatoes is pink at harvest or fades during storage, it can result in potatoes with lower value. Anthocyanin concentrations can change during growth and development due to environmental and growth conditions such as temperature, disease, and nutrition levels. However, when harvested, factors such as handling conditions during harvest, storage conditions and duration may also alter anthocyanin concentrations.

Anthocyanins can be degraded in plant tissues due to the following enzymes: glycosidases, polyphenoloxidase (PPO), and peroxidase (Francis, 1989; Macheix et al., 1990). Glycosidase hydrolyzes the anthocyanin to the anthocyanidin (aglycone) and glycoside after which the anthocyanidin is more prone to further degradation (Huang, 1955). Peroxidase is found to enhance the degradation of the aglycone. As senescence continues during storage, cells will become more vulnerable to degradation of anthocyanins as a result of the interaction of these enzymes with anthocyanins (Mayer and Harel, 1979; Walker and Ferrar, 1988).

Lewis et al. (1999) found anthocyanins to increase in 'Arran Victory' (purple skin/ white flesh), 'Desirée' (pink skin/ white flesh), 'Red Flesh' (red skin/

pink flesh), and 'Urenika' (purple skin/ purple flesh) varieties stored at 4 °C. Conversely, Andersen et al., 2002 found that anthocyanins decreased in 'Norland' (red skin/ white flesh) after 1 month in storage at 4 °C. This discrepancy in results is probably due to the different responses of varieties to cold-storage. The response of 'Michigan Purple' pigment content to low temperature storage is not known.

Hung et al. (1997) found anthocyanin concentrations and chroma of 'Norland' potatoes to decrease with increasing tuber size. Hung et al. (1997) did not find a change in hue with increase in tuber size. Lewis et al. (1999) found a slight increase in anthocyanin concentrations with initial tuber weight increase in 'Desiree tubers', after which anthocyanin content stabilized.

In this paper, we investigate the change in anthocyanins in the skins of 'Michigan Purple' during development of the tubers as well as during storage at 4°C and 10 °C for 6 months. We compare the behavior of purple 'Michigan Purple' tubers in storage with red cultivars 'Dakota Rose', 'California Red', 'Chieftain', and 'Norland'. We hypothesize that storage of potatoes at low temperatures would result in an increase in anthocyanin concentration in the tuber skins.

MATERIALS AND METHODS

Site description and experimental design. In 2002 'Michigan Purple', 'Dakota Rose', 'California Red' and 'Norland' were grown in black organic soil at Krummrey & Sons, Inc., Stockbridge, Michigan. The area is classified as an OsB-Oshtemo sandy loam with a 0-6 percent slope (USDA, 1979). In 2003, 'Michigan Purple', 'Dakota Rose', and 'Chieftain' tubers were grown in Montcalm/McBride sandy loam soil at Montcalm Research Farm, Entrican, Michigan. In addition, we implemented a developmental study in 2003 with the goal of determining whether intensity of color is affected by the stage of tuber development.

Storage conditions. In 2002, 40 tubers of each cultivar were washed, air dried, and placed into trays. A circle, 1.5 cm in diameter, was drawn on each potato to indicate the area where color was to be measured. The tubers were stored at 10 °C and 80-90% relative humidity (RH) for 6 months. In 2003, 20 tubers of each cultivar were numbered, marked with a circle, and randomly placed into 3 trays to be stored at 4 °C and 85-90% RH for 6 months. In addition, extra tubers were stored for extraction purposes at 0, 2, 4 and 6 months: three replicates of 5-10 tubers of each cultivar were placed into brown paper bags immediately after harvest and stored under same conditions as trays in both years.

Color measurement. In 2002, color was measured every 2 weeks in circled areas with a colorimeter (Colorimeter CR 300, Minolta, Japan). Chroma, hue angle, and lightness values were recorded. Chroma refers to the intensity of the color and hue angle ($h° = \arctan[b/a]$) refers to the angle formed by line from the origin to the intercept of the a (x-axis) and b (y-axis) co-ordinates, where

0° = red-purple, 90° = yellow, 180° = bluish-green, and 270° = blue (McGuire, 1992).

Anthocyanin extraction: In 2002, an extraction protocol was followed for quantification and identification of anthocyanins with high-performance liquid chromatography (HPLC). Tubers were taken from storage at the appropriate times and the periderm removed by scraping with a scalpel. The skin was immediately frozen in liquid nitrogen and stored at -80 °C until analyzed. Two grams of potato periderm were ground to a powder in a mortar and pestle using liquid nitrogen. Samples were extracted in 20 mL of 2.5% (v/v) formic acid in methanol and placed in a shaker for 60 min at 4 °C. Samples were centrifuged at 3000 rpm for 15 min at 4 C° and the supernatant decanted. The residue was extracted three more times with 10 mL extraction solvent, centrifuging for 10 min at 3000 rpm, and decanting the supernatant.

The combined extracts were filtered with a 0.8 µm filter into a clean test tube. Samples were evaporated to dryness at 35 °C and redissolved in 2 mL 2.5% (v/v) formic acid in water. Filter cartridges (Sep Pak® Plus C18, Waters, Ireland) were prepared by dripping 5 mL 100% methanol through and rinsing with 5 mL 2.5% (v/v) formic acid in water. The sample was slowly loaded onto the filter cartridge and rinsed twice with 5 mL 2.5% (v/v) formic acid in water. The sample was eluted with 1.8 ml 2.5% (v/v) formic acid in methanol and filtered through a 0.2 filter into an auto-sampling vial (Alltech associates, Inc., IL).

Anthocyanins were analyzed using analytical reversed-phase HPLC with a separations module (Waters 2690) with a photodiode array detector (Waters

2994) and a 4.6 x 150 mm column (XTerra® RP-18 column), maintained at 30 °C. Sample volume was 15-20 µL. Elution solvents used were: A, 2.5% formic acid in methanol and B, 2.5 % formic acid in water. A flow rate of 1 $mL.min^{-1}$ was maintained. Samples were eluted using the following program: 70% A to 35% A and 30% B to 65 % B from 0 min to 17 min; 0% A and 100% B at 19 min and 70% A and 30 % B at 21 min. The eluted compounds were monitored at 530 nm for anthocyanins.

Four standards were prepared and run on the HPLC for our identifications properties: petunidin chloride (Apin chemicals, UK), pelargonidin 3,5-diglucoside (Apin chemicals, UK), cyanidin 3-glucoside, and cyanidin 3-rutinoside (Sigma, St. Louis, US).

For additional identification purposes, HPLC analysis was done with a 4.6 x 150 mm Capsell RP-18 column. The elution solvents were on a v/v basis: A, 0.1 % trifluroacetic acid in water and B, 50.4 % water, 48.5 % acetonitrile, 1 % acetic acid and 0.1 % trifluroacetic acid. The samples were eluted using the following program: 80% A to 40 % A and 20 % B to 60 % B from 0 min to 26 min; 80% A and 20% B at 30 min, held until 45 min.

Based on their retention times, spectra, and a library comparison, peaks 3 and 4 were identified as the anthocyanidins delphinidin and cyanindin, respectively. Peak 1 was identified as an anthocyanin. Peak 1 had the same retention time as pelargonidin 3, 5-diglucoside.

In 2003, extractions were based on the procedure of Siegelman and Hendricks (1958). Skin samples were frozen and ground as described above. 0.5

g of ground sample was added to 15 mL of 1% (v/v) HCl in methanol solution and left for 18 hours at 5 °C in the dark. Samples were centrifuged for 10 min at 3000 rpm. Absorbance of the supernatant was measured at 530 nm on a spectrophotometer (U-3000, Hitachi, Japan) and compared to the standards pelargonidin 3, 5-diglucoside and petunidin chloride (Apin chemicals, UK).

Development experiment. In 2003, starting eight weeks after planting, three 'Michigan Purple' plants were randomly selected from the plots at Montcalm Research Farm, Entrican, Michigan on July 25, August 7 and 28. All tubers of every plant were harvested and divided into four classes according to their weight: ≤5 g; 5-24.9g; 25-49.9 g; >50 g (Hung et al., 1997). Color measurements and anthocyanin extractions were done as described above.

Statistical analysis. Data was analyzed using a Statistical Analysis System (SAS Institute, Inc., Version 8.0, 2000) to conduct least significant differences between treatments using PROC MIXED and PROC GLM. Slope analyses were done using PROC MIXED.

RESULTS AND DISCUSSION

Effect of storage at 10 °C. There was considerable variation in the total pigment concentration (anthocyanin and anthocyanidins) (5 peaks combined) of the skins of the four cultivars during storage and no consistent change in concentration could be measured over the 6 months storage period (Figure 1). (See Appendix

A Tables 2 and 3 for complete analysis of HPLC peaks and Figures 1-4 for HPLC chromatogram with spectra of the individual peaks of each cultivar).

'California Red' had the highest concentration of total anthocyanins at 0 and 6 months, differing significantly from the other cultivars. After two months of storage, 'Michigan Purple' had a statistically lower concentration, but there was no statistical difference between the other three cultivars. However, after four months both 'California Red' and 'Dakota Rose' had significantly higher pigment concentrations than the other cultivars and 'Michigan Purple' was significantly lower than 'Norland'. After six months in storage, 'Michigan Purple' was significantly lower in pigment concentration than 'California Red' and 'Dakota Rose', but not 'Norland'. These data suggest that storage duration, when the storage temperature was 10 °C did not affect the anthocyanin content of potatoes, even after six months of storage.

Since the anthocyanin concentration of 'Michigan Purple' was quantified using petunidin chloride (an anthocyanidin) and the other three potato cultivars were quanitified using pelargonidin 3,5-diglucoside (an anthocyanin), direct comparisons on their concentration are difficult. Different anthocyanin standards can result in very different readings (Holcroft and Kader, 1999). Consequently, a comparison of peak areas (AU) at 530 nm is included to give a better indication of the anthocyanin intensity of 'Michigan Purple' (Appendix A Table 1). There was no change in AU values in any of the cultivars between months 0 and 6. 'Michigan Purple' samples had significantly higher AU values than 'Norland' at

month 0, but were not different from 'California Red' or 'Dakota Rose'. 'California Red' had the highest AU value after 4 months of storage.

Hue angle (h°) increased over the six month storage period at 10 °C in all cultivars except 'Norland' (Figure 2; Appendix A Table 1), indicating a reddening during storage. As expected, 'Michigan Purple' had a consistently lower h° value than the red cultivars which reflected its purple coloration. The slope of the h° curve over months in storage was used as a means to describe the rate of change in skin color of the stored potato (Table 1). The slope of 'Michigan Purple' was steeper (0.81 hue. $week^{-1}$) than the red cultivars (0.41, 0.41 and 0.18 hue. $week^{-1}$), indicating a faster rate of change. However, since the h° of 'Michigan Purple' was initially lower, the h° after storage was still significantly lower (more purple) than the red cultivars. The slope of the red cultivars did not differ from one another, indicating that there was no difference in rate of color change.

Lightness increased and remained significantly higher after two months in storage in the cultivars 'Michigan Purple' and 'Dakota Rose' (Figure 2; Appendix A Table 1). No significant changes were recorded for 'California Red' and 'Norland'. Significant differences in lightness were recorded at harvest (month 0) between 'Michigan Purple', 'California Red' and 'Norland' and after two months between 'Michigan Purple' and all three red cultivars; however, after four and six months in storage, no significant differences were recorded. These changes in lightness, though significant, were still very small and probably did not contribute to major color changes.

Chroma did not change significantly after six months in storage in any of the cultivars. 'Michigan Purple' had a lower chroma value than the red cultivars at all times (Figure 2; Appendix A Table 1). The chroma of the red cultivars did not differ between 0 and 6 months although 'Dakota Rose' differed from 'Norland' at months four and six. These data indicate that storage at 10 °C did not influence chroma or anthocyanin concentration, but with time a change in perceived color of red and purple potatoes was observed.

Effect of storage at 4 °C. Of the four cultivars tested, 'Dakota Rose' was the only cultivar that showed significant increases in pigment (anthocyanidin and anthocyanin) concentration after two, four, and six months (Figure 3; Appendix A Table 4). A similar response of potatoes to cold storage has been reported by Lewis et al. (1999) who suggested that this increase in anthocyanin concentrations at 4 °C is due to the conversion of starch to sugars in the tubers. Since sugars are the precursors of anthocyanins, this leads to increased anthocyanin synthesis. 'Michigan Purple' had the lowest anthocyanin concentration at all times and there was no increase in storage at 4 °C. This data agrees with Andersen et al. (2002) who found that anthocyanin concentration in 'Norland' decreased in storage at 4 °C. This discrepancy in results could be due to the response of different cultivars to temperature.

Hue angle values of all the cultivars were significantly higher after 2 months in storage at 4 °C and remained so for the duration of the storage time (Figure 4, Appendix Table 4). 'Chieftain' had the highest hue angle throughout the storage duration, while the hue angle values of 'Michigan Purple' and 'Dakota

Rose' were significantly lower than 'Chieftain'. The hue angle of 'Michigan Purple' increased during storage and reached the same value as 'Dakota Rose' after 6 months. Interestingly, the skin color, as judged by the human eye, did not resemble each other suggesting the colorimeter was responding to pigments not readily seen by the human eye, perhaps located beneath the surface layer. The rate change in hue was greatest in 'Michigan Purple' during 6 months of storage (Table 2). It appears that the color of the potato cultivars stabilized after 3 months of storage as indicated by the lower slope values (Table 2).

The hue angle of 'Michigan Purple' increased from 1.09° to 26.44°after six months at 4 °C, and from 359.97° to 6.45 °after 6 months at 10 °C. These data suggest that storage at higher temperature of 10 °C is better for maintaining a more purple color.

All three cultivars became significantly darker after 2 months of storage but of these, 'Chieftain' was significantly lighter than 'Michigan Purple' and 'Dakota Rose' after 2 months of storage. All cultivars had significantly higher chroma values after 2 months in storage indicating a more intense color.

Development experiment. Tubers in stage 3 (5 g to 25 g) had the highest concentration of pigments (Figure 5). All stages differed significantly from each other. Anthocyanin and anthocyanidin content underwent a decreasing trend as tuber weight increased similar to observations by Hung et al. (1997). There was a negative correlation between pigment concentration and h°, lightness and chroma measurements.

CONCLUSIONS

From this study we now know that 'Michigan Purple' potatoes have both anthocyanin and anthocyanidin pigments. We concluded that storage at low temperatures did not affect anthocyanin concentrations in 'Michigan Purple' potato tubers up to six months in storage at 4 or 10 °C. However, color (hue) change was cultivar-dependant and was faster at 4 °C than at 10 °C, suggesting that reducing storage temperature, while not benefiting anthocyanin content, does improve color stability.

In addition, we found anthocyanin concentrations to be different in tubers from different developmental stages, with the smaller tubers having the highest concentrations. Hence, 'Michigan Purple' might be best marketed as new potatoes since they have the highest anthocyanin concentrations when tubers are small, and thus the most health benefits.

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Table 1. A comparison between the rates of change in hue angle values of over 6 months of storage in 'Michigan Purple', 'Dakota Rose', 'California Red', and 'Norland' potatoes stored at 10 °C. Standard errors are given in parentheses.

Cultivar	Slope hue.week^{-1}	
Michigan Purple	0.81 (0.19)	a
Dakota Rose	0.41 (0.10)	b
California Red	0.41 (0.10)	b
Norland	0.18 (0.08)	b

Means separation within column ($P< 0.05$, LSD, n=30).

Table 2. A comparison between the rates of change in hue angle values in the first 3 months, the last 3 months and the overall 6 months of storage at 4 °C in 'Michigan Purple', 'Dakota Rose', and 'Chieftain' potatoes. Standard errors are given in parentheses

Cultivar	Date	Slope hue.week^{-1}	
Michigan Purple	First 3 months	1.92 (0.02)	a
Chieftain		0.70 (0.02)	b
Dakota Rose		0.76 (0.02)	b
Michigan Purple	Last 3 months	0.72 (0.06)	a
Chieftain		0.37 (0.05)	b
Dakota Rose		0.22 (0.03)	c
Michigan Purple	Overall 6 months	1.33 (0.08)	a
Chieftain		0.57 (0.03)	b
Dakota Rose		0.46 (0.03)	b

Means separation within column ($P< 0.05$, LSD, n=30).

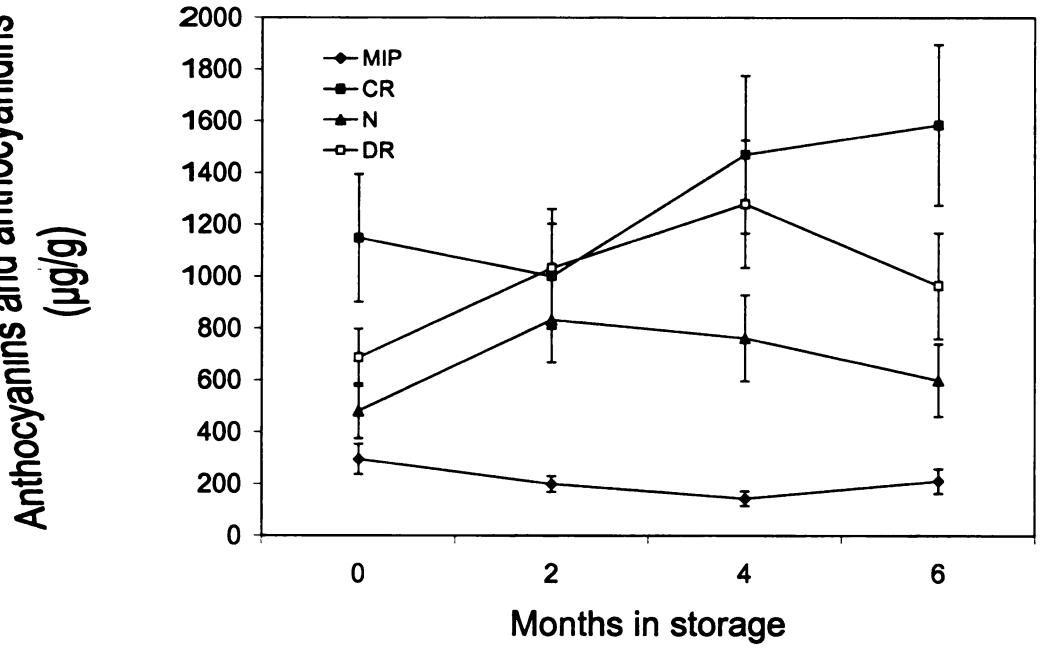
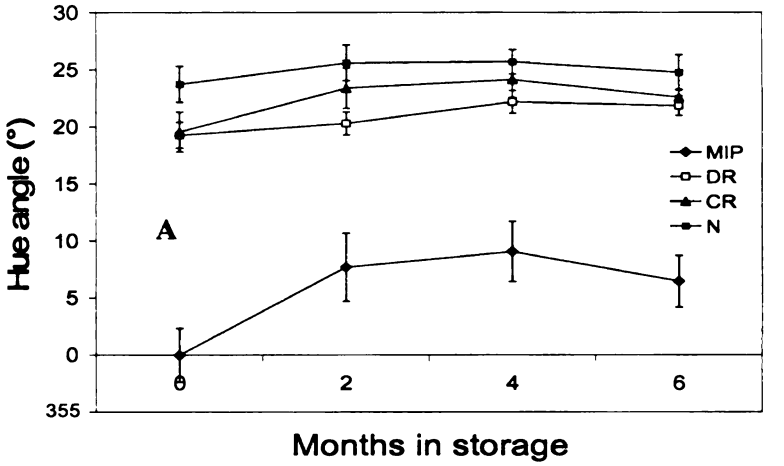
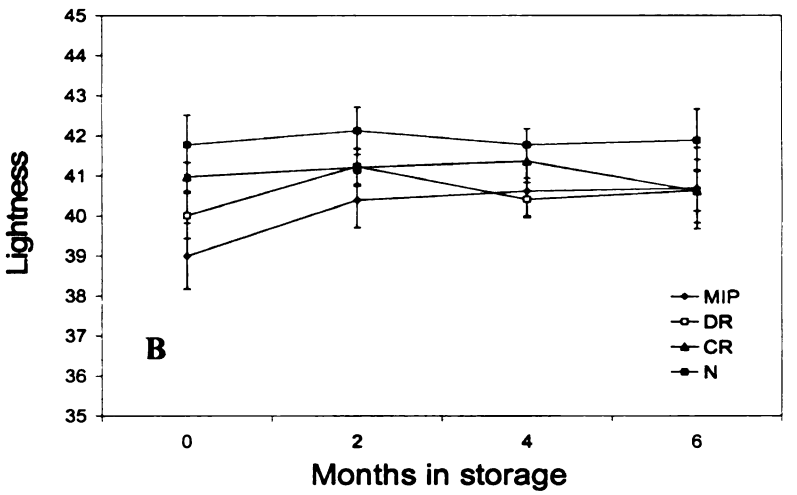


Figure 1. The effect of storage at 10 °C on the pigment (anthocyanin and anthocyanidin) concentrations of the cultivars 'Michigan Purple' (MIP), 'Dakota Rose' (DR) 'California Red' (CR), and 'Norland' (N). Vertical bars refer to standard error of the means (n=20). Anthocyanin analyses were done by using a spectrophotometer.

Figure 2. The effect of storage at 10 °C on (a) the hue angle values, (b) the lightness and (c) chroma of the skin of 'Michigan Purple' (MIP), 'Dakota Rose' (DR), 'California Red' (CR), and 'Norland' (N) potatoes. Vertical bars refer to standard error of the means (n=20).


30
25
20
15
10
5
0
355
Hue angle (°)
A
MIP
DR
CR
N
0
2
4
6
Months in storage


45
44
43
42
41
40
39
38
37
36
35
Lightness
B
MIP
DR
CR
N
0
2
4
6
Months in storage

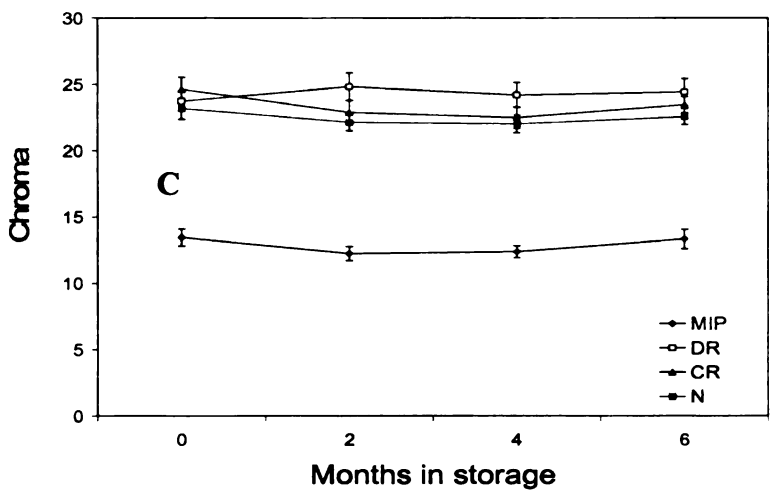

30
25
20
15
10
5
0
Chroma
C
MIP
DR
CR
N
0
2
4
6
Months in storage

Figure 2

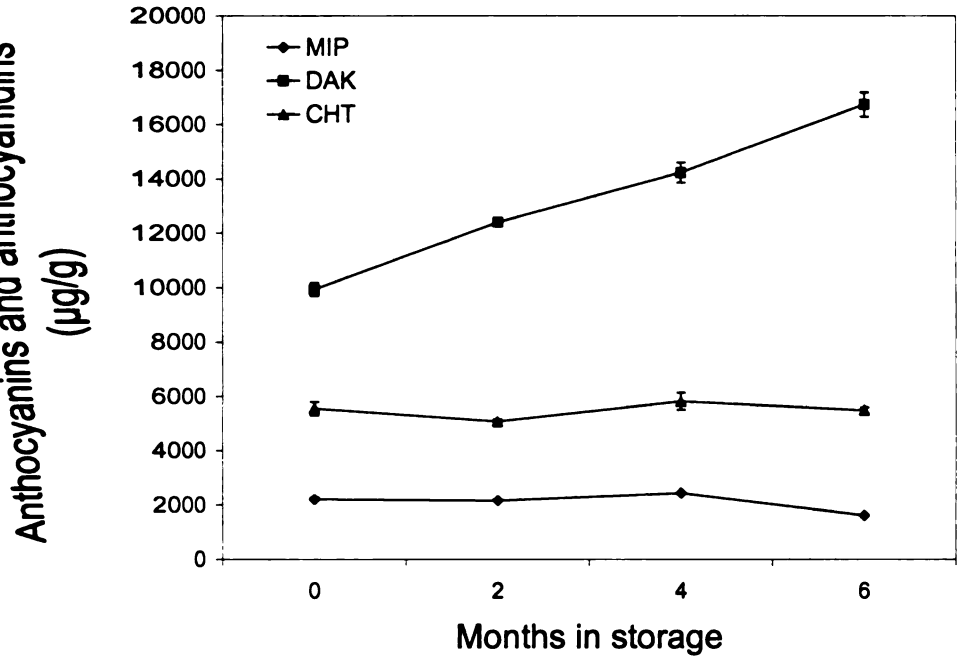


Figure 3. The effect of storage at 4 °C on the pigment (anthocyanin and anthocyanidin) concentrations in the skin of 'Michigan Purple' (MIP), 'Dakota Rose' (DR), and 'Chieftain' (CHT) tubers. Vertical bars refer to standard error of the means (n=30). Anthocyanin analyses were done by using a spectrophotometer.

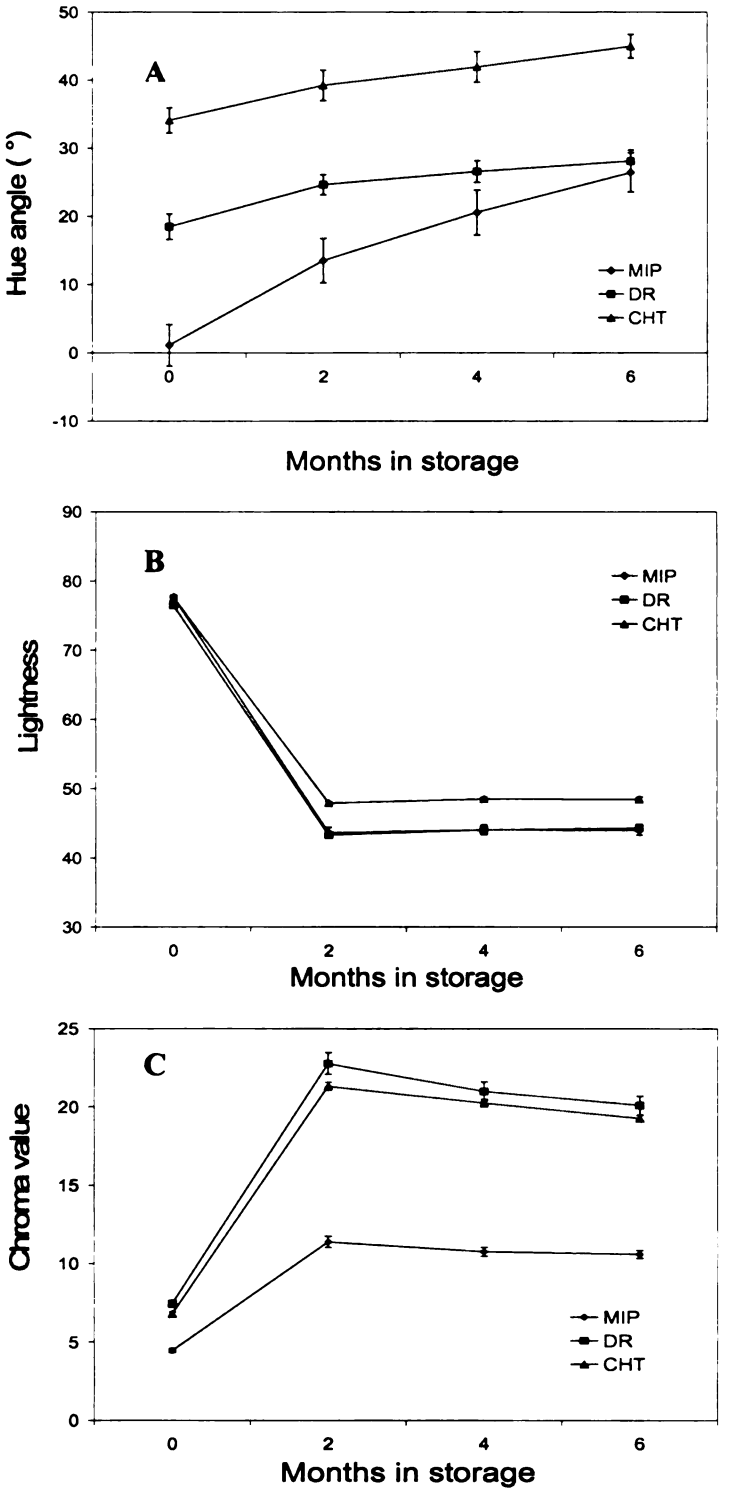


Figure 4. The effect of storage at 4 °C on the (a) hue angle values, (b) lightness, and (c) chroma of the skin of 'Michigan Purple' (MIP), 'Dakota Rose' (DR), and 'Chieftain'(CHT) potatoes. Vertical bars refer to standard error of the means (n=30).

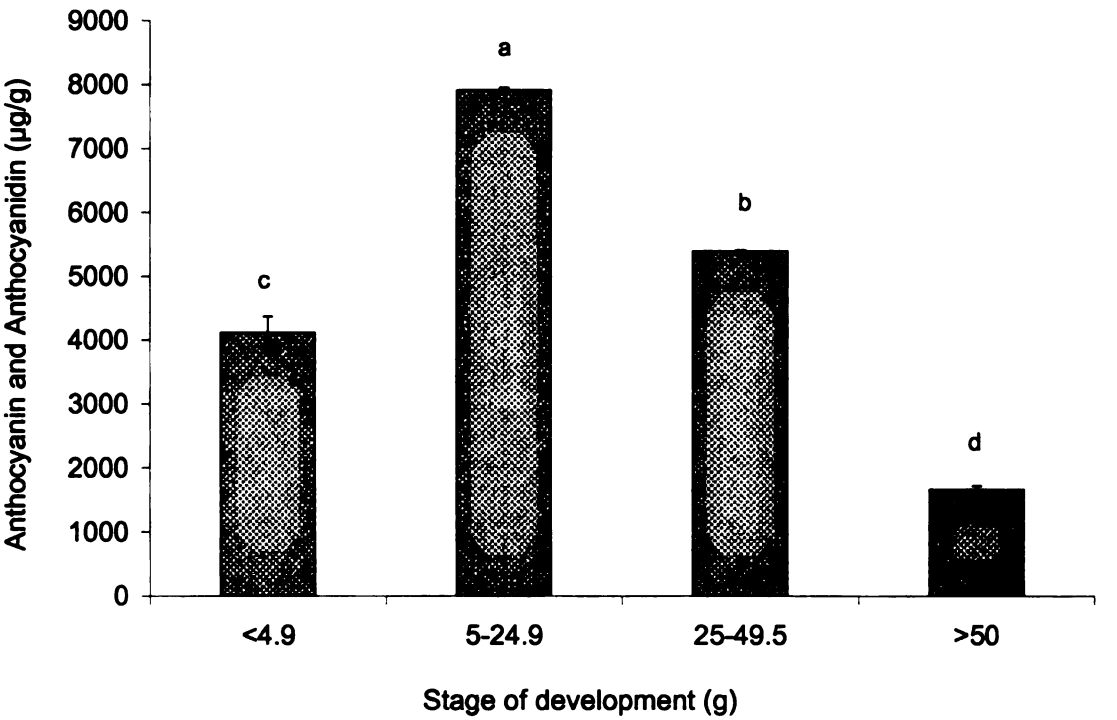


Figure 5. The effect of stage of development of the pigment content (µg/g) in the skin of 'Michigan Purple' tubers. Vertical bars refer to standard errors of the means ($P < 0.05$, LSD). Anthocyanin analyses were done by using spectrophotometer.

CHAPTER II

PREHARVEST TECHNIQUES TO IMPROVE PIGMENT CONCENTRATION AND COLOR OF 'MICHIGAN PURPLE' POTATOES AT HARVEST AND IN STORAGE

INTRODUCTION

Intense tuber skin-color is an important quality factor for growers who wish to obtain high prices for their niche market potatoes (*Solanum tuberosum* L.). Three preharvest tools that could improve the outcome of tuber skin-color at harvest are soil type and plant spacing, the application of different sources of nitrogen (inorganic and organic), and the application of different rates of 2,4-dichlorophenoxyacetic acid (2,4-D).

We investigated the difference in skin color of 'Michigan Purple' potatoes grown in mineral and organic soils at harvest and in storage. Mineral and organic soils differ markedly in their physical and nutritional make up. Mineral soils form from rock, sediment and a mixture of hundreds of minerals, and are made up from organic matter, mineral matter, water, and air. Organic soils, also known as muck or peat soils, have much more organic carbon (usually > 3-10%), are made from peat and plant remains, and usually have a darker color than mineral soils (Miller and Donahue, 1995; Pierzynski et al., 1994)

In addition, we investigated whether different planting spaces could have an effect on skin-color. Deficiencies in nutrients such as phosphorus have been found to increase anthocyanin biosynthesis in grapes (Dedaldechamp et al. 1995), carrots (Rajendran et al. 1992), and *Arabidopsis thaliana* (Trull et al., 1997), and deficiencies in nitrogen have been found to increase anthocyanin biosynthesis in tomato (Bongue-Bartelsman and Philips, 1995), and grape (Do and Cormier, 1991). Therefore, we hypothesize that plants grown closer together

would compete for nutrients and sunlight and hence be under more nutrient stress, resulting in increased anthocyanin concentrations in tubers. To our knowledge, the effect of soil type and plant spacing on potato tuber skin-color has not been investigated.

Nitrogen (N) is a critical nutrient for potato plants since it has a great effect on yield and tuber quality. Inadequate amounts of N will reduce yields, whereas an excess can reduce tuber quality and tuber yield. A nitrogen deficiency has also been found to increase anthocyanin synthesis in other commodities such as grape (Do and Cormier, 1991); however, it is not clear whether excessive amounts of nitrogen could suppress anthocyanin formation and thus contribute to less intensely colored tubers. We investigated the application of different rates and sources of nitrogen within the range of commercially acceptable fertilization levels on the tuber skin-color of 'Michigan Purple'. We expected to see the best color in the treatments with the least amount of nitrogen since nitrogen deficiencies result in an accumulation of carbohydrates. Since these carbohydrates cannot be used in the synthesis of amino acids or other nitrogen compounds, they may be used in anthocyanin production (Taiz and Zeiger, 1998). We hypothesized that tubers receiving higher amounts of nitrogen will have lower anthocyanin concentrations than the tubers receiving lower nitrogen concentrations.

Anthocyanin synthesis is an energy expensive process and significant production only occurs during specific developmental stages of plant organs or in response to pre- or postharvest environmental stresses. Many stress responses

act through ethylene, which itself stimulates anthocyanin synthesis (Faragher and Brohier, 1984; Faragher and Chalmers, 1977; Li et al., 2002). In addition to ethylene, auxin has been shown to induce the biosynthesis of anthocyanins in cell culture (Rajendran et al., 1992; Sakamoto et al., 1994). Auxin causes the stimulation of ethylene biosynthesis through enhancing the conversion of S-Adenosyl-methionine (Adomet) to 1- Aminocyclopropane-1-carboxylic acid (ACC).

Ethylene stimulates anthocyanin biosynthesis by increasing the activity of phenylalanine ammonia-lyase (PAL) (Faragher and Brohier, 1984), chalcone isomerase (CHI) (Li et al., 2002), and UDP glucose-flavonoid 3-*O*-glucosyltransferase (UFGT) (Kereamy et al., 2002). The ethylene-generating compound, ethephon, has been used on many solanaceous fruit, including tomato (*Lycopersicon esculentum* Mill. (Burg and Burg, 1965; Edwards et al., 1984) and pepper (*Capsicum annuum L.*) (Armitage, 1989; Cantliffe and Goodwin, 1975; Knavel and Kemp, 1973 and Worku et al., 1975) to advance ripening and promote anthocyanin accumulation.

We investigated the efficacy of preharvest 2,4-D applications on the purple variety 'Michigan Purple' as a means to increase tuber skin-color. The synthetic auxin, 2,4 -D has been proven to enhance the skin-color of red potatoes when applied at pre-bloom stage at 0.4 $kg.ha^{-1}$ in sodium salt form (76% acid equivalent) (Fults and Schaal, 1948; Payne et al., 1950). 2,4-D is labeled for commercial use as a means to increase tuber color of red potato. The efficacy of 2,4-D on anthocyanin accumulation in purple-skinned cultivars has not been

demonstrated. We hypothesize that preharvest 2,4-D applications will result in tubers with higher anthocyanin concentrations.

This paper will therefore focus on three cultural tools to increase anthocyanin accumulation in 'Michigan Purple' potatoes: planting site selection (organic vs. mineral), soil nutrition (nitrogen fertilization) and growth regulator (2,4-D) application.

MATERIALS AND METHODS

Experiment 1. The effect of organic and mineral soils on tuber skin-color.

Site description. In 2002 'Michigan Purple' tubers were grown in black organic soil at Krummrey & Sons, Inc., Stockbridge, Michigan, and in mineral soils in Rogers City, Michigan. Tubers grown in organic soil were planted 0.3 m apart, those in mineral soil were planted either 0.2 or 0.33 m apart.

Storage conditions. After harvest, 40 tubers of each treatment were washed, air dried, and placed into carton trays. A circle, 1.5 cm in diameter, was drawn on each potato with indelible marker to indicate the position at which color measurements would be made. Trays were stored at 10 °C and 80-90% RH. In addition, extra tubers were stored for extraction purposes at 0, 2, 4 and 6 months: three replicates of 5-10 tubers of each cultivar were placed into brown paper bags immediately after harvest and stored under same conditions as trays.

Color measurements. Color was measured biweekly in circled areas with a colorimeter (Colorimeter, Minolta CR 300, Japan). Chroma, hue angle, and

lightness values were recorded. Chroma refers to the intensity of the color (0 being grayish, 30 dull, and 60 vivid) and hue angle (h° = arcangle[b/a]) refers to the angle formed by line from the origin to the intercept of the a (x-axis) and b (y-axis) co-ordinates, where 0° = red-purple, 90° = yellow, 180° = bluish-green, and 270° = blue (McGuire, 1992). Lightness is presented on a scale of 1-100, 1 being black and 100 being white.

Anthocyanin extraction. Approximately five grams of tuber periderm was removed from 1 to 10 tubers by scraping with a scalpel. The skin was immediately frozen in liquid nitrogen and stored at -80 °C until analyzed. Two grams of potato periderm were ground to a powder in a mortar and pestle using liquid nitrogen. Samples were extracted in 20 mL of 2.5% (v/v) formic acid in methanol and placed in a shaker for 60 min at 4 °C. Samples were centrifuged at 3000 rpm for 15 min at 4 C° and the supernatant decanted. Three more extractions were made by adding 10 mL of the extraction solvent to sample, centrifuging at 3000 rpm for 10 min and decanting the supernatant.

The combined extracts were filtered with a 50 mm filter into a clean test tube. Samples were evaporated to dryness at 35 °C and re-dissolved in 2 mL 2.5% (v/v) formic acid in water. Filter cartridges (Sep Pak® Plus C18, Waters, Ireland) were prepared by dripping 5 mL 100% methanol through the empty cartridge and rinsing with 5 mL 2.5% (v/v) formic acid in water. The sample was slowly loaded onto the filter cartridge and rinsed twice with 5 mL 2.5% (v/v) formic acid in water. The sample was eluted with 1.8 ml 2.5% (v/v) formic acid in

methanol and filtered through a 0.2 µm filter into an auto-sampling vial (Alltech associates, Inc., IL).

Anthocyanin analysis was achieved using analytical reversed-phase HPLC with a separations module (Waters 2690), a photodiode array detector (Waters 2994), and a 4.6 x 150 mm column (XTerra® RP-18 column, Waters), maintained at 30 °C. Sample volume was 15-20 µL. Elution solvents used were: A, 2.5% formic acid in methanol and B, 2.5 % formic acid in water. A flow rate of 1 mL. min^{-1} was maintained. Samples were eluted using the following program: 70% A to 35% A and 30% B to 65 % B from 0 min to 17 min; 0% A and 100% B at 19 min and 70% A and 30 % B at 21 min. The eluted compounds were monitored at 530 nm for anthocyanins.

Four standards were prepared and run on the HPLC for our identification properties: petunidin chloride (Apin chemicals, UK), pelargonidin -3,5 - diglucoside (Apin chemicals, UK), cyanidin-3-glucoside, and cyanidin-3-rutinoside (Sigma, St. Louis, US).

For additional identification purposes, HPLC analysis was done with a 4.6 x 150 mm Capsell RP-18 column. The elution solvents were on a v/v basis: A, 0.1 % trifluroacetic acid in water and B, 50.4 % water, 48.5 % acetonitrile, 1 % acetic acid and 0.1 % trifluroacetic acid. The samples were eluted using the following program: 80% A to 40 % A and 20 % B to 60 % B from 0 min to 26 min; 80% A and 20% B at 30 min, held until 45 min.

Based on their retention times, spectra, and a library comparison, peaks 3 and 4 were identified as the anthocyanidins delphinidin and cyanindin,

respectively. Peak 1 was identified as an anthocyanin. Peak 1 had the same retention time as pelargonidin -3, 5-diglucoside.

Experiment 2. The effect of nitrogen fertilizer type and rate on tuber skin-color.

Site description and experimental design. In 2003, 'Michigan Purple' tubers were grown in a commercial 'Chieftain' plot on Montcalm/McBride sandy loam soil at Montcalm Research Farm, Entrican, Michigan. Nitrogen rates used were respectively: 200 $kg.ha^{-1}$ nitrogen (in the form of urea applications), 200 $kg.ha^{-1}$slow releasing nitrogen (in the form of MeisterT102 and an urea application), 300 $kg.ha^{-1}$ nitrogen (in the form of urea applications) and 200 $kg.ha^{-1}$ poultry manure (in the form of 5600 $kg.ha^{-1}$ poultry manure and urea applications (Table 1). Nitrogen application timing was as indicated in Fig.1, in 4 splits. Potatoes were harvested after 5 months in the ground. After harvest tubers were placed in storage in order to study color changes.

Storage conditions. After harvest, twenty tubers of each treatment were washed, air dried, and randomly placed into four carton trays. A circle, 1.5 cm in diameter, was drawn on each potato. Trays were stored at 4 °C and 85-90% RH. Additional tubers were used for anthocyanin extractions after 0, 2, 4, and 6 months. Three replicates of 5-10 tubers of each treatment were placed into brown paper bags immediately after harvest and stored under the same conditions as trays.

Color measurement. Color was measured weekly as described above.

Anthocyanin extractions. Extractions were based on the procedure of Siegelman and Hendricks (1958). Tubers were taken from storage at the appropriate times and the periderm was removed by scraping with a scalpel. Skin samples were frozen and ground as described above. Periderm samples (0.5 g) were added to 15 mL of 1% (v/v) HCl in methanol solution and left for 18 hours at 5 °C in the dark. Samples were centrifuged for 10 min at 3000 rpm. Absorbance of the supernatant was measured at 510, 530 and 540 nm on a spectrophotometer (U-3000, Hitachi, Japan).

Experiment 3. The effect of 2, 4-Dichlorophenoxyacetic acid on tuber skin-color.

Site description and experimental design. The potato variety 'Michigan Purple' was grown in 11.5 cm^3 plastic pots in the greenhouse at Michigan State University. Pots were filled with mixed media, prepared from 60 % sandy soil from Sandhill research trail field in East Lansing, Michigan and 40 % perlite. Greenhouse conditions were 16 h daylight period and approximately 28 °C. Plants were fertilized at planting, 4 weeks after planting, and 8 weeks after planting with 1.45 g per pot nitrogen, 0.62 g per pot phosphate, and 2.5 g per pot potassium. Plants were sprayed with 2,4-dichlorophenoxyacetic acid (44% a.i. 2,4-D, Agrisolutions, Agriliance, LLC) using a bench-type spraying machine. A randomized complete block design was used with four treatments and 5 replications. 2,4-D active ingredient treatment concentrations were: 39.25 $g.ha^{-1}$, 78.50 $g.ha^{-1}$ (recommended dosage), and 157.0 $g.ha^{-1}$. The first application was made when plants were 7-10 inches high (pre-bud stage), and the second

application was made 14 days later. Tubers were harvested 9 weeks after planting.

Color measurements. Color was measured on harvested potatoes as described above.

Anthocyanin extraction. As described above for nitrogen experiment.

Statistical analysis. Data from all experiments were analyzed using a Statistical Analysis System (SAS Institute, Inc., Version 8.0, 2000) to conduct least significant differences between treatments using PROC MIXED and PROC GLM. Slope analyses were done using PROC MIXED.

RESULTS AND DISCUSSION

Effect of soil type on tuber skin color. Organic soil grown tubers had higher total pigments than those grown in mineral soils (Figure 1). Tubers grown 0.2 m and 0.33 m apart in mineral soils did not differ in anthocyanin concentration. The anthocyanin content in tubers grown in organic soils decreased after 2 months in storage, however, the anthocyanin content of those grown in mineral soils did not change. A complete comparison of HPLC peaks between treatments can be seen in Appendix B Tables 1 and 2.

Hue angle (h°) was lowest in tubers grown in organic soils spaced at 0.3 m compared to those grown at either spacing in mineral soils throughout the storage period. Hue angle of these tubers increased during the first three months in storage (Figure 2; Appendix B Table 3). Organic soil grown tubers were also

darkest and more intensely colored. Lightness and chroma data shows that these tubers became significantly lighter and color less intense after one month in storage.

Tubers grown in mineral soils became slightly darker and the color more intense after four months of storage. Tubers grown 0.2 m apart in mineral soils had higher h° values than those planted 0.33 m apart. Although lightness values were usually not different, the chroma values of the 0.2 m planted tubers were higher than the 0.33 m planted tubers after one month in storage, meaning that these tubers were more intensely colored. These data indicate that plants grown closer together produce purple tubers with a more reddish hue, but a more intense color. One could hypothesize that the closer spacing resulted in nutrient stress e.g. phosphorus and nitrogen which lead to increased anthocyanin synthesis since anthocyanins are synthesized in response to plant stress (Chalker-Scott, 1999, Leonchenko, 1988, Parker 1962,). However, the differences of the colorimeter readings, though significant, are very small, and since the total anthocyanin data indicate no significant difference between different planting spaces, we can conclude from this study that planting space is not an effective way of enhancing tuber color in mineral soils.

In order to establish whether tubers grown in organic soils change color at a different rate than tubers from mineral soils, slope comparisons of the change in h° over time were made (Table 2). Slopes of organic grown treatments from both the first 2 months and the last 2 months were significantly higher than mineral soil grown treatments. However, slopes, thus rate of change in hue, did

not differ between 0.2 m and 0.33 m grown treatments, indicating that different planting spaces do not have an effect on the rate of color change in storage.

Effect of different nitrogen application rates. Tubers receiving 200 $kg.ha^{-1}$ N achieved the highest anthocyanin concentration after six months in storage at 4 °C (Figure 3, Appendix B Table 4). The other three treatments reached maximum anthocyanin concentrations after four months of storage, but decreased thereafter as can be seen at six months. There was no difference in anthocyanin concentration between treatments at harvest. After two and four months tubers supplied with 200 $kg.ha^{-1}$ slow-releasing N had the highest anthocyanin concentration. At six months in storage, the 200 $kg.ha^{-1}$ treatment had a significantly higher anthocyanin concentration than the other treatments.

Monthly h° values indicate that all treatments affected color significantly as early as two months in storage (Figure 4, Appendix B Table 4). Initially and after six months, h° of tubers grown with 200 $kg.ha^{-1}$ N were higher than those grown with 200 $kg.ha^{-1}$ poultry manure and 200 $kg.ha^{-1}$ slow-releasing N, however, there was no significant difference in lightness and chroma (intensity) between treatments after six months in storage.

In order to investigate rate of color change between treatments, a slope comparison of change in h° was made (Table 3). No significant changes were noted in the first 3 months of storage or the last 3 months, implying that rate of color change in storage at 4 ° was not different between treatments.

Effect of different 2, 4-D application rates. All plants showed signs of 2,4-D damage, including the control plants due to drift of 2,4-D. Chlorosis of the leaves, flower abscission, and foliar damage (e.g. leaf curling), were observed.

Plants that received the recommended rate of 78.50 $g.ha^{-1}$ a.i. of 2, 4-D had the highest anthocyanin concentration at harvest (Table 4). Although 78.50 $g.ha^{-1}$ 2, 4-D differed from the control, it was not significantly different from the other treatments. The 157.0 $g.ha^{-1}$ a.i. treatment was not significantly different from the control or any of the treatments (Table 4).

These results agree with the findings of Fults and Schaal (1948), and Payne et al. (1950) for red-skinned varieties. This means that anthocyanins can be increased through the preharvest addition of a synthetic auxin in purple potatoes as well as red potatoes. The performance of these treated potatoes in storage was not tested, however, Payne et al. (1950) found that 2,4-D treated 'Red McClure' tubers retained their color in 6 months of storage at 5 °C. It is known that auxin can induce ethylene production since auxin enhances the conversion of S- adenosyl methionine (Adomet) to 1-aminocyclopropane-1-carboxylic acid (ACC) which in turn leads to the production of enthylene. Ethylene has been found to enhance the biosynthesis of PAL and CSI, which are important enzymes in the anthocyanin biosynthesis pathway (Faragher and Chalmers, 1977; Faragher and Brohier, 1984; Li et al., 2002).

There was no difference in h° between treatments, but the 39.25 and 78.50 $g.ha^{-1}$ treatments were darker than the control (Table 4). Chroma data

indicates that the control had a more intense color than any of the treatments (Table 4).

CONCLUSIONS

Our study demonstrates that preharvest cultural tools can be used to successfully modify the anthocyanin concentration and color of potato tubers. We recommend growing 'Michigan Purple' potatoes in organic soils rather than mineral soils, to enhance color and anthocyanin concentrations (approximately 500 $\mu g.g^{-1}$ compared to 100 $\mu g.g^{-1}$ in mineral soils). Plant spacing did not result in differences in anthocyanin concentration, color (hue), or rates of color change in storage. The effectiveness of different planting spaces in organic soils on tuber color has not been determined.

Nitrogen fertilizer type and rate did not affect anthocyanin concentration at harvest. From a nutritional point of view this implies that type of fertilizer will not affect the health benefits of these colored potatoes. In addition, this gives growers the freedom to decide on the type of fertilizer to use since there were no differences between them. Different nitrogen types and rates did affect the color (hue) of the tubers, but did not affect the rate of color change in skins of stored potatoes.

Application of 2,4-D altered tuber anthocyanin concentrations in the skins of 'Michigan Purple' tubers in a concentration-dependant manner. The recommended 2,4-D rate of 78.50 $g.ha^{-1}$ provided a concentration 2-fold higher

than the control. However, 2,4-D resulted in damaged plants. Based on these greenhouse trials, we recommend using the lowest rate of 38.25 $g.ha^{-1}$ since this rate resulted in anthocyanin concentrations not significantly different than the recommended rate, and it could result in a decreased level of damage to the plant.

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Table 1. Four nitrogen schemes with different fertilizer or amendment concentrations ($kg.ha^{-1}$) applied at given times to 'Michigan Purple' potato plots. Amount of actual N applied ($kg.ha^{-1}$) given in parentheses.

Fertilizer or Amendment	Nitrogen Treatment			
	200 kg Conventional	200 kg Manure	200 kg Slow-release	300kg Conventional
Meister T102 (40%N, pre-plant)			400 (160)	
Poultry manure compost		5600 (56)		
Urea (46-0-0) (N credit)				
- at planting-13 May	100 (46)	100 (46)	100 (46)	100 (46)
-at hilling- 28 May	135 (62)	70 (32)		185 (85)
-23 June	135 (62)	70 (32)		185 (85)
-18 July	70 (32)	70 (32)		185 (85)
Total N	200	200	200	300

(adapted from Snapp et. al., 2003)

Table 2. Effect of soil type and plant spacing on rate of hue angle change in 'Michigan Purple' potato tubers grown in organic and mineral soils at 0.3, 0.2: and 0.33 m respectively during first two months and last two months of 4 months storage at 10 °C. Standard errors are given in parentheses.

Time	Treatment		
	Organic (0.3 m)	Mineral (0.2 m)	Mineral (0.33 m)
First 2 months	1.54 (0.08) a	0.61 (0.07) b	0.72 (0.11) b
Last 2 months	0.47 (0.12) a	-0.08 (0.06) b	-0.17 (0.04) b

Mean separation within rows ($P < 0.05$, LSD, n=40).

Table 3. A comparison between the rates of change in hue angle in 'Michigan Purple' potatoes treated with four different rates of nitrogen: 200 kg.ha^{-1}, 300 kg.ha^{-1}, 200 kg.ha^{-1} poultry manure, and 200 kg.ha^{-1} slow releasing nitrogen. Standard errors are given in parentheses.

Cultivar	Slope in first 3 months (hue.week^{-1})		Slope in last 3 months (hue.week^{-1})	
200 kg.ha^{-1}	2.65 (0.26)	a	2.52 (0.09)	a
300 kg.ha^{-1}	2.87 (0.19)	a	2.73 (0.11)	a
200 kg.ha^{-1} poultry manure	2.52 (0.20)	a	2.42 (0.13)	a
200 kg.ha^{-1} slow releasing	2.86 (0.22)	a	2.67 (0.08)	a

Mean separation within a column ($P < 0.05$, LSD, n=20).

Table 4. Effect of foliar applications of 2,4-D concentrations on the anthocyanin concentration, hue, lightness, and chroma of the skin of 'Michigan Purple' potatoes. Tubers were harvested 12 days after the second 2,4-D application. Anthocyanin analyses were done by using a spectrophotometer. Standard errors are given in parentheses.

Treatment (g.ha^{-1} a.i.)	Anthocyanins (µg/g)	Hue angle (°)	Lightness	Chroma
control	2350 b (46)	346.3 a (2.9)	38.7 a (1.1)	22.5 a (1.2)
39.25	3920 a (345)	354.9 a (4.2)	35.7 b (0.7)	14.1 b (1.6)
79.5	4360 a (290)	349.8 a (3.7)	34.8 b (0.6)	12.7 b (1.3)
157	3210 ab (182)	348.1 a (3.0)	36.7 ab (0.6)	15.6 b (1.5)

Mean separation within columns ($P < 0.05$, LSD).

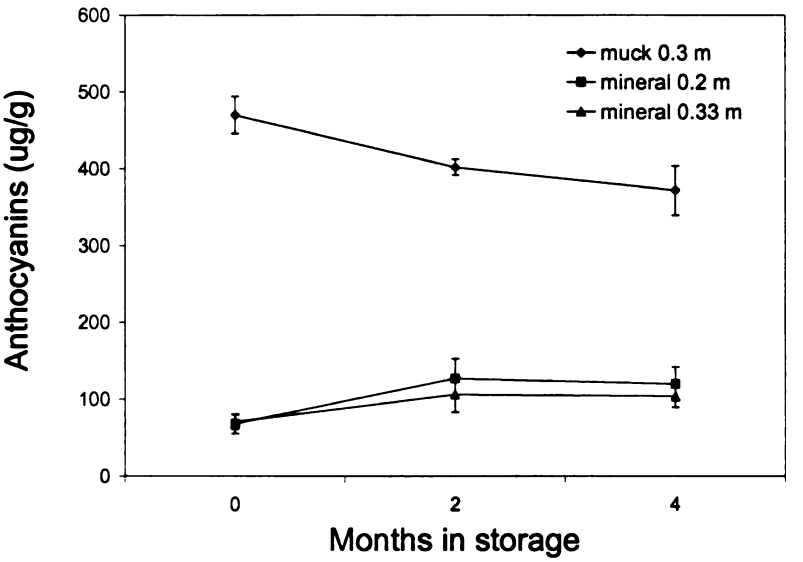
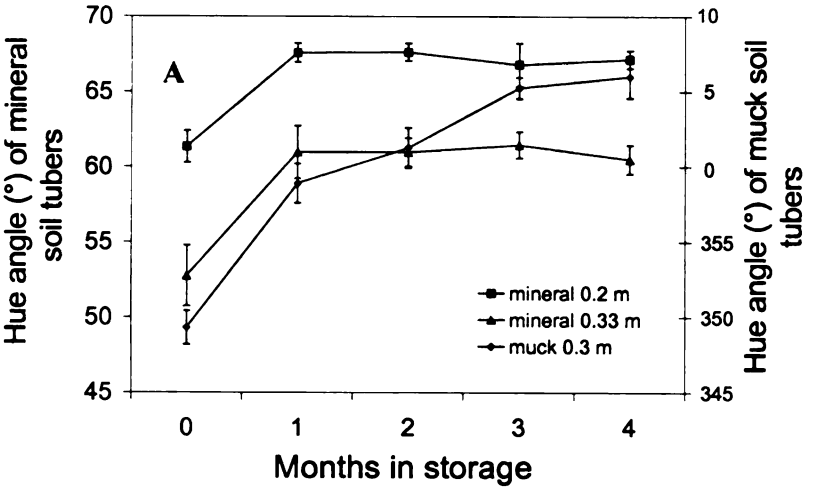
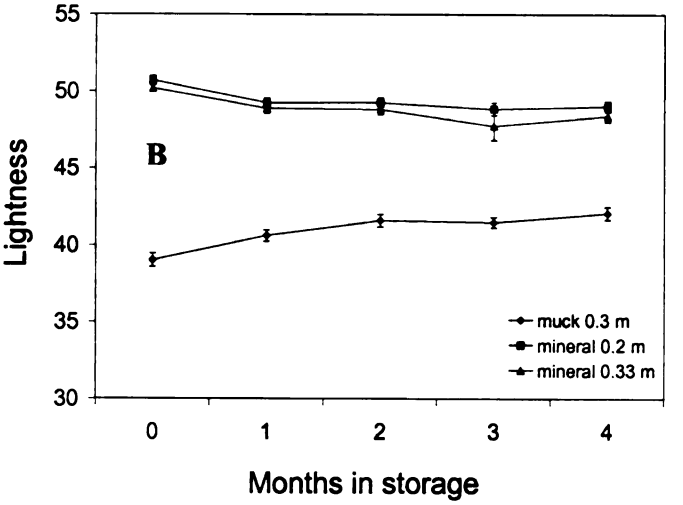


Figure 1. The effect of storage at 10 °C on the anthocyanin and anthocyanidin (μg/g) concentration in the skins of 'Michigan Purple' potato tubers grown in organic soil, 0.3 m apart, and in mineral soil, 0.2 and 0.33 m apart. Vertical bars refer to standard errors of the means (n=40). Anthocyanin analyses were done by using HPLC.

Figure 2. The effect of storage at 10 °C on the (a) hue angle, (b) lightness, and (c) chroma of the skins of 'Michigan Purple' tubers grown in organic soil, 0.3 m apart, and in mineral soil, 0.2 and 0.33 m apart. Vertical bars refer to standard errors of the means (n=40).


A
Hue angle (°) of mineral soil tubers
Hue angle (°) of muck soil tubers
70
65
60
55
50
45
10
5
0
355
350
345
mineral 0.2 m
mineral 0.33 m
muck 0.3 m
0
1
2
3
4
Months in storage


B
Lightness
55
50
45
40
35
30
muck 0.3 m
mineral 0.2 m
mineral 0.33 m
0
1
2
3
4
Months in storage

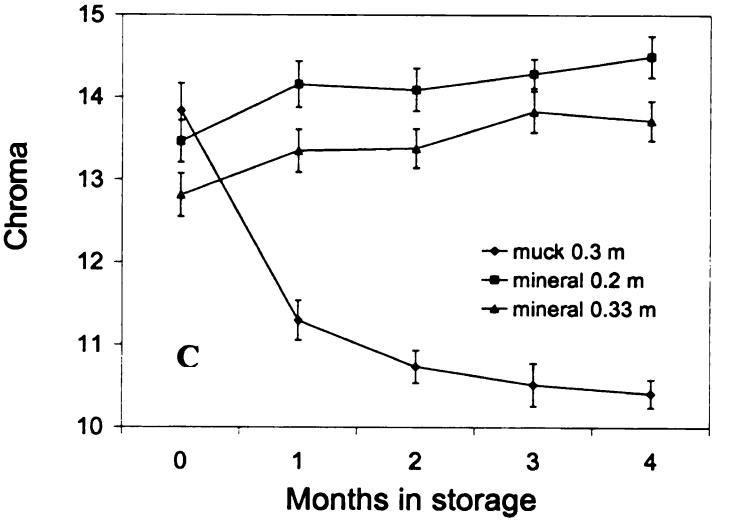

C
Chroma
15
14
13
12
11
10
muck 0.3 m
mineral 0.2 m
mineral 0.33 m
0
1
2
3
4
Months in storage

Figure 2

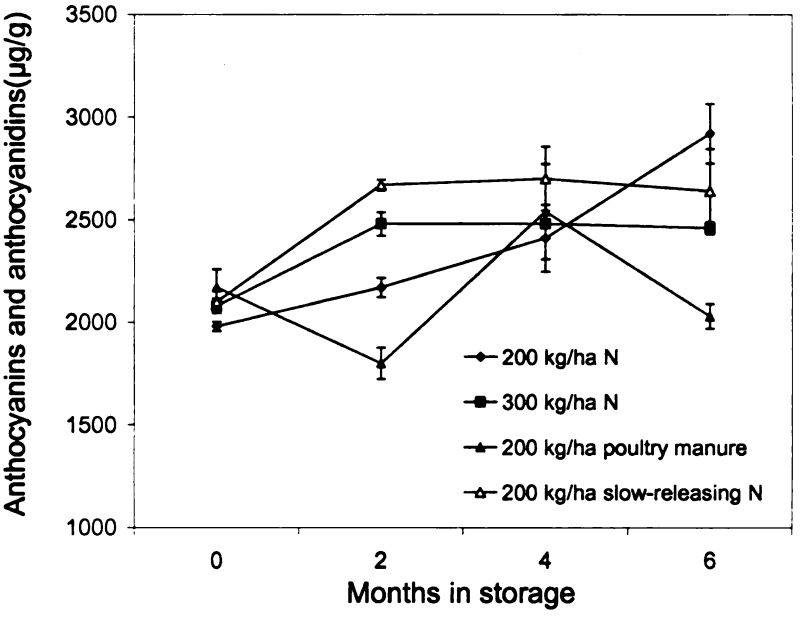
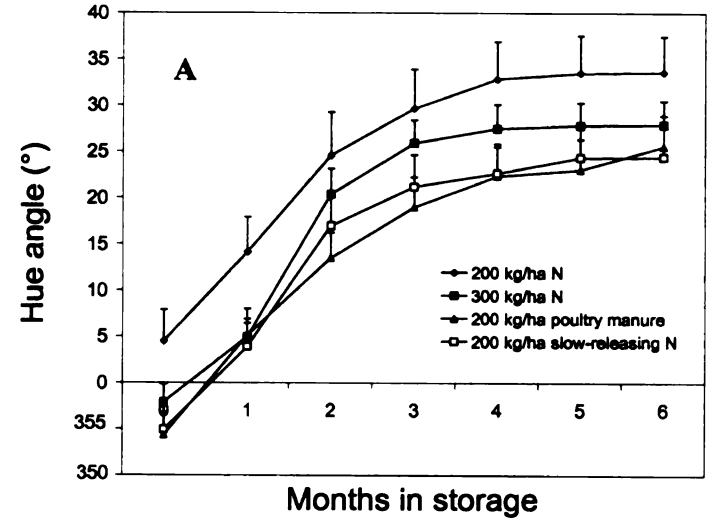
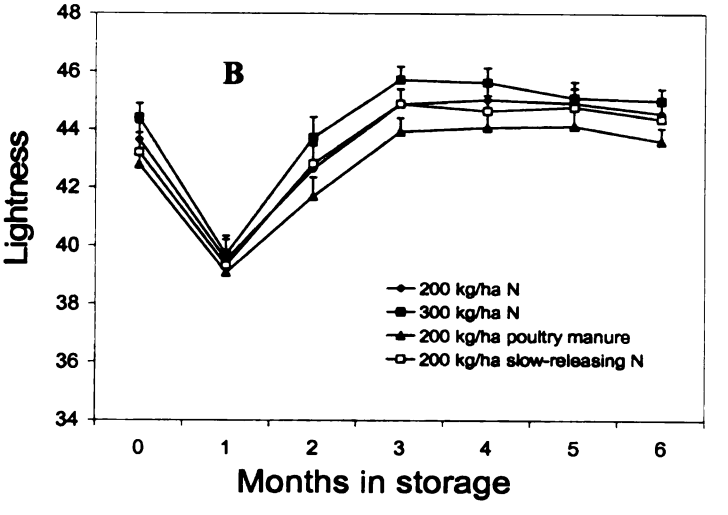


Figure 3. The effect of different nitrogen treatments: 200 kg.ha^{-1}, 300 kg.ha^{-1}, 200 kg.ha^{-1} poultry manure, and 200 kg.ha^{-1} slow-releasing nitrogen, on the anthocyanin and anthocyanidin (µg.g^{-1}) content in the skins of 'Michigan Purple' potato tubers at harvest and in storage. Vertical bars refer to standard error of the means (n=20). Anthocyanin analyses were done by using a spectrophotometer.

Figure 4. The effect of different nitrogen treatments: 200 kg.ha^{-1}, 300 kg.ha^{-1}, 200 kg.ha^{-1} poultry manure, and 200 kg.ha^{-1} slow-releasing nitrogen, on the (a) hue angle, (b) lightness and (c) chroma of the skins of 'Michigan Purple' tubers at harvest and in storage. Vertical bars refer to standard error of the means (n=20).


A
Hue angle (°)
40
35
30
25
20
15
10
5
0
355
350
1
2
3
4
5
6
Months in storage
200 kg/ha N
300 kg/ha N
200 kg/ha poultry manure
200 kg/ha slow-releasing N


B
Lightness
48
46
44
42
40
38
36
34
0
1
2
3
4
5
6
Months in storage
200 kg/ha N
300 kg/ha N
200 kg/ha poultry manure
200 kg/ha slow-releasing N

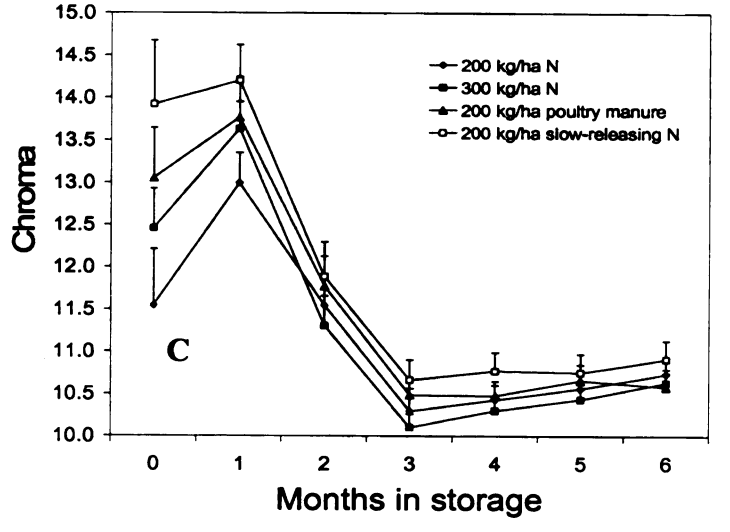

C
Chroma
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
0
1
2
3
4
5
6
Months in storage
200 kg/ha N
300 kg/ha N
200 kg/ha poultry manure
200 kg/ha slow-releasing N

Figure 4

CHAPTER III

THE EFFECT OF POSTHARVEST STORAGE TEMPERATURE REGIMES AND ETHYLENE EXPOSURE ON THE COLOR AND ANTHOCYANIN CONTENT OF 'MICHIGAN PURPLE' POTATO TUBERS

INTRODUCTION

Appropriate color and visual appearance of stored potatoes is critical to consumer purchasing decisions (Maskan, 2001).Temperature is one of the most important storage factors in postharvest quality control of potatoes. Ineffective temperature management can lead to postharvest decay and sugar accumulation as a consequence of excessively low storage temperatures (Kazami et al., 2000; Spychalla and Desborough, 1990). The colored cultivar 'Chieftain' has been reported to become softer and darker in storage at 4, 8, 12, 16 and 20 °C, but storage temperatures of 16 and 20 °C resulted in more rapid changes in quality parameters (Nourian et al., 2003). Lewis et al. (1999) reported that anthocyanin concentrations increased in the skins of colored potatoes stored at 4 °C, but not at 10, 18, and 26 °C. Conversely, Andersen et al. (2002) reported a decrease in the anthocyanin concentrations of potatoes stored at 4 °C. It is likely that this discrepancy is due to differences in response of different varieties to temperature.

Lewis et al. (1999) argued that cold temperature induction of sweetening i.e. the conversion of starch to sugars causes the increase in anthocyanins, since sugar is an anthocyanin precursor. Hara et al. (2003) found that in the hypocotyls of radish (*Raphanus sativus* L.), sugars act as signal molecules that activate the *CHS* (chalcone synthase) and the *ANS* (anthocyanin synthase) genes to accumulate anthocyanins. Similar results were also found for *CHS-A* gene from petunia in transgenic *Arabidopsis* (Tsukaya et al., 1991). On the other hand, it has also been found that anthocyanin concentrations can increase when plant

tissue is exposed to temperatures high enough to activate heat shock proteins (Takeda et al., 2003).

Anthocyanin synthesis is an energy expensive process that is usually influenced by pre- or postharvest environmental stresses. Many of these stress responses act through ethylene (Faragher and Chalmers, 1977; Faragher and Brohier, 1984; Li et al., 2002). Ethylene stimulates anthocyanin biosynthesis by increasing the activity of phenylalanine ammonia-lyase (PAL) (Faragher and Brohier, 1984), chalcone isomerase (CHI) (Li et al., 2002), and UDP glucose-flavonoid 3-*O*-glucosyltransferase (UFGT) (Kereamy et al., 2002). The ethylene-generating compound, ethephon, has been used on many Solanaceous fruit to enhance color, including tomato (*Lycopersicon esculentum* Mill). (Burg and Burg, 1965; Edwards et al., 1984) and pepper (*Capsicum annuum L.*) (Cantliffe and Goodwin, 1975; Armitage, 1989). It is also used on apples (*Malus x domestica*) to enhance color (Awad and de Jager, 2002; Li et al., 2002).

In this paper we investigate the effects of postharvest storage temperature manipulation on the skin color of 'Michigan Purple' potatoes. We hypothesize that alternating low (4 °C) and high (20 °C) temperatures will result in higher anthocyanin concentrations than continuous low temperature storage. Our rationale is based on the idea that enzymes active in anthocyanin biosynthesis will be synthesized at lower temperatures, and activated at the higher temperatures. In addition, we investigate the effect of postharvest ethylene application on the tuber skin-color of 'Michigan Purple' potatoes. We hypothesize

that postharvest ethylene will enhance the activity of enzymes such as PAL, and therefore stimulate anthocyanin biosynthesis in the tuber skins.

METHODS AND MATERIALS

Temperature experiment:

Site description and experimental design. 'Michigan Purple' potato tubers were obtained from Montcalm Research Farm, Entrican, Michigan. After harvest, tubers were washed, air dried and divided into replicates. Following initial color measurement, tubers were placed into trays in coldrooms. Three replicates of ten tubers were used for each of the treatments. Treatments were: (1) 60 days at 4 °C, (2) 60 days at 20 °C, (3) 15 days at 4 °C followed by 45 days at 20 °C, (4) 30 days at 4 °C followed by 30 days at 20 °C, (5) 45 days at 4 °C followed by 15 days at 20 °C, and (6) 7 days at 30 °C followed by 53 days at 20 °C. Relative humidity was kept at 80-90%.

Color measurements: A circle, 1.5 cm in diameter, was drawn on each potato before placed into storage. Color was measured weekly on the circled area with a colorimeter (Colorimeter CR 300, Minolta, Japan) in order to trace color change in the particular area. Chroma, hue angle, and lightness values were recorded. Colorimeter was calibrated using a standard white plate (lightness 97.99, chroma 2.03, hue angle 101.0).

Anthocyanin extractions. Extractions were based on the procedure of Siegelman and Hendricks (1958). Tubers were taken from storage at the appropriate times and the periderm removed by scraping with a scalpel. The skin

was immediately frozen in liquid nitrogen and stored at -80 °C until analyzed. The periderm was ground to a powder with a mortar and pestle. Periderm samples (0.5 g) were added to 15 mL of 1% HCl in methanol solution and left for 18 hours at 5 °C in the dark. Samples were centrifuged for 10 min at 12000 x g. Absorbance of the supernatant was measured at 510, 530 and 540 nm on a spectrophotometer (U-3000, Hitachi, Japan).

Ethylene experiment: Freshly harvested potatoes were divided into eight replicates of five potatoes. A circle, 1.5 cm in diameter, was drawn on each potato and the potato was numbered. Color was measured as described above and tubers were placed into glass jars. Four jars were exposed to 100 ($\mu L.L^{-1}$) ethylene for seven days at room temperature while the control was exposed to air at room temperature. After seven days, tubers were removed from the glass jars and color was measured every week for the next five weeks.

Statistical analysis. Data was analyzed using commercial Statistical Analysis System software (SAS Institute, Inc., Version 8.0, 2000) to calculate least significant differences between treatments using PROC MIXED and PROC GLM. Slope analyses were done using PROC MIXED.

RESULTS AND DISCUSSION

Temperature experiment. After 60 days of storage, the amount of anthocyanin in the skin of the 'Michigan Purple' tubers was the highest in the tubers that were stored at 4 °C for 60 days (Table 1). While exposure to elevated

temperatures during the storage period did not always result in significantly lower anthocyanin levels, treatments 2, 5, and 6 were significantly lower than treatment 1 after 60 days.

Hue angle was not affected by treatments at harvest, and even though there were differences between treatments after week one and six, after eight weeks there was still no difference in h° (Table 2; Appendix C Figure 1). Hue angle increased significantly within every treatment in storage. The color of tubers stored at 4 °C for 60 days changed significantly after four weeks in storage and remained at this higher h° until taken out of storage after 8 weeks. Tubers stored at 20 °C for 60 days changed after one week from 0.90° to 13.69°, and remained at this higher hue angle from weeks 4 to 8. Tubers receiving the heat shock treatment at 30 °C increased significantly in hue angle from 1.75° to 21.79° after one week and also remained higher than the initial for the 60 days in storage.

All treatments resulted in darker tubers (decreased lightness) after 8 weeks in storage (Table 2). Although treatments did not differ in lightness at harvest, tubers receiving treatments 1 and 5 were darker than tubers receiving treatments 3 and 4. Treatments did not differ in skin-color intensity at harvest, but after 8 weeks in storage treatment 1 resulted in significantly more intense tubers. All treatments resulted in more intensely colored tubers after two weeks and remained more intense until removal from storage. The hue angle of the tubers did not show a specific shift at this time. Tubers with higher anthocyanin content were the most intensely colored at the end of the experiment.

Ethylene experiment. Initially, when tubers were placed into jars, the control and the ethylene treatment had significantly different hue values; however, after two weeks there was no difference between treatments (Figure 1). Even after 5 weeks of close observation, there was still no difference in color. Chroma and lightness values did not change over the storage period (data not shown). We found 'Michigan Purple' tubers to be insensitive to postharvest ethylene application.

CONCLUSIONS

We found that the highest pigment concentration was maintained under continuous cold temperature of 4 °C, possibly as a result of the conversion of starch to sugars, which then served as precursors to anthocyanins. Continuous storage at 20 °C yielded significantly lower pigment concentrations. It seems that timing of removal of tubers from storage was also important since removal from 4° C after 45 days yielded significantly lower pigment concentration that the control at 4°C. We suggest that the last 15 days at 20 °C could have resulted in the deterioration of the pigment, or the initial pigment concentration of the treatment could have been lower. The heat-shock treatment (7 days at 30 °C) did not result in higher pigment concentrations. The 30 °C might have been too low for the activation of the heat-shock proteins. Since the trend in the change of hue angle values could not be explained by the anthocyanin concentrations after

storage, we conclude that hue angle shifts are not entirely associated with changes in anthocyanin concentrations.

Further studies should be done to determine the pigment concentration at the point of removal from the initial lower temperature before placement at the higher temperature since it will be an accurate way of determining when the changes in pigment concentrations take place.

Postharvest ethylene treatment did not increase or change the color of the tubers. Further study could include preharvest ethephon applications to determine if tubers perceive preharvest ethylene and whether pigment accumulation is enhanced and if so, at which stage they are most responsive.

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Table 1. Anthocyanin (µg/g FW) concentrations in the skin of 'Michigan Purple' potato tubers after 60 days of storage at different combinations of 4 °C, 20 °C and 30 °C. Analyses were done by using spectrophotometer. Standard errors are given in parentheses.

Treatment				Anthocyanin (µg/g)	
Number	Days at 4 °C	Days at 30 °C	Days at 20 °C		
1	60	0	0	2310 (262)	a
2	45	0	15	1402 (77)	b
3	30	0	30	1760 (146)	ab
4	15	0	45	1730 (77)	ab
5	0	0	60	1360 (50)	b
6	0	7	53	1420 (50)	b

Mean separation within columns ($P < 0.05$, LSD).

Table 2. Differences in hue angle, lightness, and chroma between 'Michigan purple' potatoes stored under different conditions. Standard errors are given in parentheses.

Treatment				Days in storage											
				0				7				14			
Number	Days at 4 °C	Days at 30 °C	Days at 20 °C												
				Hue											
1	60	0	0	3.14	(2.5)	A	c	8.12	(2.2)	AB	bc	7.05	(2.8)	A	bc
2	45	0	15	4.91	(2.4)	A	bc	2.62	(1.5)	B	bc	0.42	(3.1)	A	c
3	30	0	30	5.66	(2.8)	A	c	4.88	(1.7)	B	c	7.28	(3.4)	A	bc
4	15	0	45	11.30	(2.8)	A	b	6.32	(2.6)	B	b	9.63	(2.9)	A	b
5	0	0	60	0.90	(2.9)	A	b	13.69	(2.6)	AB	a	3.00	(3.1)	A	b
6	0	7	53	1.75	(3.2)	A	c	21.79	(2.2)	A	ab	10.99	(3.6)	A	b
				Lightness											
1	60	0	0	76.28	(0.2)	A	b	77.85	(0.2)	B	a	42.59	(0.5)	AB	c
2	45	0	15	76.76	(0.1)	A	a	77.44	(0.1)	B	a	42.44	(0.5)	AB	c
3	30	0	30	76.75	(0.2)	A	a	77.43	(0.2)	B	a	42.32	(0.4)	B	c
4	15	0	45	76.81	(0.3)	A	a	77.34	(0.3)	B	a	43.38	(0.5)	AB	c
5	0	0	60	76.10	(0.2)	A	b	79.73	(0.2)	A	a	43.50	(0.5)	AB	d
6	0	7	53	76.61	(0.2)	A	b	80.60	(0.2)	A	a	44.22	(0.6)	A	d
				Chroma											
1	60	0	0	4.69	(0.1)	A	c	4.64	(0.1)	A	c	12.02	(0.4)	A	ab
2	45	0	15	4.94	(0.1)	A	c	4.81	(0.1)	A	c	12.84	(0.4)	A	a
3	30	0	30	4.81	(0.1)	A	c	4.78	(0.1)	A	c	12.18	(0.4)	A	a
4	15	0	45	4.52	(0.1)	A	b	4.50	(0.1)	A	b	10.89	(0.3)	A	a
5	0	0	60	4.83	(0.1)	A	b	4.16	(0.1)	A	b	11.98	(0.3)	A	a
6	0	7	53	4.95	(0.1)	A	c	4.30	(0.1)	A	c	12.62	(0.5)	A	a

Capital letters refer to mean separation within treatments (within column); small letters refer means separation between treatments (within rows); ($P < 0.05$, LSD, n=30).

Table 2. (continued).

Treatment				Days in storage											
				28				42				60			
Number	Days at 4 °C	Days at 30 °C	Days at 20 °C												
								Hue							
1	60	0	0	15.29	(3.0)	A	ab	14.28	(2.8)	AB	b	22.66	(3.2)	A	a
2	45	0	15	8.72	(3.0)	A	b	10.89	(3.0)	B	b	23.11	(2.8)	A	a
3	30	0	30	14.33	(2.9)	A	b	25.21	(3.0)	A	a	28.22	(3.0)	A	a
4	15	0	45	19.73	(3.2)	A	a	23.91	(2.8)	AB	a	25.39	(2.9)	A	a
5	0	0	60	8.52	(3.1)	A	ab	11.11	(2.9)	B	a	25.11	(2.7)	A	a
6	0	7	53	15.25	(3.3)	A	b	19.26	(3.6)	AB	ab	23.84	(3.3)	A	a
								Lightness							
1	60	0	0	42.96	(0.5)	B	c	39.41	(0.6)	B	d	43.09	(0.5)	B	c
2	45	0	15	41.50	(0.5)	B	c	38.98	(0.5)	B	d	44.08	(0.5)	B	b
3	30	0	30	42.45	(0.4)	B	c	44.73	(0.4)	A	b	45.34	(0.4)	AB	b
4	15	0	45	45.19	(0.5)	A	b	45.74	(0.4)	A	b	46.26	(0.4)	A	b
5	0	0	60	44.21	(0.4)	AB	cd	44.40	(0.4)	A	cd	45.16	(0.4)	AB	c
6	0	7	53	44.35	(0.7)	AB	d	45.17	(0.7)	A	cd	46.54	(0.5)	A	c
								Chroma							
1	60	0	0	11.02	(0.3)	AB	b	13.04	(0.4)	A	a	13.06	(2.0)	A	a
2	45	0	15	11.30	(0.3)	AB	b	13.51	(0.2)	A	a	10.43	(0.2)	B	b
3	30	0	30	11.55	(0.2)	A	ab	10.75	(0.2)	BC	b	10.62	(0.2)	B	b
4	15	0	45	10.04	(0.3)	B	a	10.07	(0.3)	C	a	10.02	(0.3)	B	a
5	0	0	60	11.46	(0.3)	A	a	11.38	(0.2)	BC	a	10.9	(0.2)	B	a
6	0	7	53	12.14	(0.2)	A	ab	11.78	(0.4)	B	ab	11.30	(0.2)	B	b

Capital letters refer to mean separation within treatments (within column); small letters refer means separation between treatments (within rows); ($P < 0.05$, LSD, n=30).

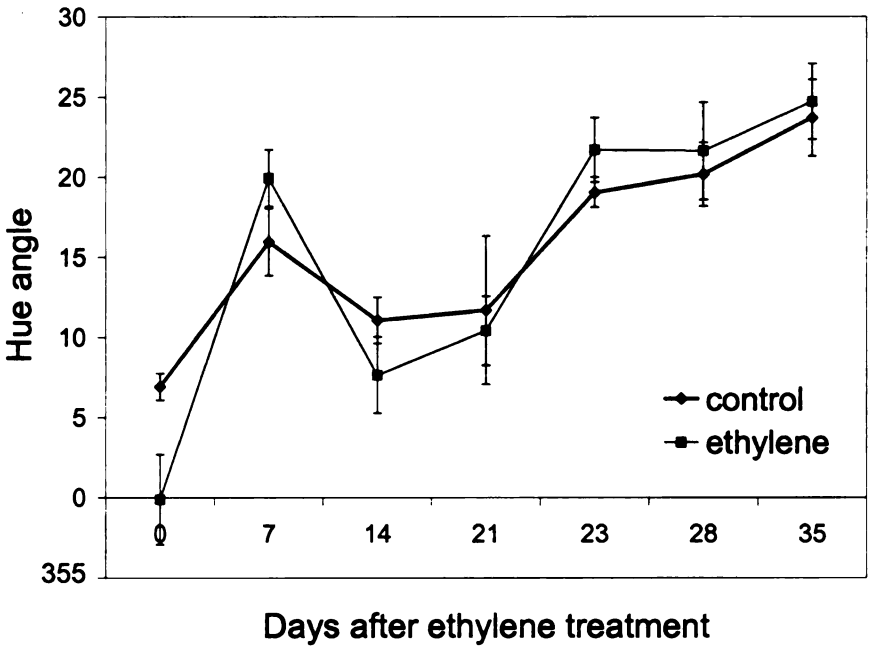


Figure 1. The effect of exposure of 100 ppm ethylene for 7 days on the hue angle of 'Michigan Purple' potatoes stored at 4 °C after receiving treatment. Vertical bars refer to the standard errors (n=25).

CHAPTER IV

SUMMARY

In conclusion, we found the most worthwhile avenue to pursue in order to achieve intense purple tuber skin-color in 'Michigan Purple' potatoes, to be the use of preharvest cultural tools. Specifically, we found planting in organic soils rather than mineral soils, and the application of 2,4-D to most dramatically intensify the tuber skin-color. Plants grown in the greenhouse showed significant 2,4-D damage, therefore, before we recommend any rates to the growers, we recommend testing 2,4-D under field conditions, since results could vary between locations.

Postharvest factors that could play a role in maintaining or even increasing anthocyanin concentrations and color (hue), are storage at low temperatures and minimizing exposure to higher storage temperatures.

Since auxin and ethylene have the potential to work in similar ways in stimulating anthocyanin biosynthesis through stimulating the enzymes in anthocyanin biosynthesis, we suggest testing preharvest foliar and soil applications of ethephon. If ethephon application is effective, the use of this product would be preferable to 2,4-D, since 2,4-D damage would be avoided.

APPENDIX A

Table 1. Impact of storage duration at 10 °C on total anthocyanins (µg/g FW), of 'Michigan Purple', 'Dakota Rose', 'California Red', and 'Norland' potatoes. HPLC absorbance values of total anthocyanins are given. HPLC absorbance values (au) at 530 nm also are given for comparison between cultivars

Table 1.

Cultivar	Months in storage at 10 °C											
	0			2			4			6		
						Total Anthocyanins						
Michigan Purple	294.4	B	a	198.7	B	ab	142.3	C	b	209.9	C	ab
Dakota Rose	686.6	B	b	1032.3	A	ab	1279.2	A	a	963.7	B	ab
California Red	1147.7	A	ab	1000.2	A	b	1471.0	A	ab	1584.7	A	a
Norland	479.8	B	a	831.9	A	a	761.4	B	a	599.1	BC	a
						Absorbance at 530 nm (AU x 1000)						
Michigan Purple	14173	A	a	10645	A	a	7560	A	a	8652	B	a
Dakota Rose	7352	AB	a	11911	A	a	13916	A	a	7392	B	a
California Red	13003	AB	a	10870	A	a	14900	A	a	18350	A	a
Norland	5870	B	a	9662	A	a	11331	A	a	7700	B	a
						Hue						
Michigan Purple	359.97	B	c	7.69	C	ab	9.07	B	a	6.45	B	b
Dakota Rose	19.28	A	b	20.30	B	ab	22.19	AB	a	21.85	A	a
California Red	19.56	A	b	23.39	AB	a	24.14	AB	a	22.58	A	a
Norland	23.74	A	a	25.6	A	a	25.7	A	a	24.76	A	a
						Lightness						
Michigan Purple	39.00	B	b	40.39	B	a	40.62	A	a	40.69	A	a
Dakota Rose	40.01	B	b	41.24	AB	a	40.41	A	ab	40.63	A	ab
California Red	40.97	AB	a	41.21	AB	a	41.36	A	a	40.62	A	a
Norland	41.77	A	a	42.13	A	a	41.77	A	a	41.89	A	a
						Chroma						
Michigan Purple	13.46	B	a	12.24	C	a	12.38	C	a	13.34	B	a
Dakota Rose	23.72	A	a	24.82	A	a	24.19	A	a	24.43	A	a
California Red	24.62	A	a	22.88	AB	b	22.48	AB	b	23.46	A	ab
Norland	23.16	A	a	22.13	B	a	22.01	B	a	22.57	A	a

Small letters refer to means separation within a row; capital letters refer to mean separation within column ($P < 0.05$, LSD, n=20).

Table 2. Impact of storage duration on anthocyanidin content of 'Michigan Purple', 'California Red', 'Norland', and 'Dakota Rose' potato tubers stored at 10 °C.

Treatment	HPLC peak	Months in storage							
		0		2		4		6	
MI Purple	1	15.7	a	14.7	a	10.8	a	23.9	a
	2	6.8	a	5.5	a	4.4	a	NA	
	3	39.7	a	15.4	b	7.8	b	12.6	b
	4	275.9	a	184.3	b	134.6	b	207.9	ab
	5	8.3	a	7.1	a	5.2	a	5.3	a
Cal. Red	1	99.4	b	106.0	b	52.3	b	164.1	a
	2	19.9	b	19.3	b	48.3	a	30.5	ab
	3	NA		12.5	a	19.1	a	13.7	a
	4	924.0	ab	788.3	b	1236.4	ab	1241.7	a
	5	104.4	a	82.4	a	114.8	a	139.3	a
Norland	1	32.5	a	52.2	a	42.6	a	45.1	a
	2	13.1	a	23.4	a	22.6	a	13.1	a
	3	23.3	a	12.1	a	11.8	a	NA	
	4	404.0	a	657.7	a	651.3	a	496.1	a
	5	15.7	b	94.3	a	44.6	ab	67.3	ab
Dak. Rose	1	34.0	a	43.2	a	73.0	a	81.4	a
	2	22.4	a	33.4	a	38.2	a	31.2	a
	3	13.9	a	17.4	a	33.8	a	13.4	a
	4	512.7	b	871.1	ab	1015.7	a	787.8	ab
	5	197.5	a	78.8	b	118.5	ab	58.8	b

Letters refer to means separation within a column ($P < 0.05$, n=30), NA=not available

Table 3. Impact of storage duration on the anthocyanin and anthocyanidin content of 'Michigan Purple', 'California Red', 'Norland', and 'Dakota Rose' potato tubers stored at 10 °C.

Months in storage	Cultivar	HPLC peak									
		1		2		3		4		5	
0	MI Purple	15.7	b	6.8	a	39.7	a	275.9	b	8.2	c
	Cal. Red	99.4	a	19.9	a	NA		924.0	a	104.4	b
	Norland	32.5	b	13.1	a	23.3	a	404.0	b	15.7	c
	Dak. Rose	34.0	b	22.4	a	13.9	a	512.7	b	197.5	a
2	MI Purple	14.7	b	5.5	a	15.4	a	184.3	b	7.1	b
	Cal. Red	106.0	a	19.3	ab	12.5	a	788.3	a	82.4	a
	Norland	52.2	b	23.4	ab	12.1	a	657.7	a	94.3	a
	Dak. Rose	43.2	b	33.4	a	17.4	a	871.1	a	78.8	a
4	MI Purple	10.8	b	4.4	b	7.7	a	134.7	c	5.2	b
	Cal. Red	52.3	ab	48.3	a	19.1	ab	1236.4	a	114.8	ab
	Norland	42.6	ab	22.6	b	11.8	ab	651.3	b	44.5	b
	Dak. Rose	73.0	a	38.2	ab	33.8	a	1015.7	ab	118.5	a
6	MI Purple	24	b	NA		12.6	a	208.0	b	5.3	b
	Cal. Red	164.1	a	30.5	a	13.7	a	1241.7	a	139.3	a
	Norland	45.1	b	13.1	a	NA		496.1	b	67.3	b
	Dak. Rose	81.4	b	31.2	a	13.4	a	787.8	b	58.8	b

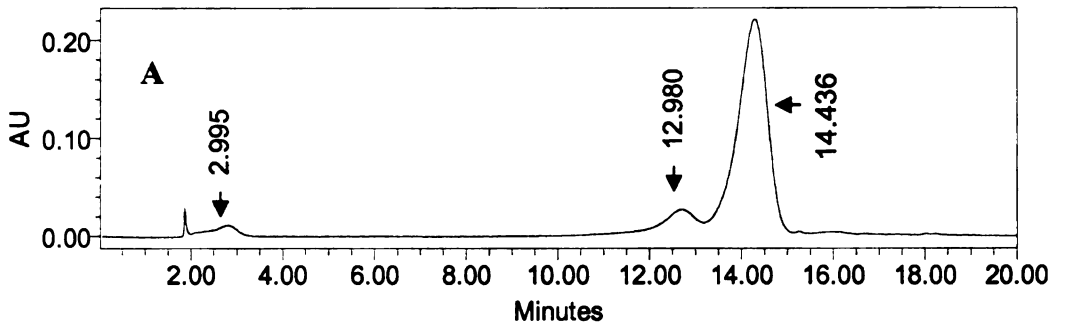
Letters refer to means separation within a column ($P < 0.05$, n=30), NA=not available.

Table 4. The effect of storage duration on the pigment (anthocyanin and anthocyanidin) concentration (µg/g), hue, lightness, and chroma of the skin of potato cultivars 'Chieftain', 'Dakota Rose' and 'Michigan Purple' stored at 4 °C in 2003.

Cultivar	Months in storage 0			2			4			6		
						Total Anthocyanins						
Michigan Purple	2184.5	C	a	2146.9	B	a	2426.5	C	a	1612.1	C	a
Chieftain	5526.5	B	a	5076.9	B	a	5758.4	B	a	5499.0	B	a
Dakota Rose	9747.2	A	d	12005.2	A	c	14008.3	A	b	16674.1	A	a
						Hue						
Michigan Purple	1.09	C	d	13.5	C	c	20.56	B	b	26.44	B	a
Chieftain	34.08	A	d	39.25	A	a	41.96	A	b	44.98	A	a
Dakota Rose	18.49	B	c	24.63	B	b	26.57	B	ab	28.10	B	a
						Lightness						
Michigan Purple	77.70	A	a	43.62	B	b	44.07	B	b	44.01	B	b
Chieftain	77.62	A	a	47.92	A	b	48.51	A	b	48.48	A	b
Dakota Rose	76.47	A	a	43.32	B	c	44.02	B	b	44.34	B	b
						Chroma						
Michigan Purple	4.46	B	c	11.38	C	a	10.76	B	b	10.60	B	b
Chieftain	6.79	A	d	21.32	B	a	20.24	A	b	19.25	A	c
Dakota Rose	7.44	A	d	22.78	A	a	21.00	A	b	20.09	A	c

Small letters refer to means separation within a row; capital letters refer to mean seperation within column ($P < 0.05$, LSD, n=30).

Figure 1. The HPLC chromatogram of 'Michigan Purple' potato (a) and the spectra of (b) peak 1, (c) peak 2, (d) peak 3 and (e) the standard, petunidin-chloride (0.25 µg/µl), used to quantify the pigment content in 'Michigan Purple' potatoes.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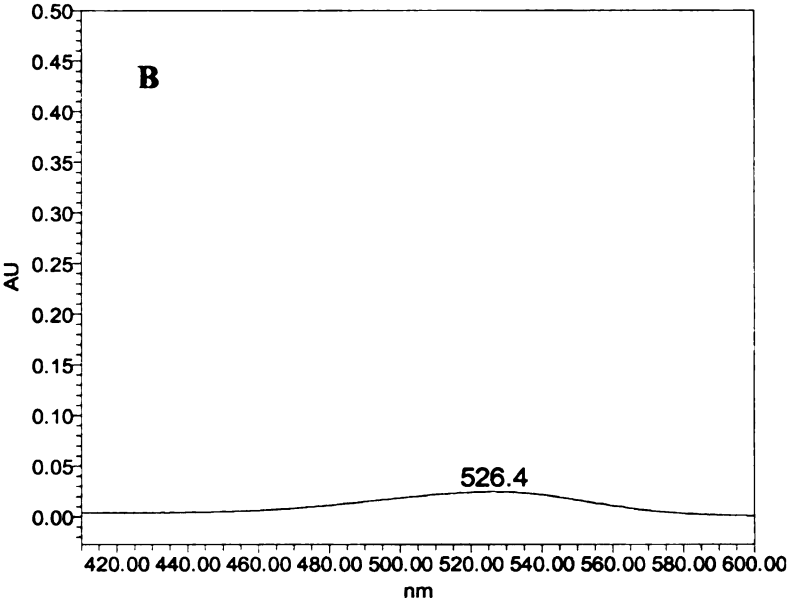
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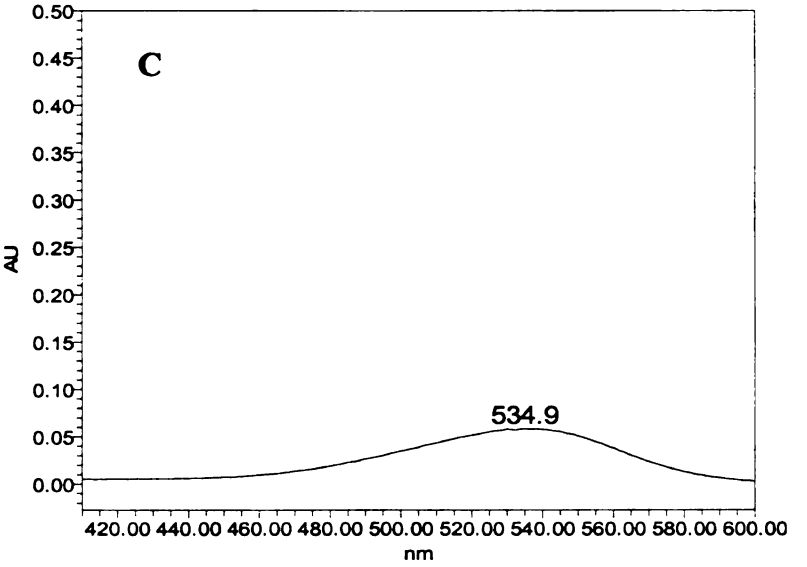
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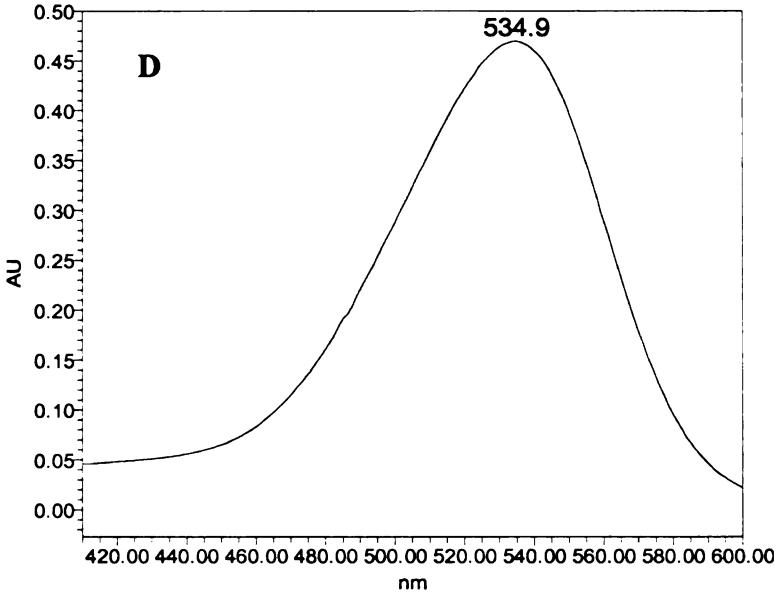
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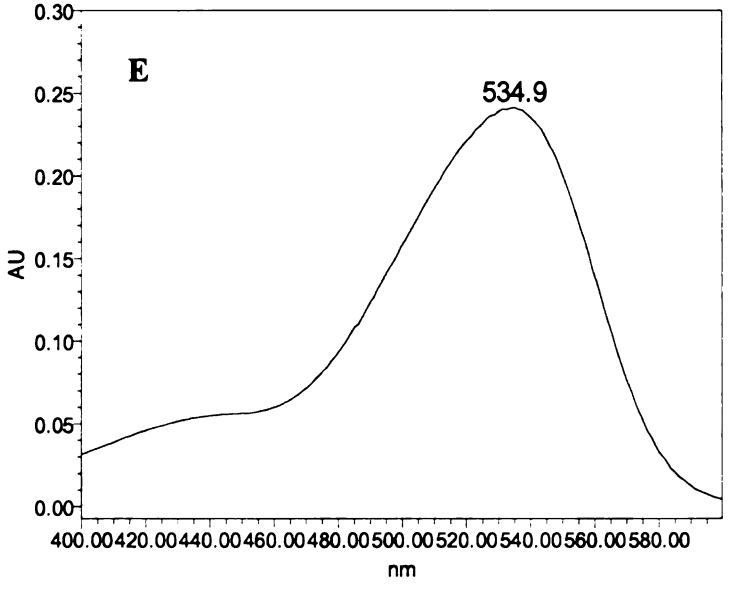
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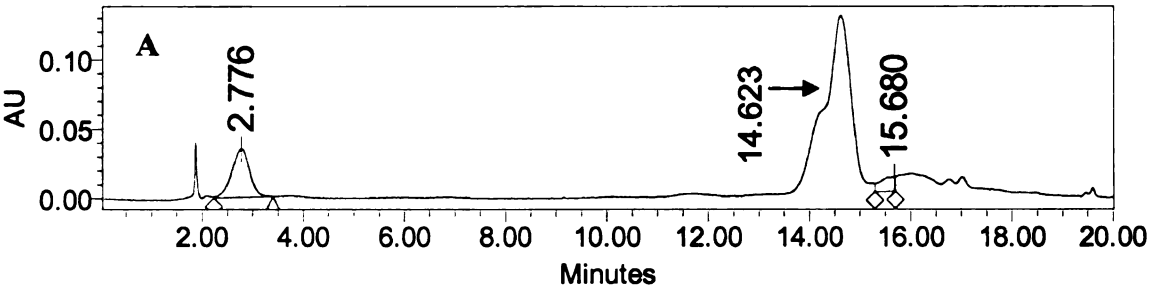
Figure 2. The HPLC chromatogram (a) of 'California Red' potato with the spectra of (b) peak 1, (c) peak 2 and (d) peak 3.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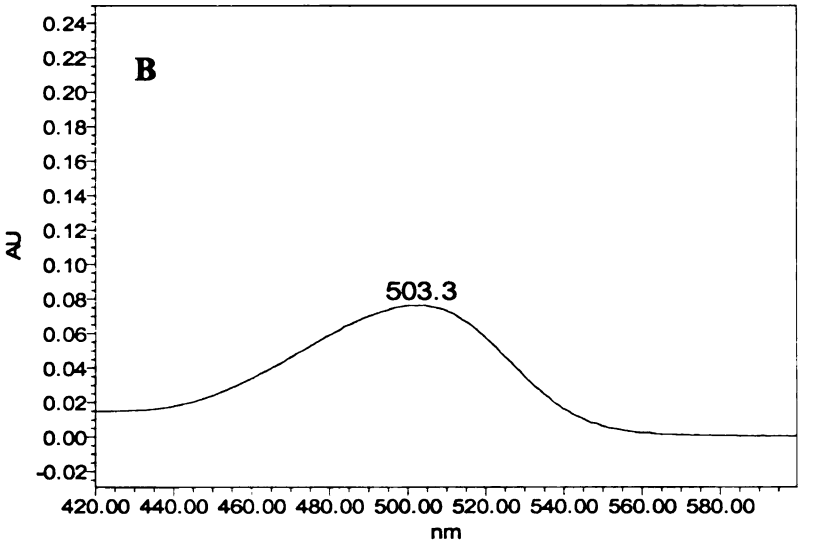
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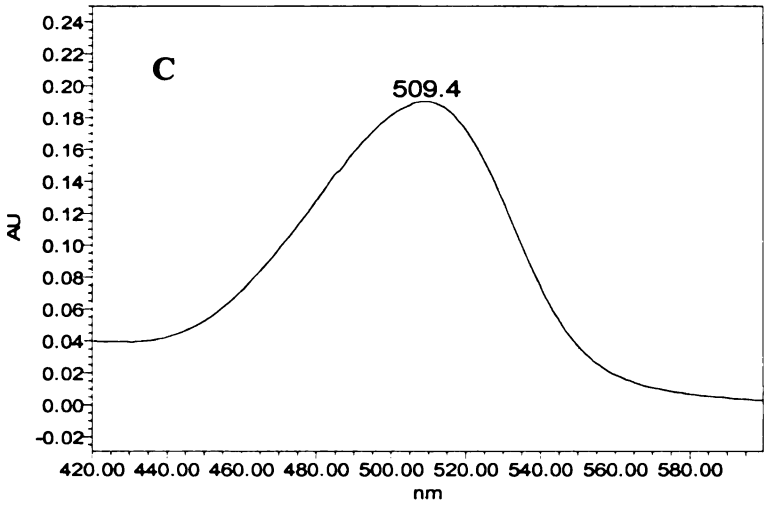
440.00
460.00
480.00
500.00
520.00
540.00
560.00
580.00
nm


C
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AU
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
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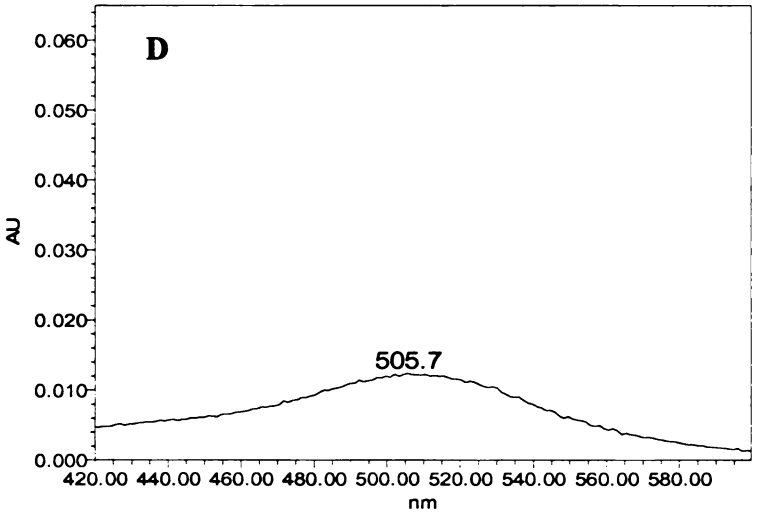
nm


D
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AU
0.060
0.050
0.040
0.030
0.020
0.010
0.000
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nm

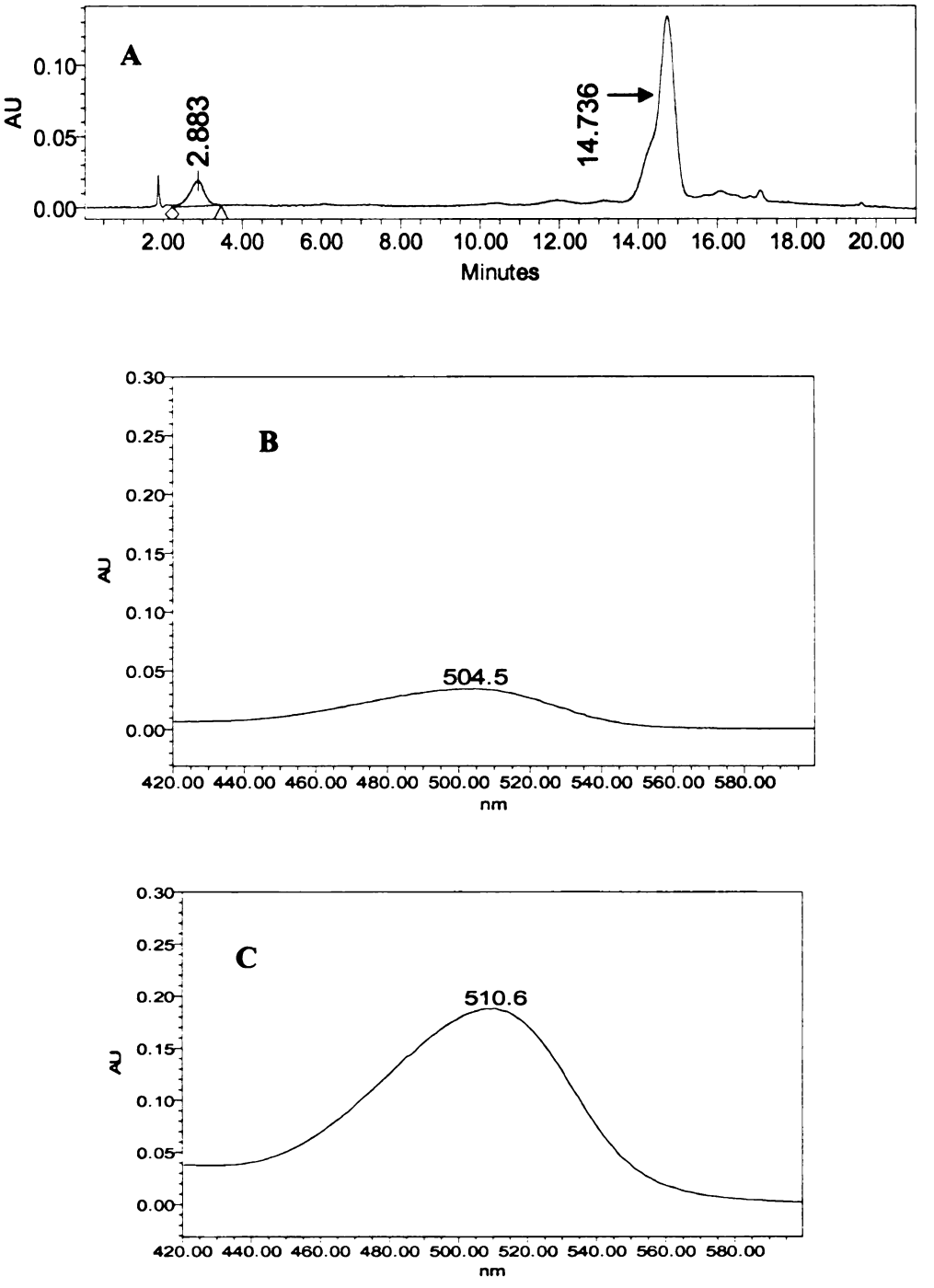
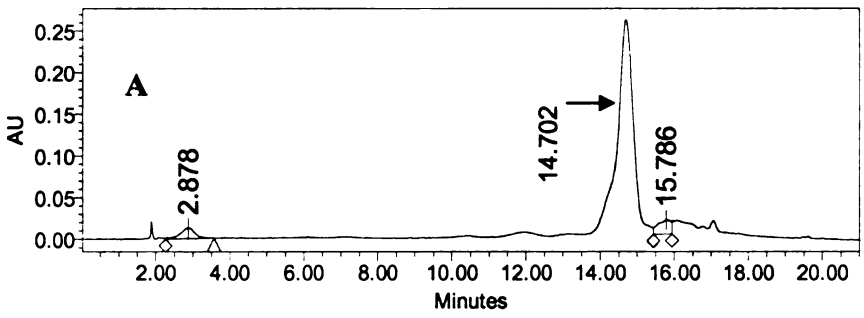


Figure 3. The HPLC chromatogram (a) of 'Norland' potato with the spectra of (b) peak 1 and (c) peak 2.

Figure 4. The HPLC chromatogram of 'Dakota Rose' potato (a) and the spectra of (b) peak 1, (c) peak 2, (d) peak 3 and (e) the standard, pelargonidin 3,5-diglucoside (1.5 $\mu g.\mu l^{-1}$) used to quantify the pigment content in 'Norland', 'California Red', and 'Dakota Rose' potatoes.


A
AU
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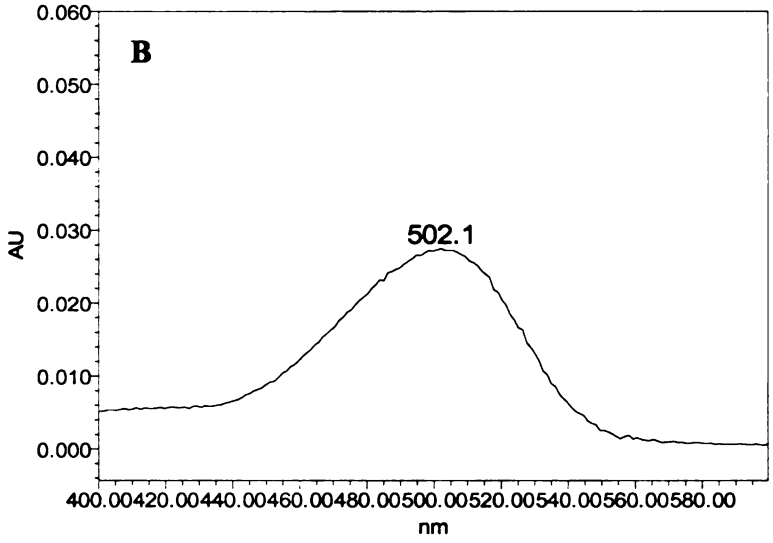
0.20
0.15
0.10
0.05
0.00
2.878
14.702
15.786
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes


B
AU
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0.040
0.030
0.020
0.010
0.000
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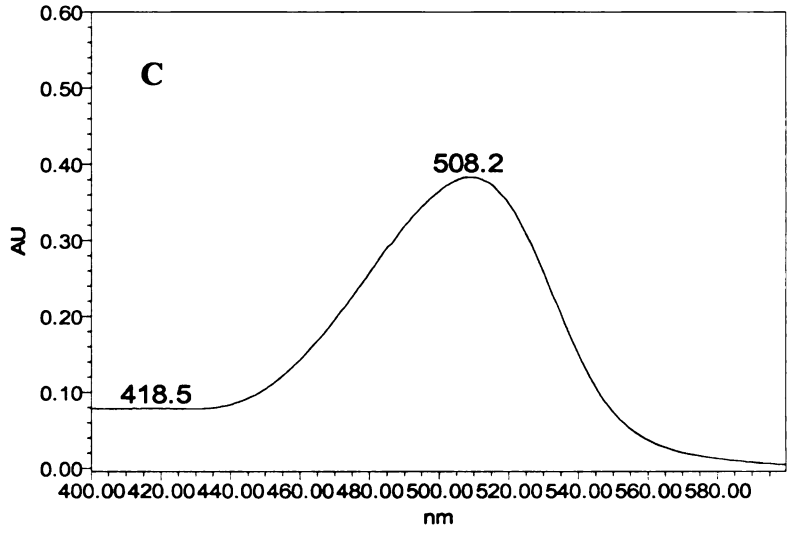
nm


C
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0.40
0.30
0.20
0.10
0.00
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418.5
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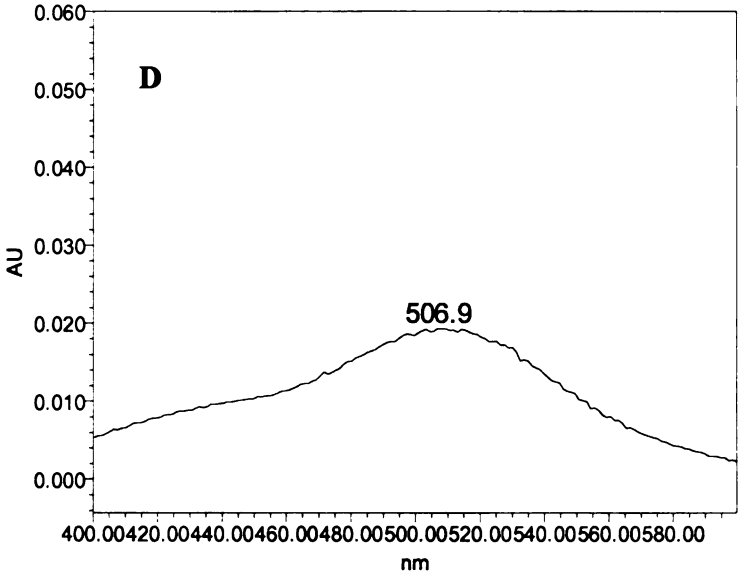
nm

Figure 4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0.040
0.030
0.020
0.010
0.000
400.00 420.00 440.00 460.00 480.00 500.00 520.00 540.00 560.00 580.00
nm

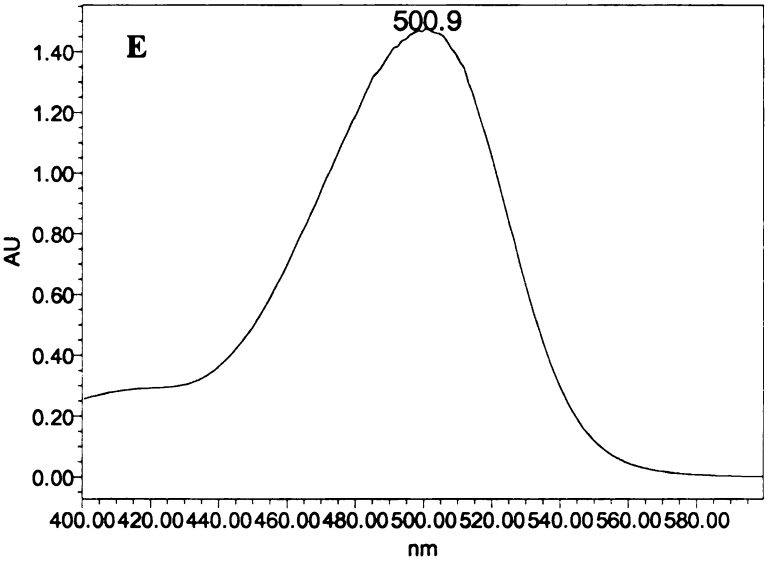
E
500.9
AU
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
400.00 420.00 440.00 460.00 480.00 500.00 520.00 540.00 560.00 580.00
nm

Figure 4

APPENDIX B

Table 1.The effect of soil type and plant spacing on anthocyanin and anthocyanidin (µg/g) concentrations in the skin of 'Michigan Purple' potatoes at harvest and after 2 and 4 months of storage at 10 °C.

Time	Treatment		HPLC peak							
	Soil	Spacing (m)	1		2		3		4	
Month 0	Muck	0.3	13.1	a	12.4	a	57.4	a	362.3	a
	Mineral	0.2	5.4	b	1.5	b	4.8	b	60.0	b
	Mineral	0.33	5.5	b	2.9	b	NA		61.6	b
Month 2	Muck	0.3	8.8	a	35.9	a	46.8	a	310.4	a
	Mineral	0.2	12.3	a	7.2	b	10.4	b	101.5	b
	Mineral	0.33	12.5	a	NA		10.4	b	85.6	b
Month 4	Muck	0.3	10.3	a	NA		31.8	a	299.3	a
	Mineral	0.2	14.6	a	3.0	a	10.8	a	90.6	b
	Mineral	0.33	11.9	a	2.0	a	9.5	a	82.0	b

Mean separation within column ($P < 0.05$, LSD, n=40, NA=not available).

Table 2. The effect of soil type and plant spacing on anthocyanin and anthocyanidin (µg/g) concentrations in the skin of 'Michigan Purple' potatoes at harvest and after 2 and 4 months of storage at 10 °C.

Treatment		HPLC peak	Months in storage					
Soil	Planting space (m)		0		2		4	
Muck	0.3	1	13.1	a	8.8	a	10.26	a
		2	12.4	a	10.8	a	NA	
		3	57.42	a	46.8	ab	35.3	b
		4	362.3	a	310.4	ab	332.6	b
Mineral	0.2	1	5.4	b	12.3	a	16.2	a
		2	1.5	a	2.2	a	3.3	a
		3	4.8	a	10.4	a	12.0	a
		4	60	a	101.5	a	100.6	a
Mineral	0.33	1	5.5	b	13.9	a	13.2	ab
		2	3.0	a	NA		2.3	a
		3	NA		10.4	a	10.5	a
		4	61.6	a	85.6	a	91.4	a

Mean separation within column ($P < 0.05$, LSD, n=40, NA=not available).

Table 3. The effect of different soil types on the total pigments (anthocyanin and anthocyanidin) concentration, total absorbance at 530 nm, hue (h°), lightness, and chroma of the skin of 'Michigan Purple' tubers stored at 10 °C.

Treatments		Months in storage				
		0	1	2	3	4
Soil	Planting space (m)					
		Anthocyanins and anthocyanidins (μg/g)				
Muck	0.3	460.1 A a	NA	397.8 A b	NA	368.1 A b
Mineral	0.2	66.7 B a	NA	125.7 B a	NA	118.8 B a
Mineral	0.33	70.2 B a	NA	105.0 B a	NA	103.0 B a
		Absorbance at 530 nm (AU x 1000)				
Muck	0.3	35990 A ab	NA	44270 A b	NA	25380 A b
Mineral	0.2	3646 B a	NA	6616 B a	NA	8566 B a
Mineral	0.33	3727 B a	NA	5697 B a	NA	5432 B a
		Hue				
Muck	0.3	349.3 C b	358.9 C b	1.3 C b	5.3 C a	6.0 C a
Mineral	0.2	61.4 A b	67.6 A a	67.6 A a	66.8 A a	67.2 A a
Mineral	0.33	52.8 B b	61.0 B a	60.9 B a	61.5 B a	60.5 B a
		Lightness				
Muck	0.3	39.0 B d	40.6 B c	41.6 B ab	41.5 C b	42.08 B a
Mineral	0.2	50.1 A a	49.25 A b	49.3 A b	48.9 A b	49.07 A b
Mineral	0.33	50.2 A a	48.90 A b	48.8 A b	47.8 B c	48.40 A b
		Chroma				
Muck	0.3	13.8 A a	11.3 C b	10.7 C c	10.5 B cd	10.4 C d
Mineral	0.2	13.5 AB c	14.2 A b	14.1 A b	14.3 A a	14.5 A a
Mineral	0.33	12.8 B c	13.4 B b	13.4 B b	13.8 A a	13.7 B a

Small letters refer to mean separation within a row; capital letters refer to mean separation within a column ($P < 0.05$, LSD, n=40).

Table 4. The anthocyanins concentration and color of the skin of 'Michigan Purple' tubers as a function of nitrogen source and rate, and storage duration at 4 °C. Treatments were 1 = 200 kg.ha^{-1} N, 2 = 300 kg.ha^{-1} N, 3 = 200 kg.ha^{-1} poultry manure and 4 = 200 kg.ha^{-1} slow releasing N.

Treat-ment	Months in storage																							
	0			1			2			3			4			5			6					
	Anthocyanins (µg/g)																							
1	1980	A	b	---			2170	B	b	---			2410	A	b	---			2920	A	a			
2	2090	A	b	---			2480	AB	a	---			2480	A	a	---			2460	B	a			
3	2170	A	b	---			1800	C	d	---			2540	A	a	---			2030	C	c			
4	2100	A	b	---			2670	A	a	---			2700	A	a	---			2640	AB	a			
	Hue																							
1	4.46	A	e	14.13	A	d	24.57	A	c	29.65	A	b	32.83	A	a	32.85	A	ab	33.57	A	a			
2	357.9	AB	d	4.98	B	c	20.38	AB	b	25.90	AB	a	27.47	AB	b a	27.45	AB	a	27.91	AB	a			
3	354.34	B	e	5.11	B	d	13.49	B	c	18.99	B	b	22.33	B	a	23.06	B	a	25.56	B	a			
4	354.94	B	d	3.91	B	c	16.96	AB	b	21.20	AB	a	22.64	B	a	24.13	AB	a	24.43	B	a			
	Lightness																							
1	43.65	AB	b	39.51	A	d	42.65	AB	c	44.88	AB	a	45.03	A	a	44.92	AB	a	44.57	A	ab			
2	44.4	A	bc	39.69	A	d	43.72	A	c	45.72	A	a	45.63	A	a	45.48	A	a	45.00	A	ab			
3	42.79	B	b	39.07	A	d	41.69	B	c	43.94	B	a	44.08	A	a	43.81	B	a	43.61	A	ab			
4	43.21	AB	b	39.32	A	c	42.82	AB	b	44.89	AB	a	44.65	A	a	44.77	AB	a	44.38	A	a			
	Chroma																							
1	11.55	B	b	12.99	B	a	11.54	A	b	10.30	A	c	10.43	A	c	10.50	A	c	10.73	A	bc			
2	12.46	B	b	13.63	AB	a	11.31	A	c	10.10	A	d	10.30	A	d	10.32	A	d	10.63	A	cd			
3	13.05	AB	b	13.77	AB	a	11.77	A	c	10.49	A	d	10.47	A	d	10.49	A	d	10.58	A	d			
4	13.92	A	a	14.2	A	a	11.89	A	b	10.66	A	c	10.77	A	c	10.70	A	c	10.91	A	c			

Small letters refer mean separation within rows; capital letters refer to mean separation within columns ($P < 0.05$, LSD, n=20).

APPENDIX C

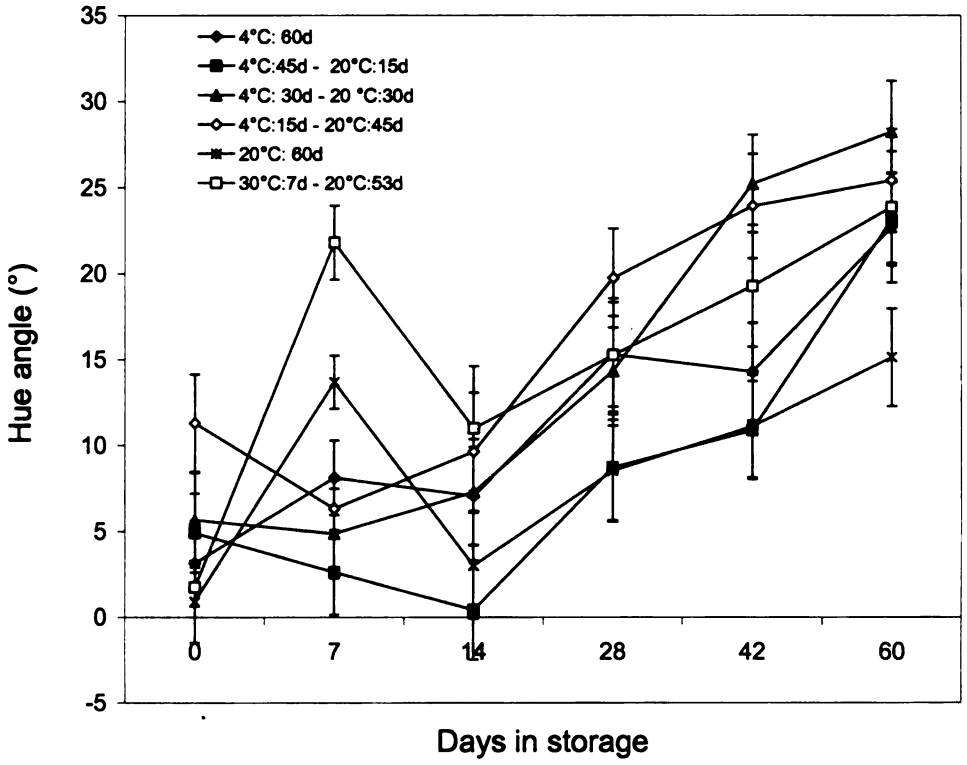

Figure 1. The effect of 60 days storage at different temperatures on the hue angle of 'Michigan Purple' potato tuber skins. Vertical bars refer to standard errors of the means (n=30).