THESIS

INFLUENCE OF STORAGE TEMPERATURE AND TIME IN STORAGE ON PIGMENT CONTENT OF POTATO (*SOLANUM TUBEROSUM L.*)

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MEKHLED M. ALENAZI ENTITLED INFLUENCE OF STORAGE TEMPERATURE AND TIME IN STORAGE ON PIGMENT CONTENT OF POTATO *(SOLANUM TUBEROSUM L.)* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

INFLUENCE OF STORAGE TEMPERATURE AND TIME IN STORAGE ON PIGMENT CONTENT OF POTATO (*SOLANUM TUBEROSUM L.*)

Anthocyanins are responsible for different colors in many plant species including potato tubers. They are also one of the important components in human health as antioxidants. Important factors that can affect anthocyanin concentration in potato tubers are storage temperature and the length of storage. The influence of storage temperature and time in storage on tuber anthocyanin concentration was investigated in seven potato genotypes. These genotypes were cultivars All Blue and Yukon Gold as well as VC0967-SR/Y, Purple Majesty (CO94I6S-3P/P), Mountain Rose (CO94183-1R/R), VC1002-3W/Y and CO97232-2R/Y. Tubers of the seven genotypes were stored at 4°C and 10°C for 0, 4, 6, 8, 10, 12, 16, 20 and 24 weeks. Both fresh and freeze-dried samples of the tubers were evaluated for each of the temperature and time treatment combinations. Extractable anthocyanins were obtained in only three genotypes, CO94183-1R/R, CO94165-3P/P and "All Blue", as determined by the UV/Vis Molecular Device Spectra Max Plus 384 spectrophotometer. There was an increase in anthocyanin concentration with increased time in storage for both fresh and freeze-dried samples ($P<0.0001$). However, tubers stored in the cooler at (4°C) had much higher levels of anthocyanin than those tubers stored at 10°C. Increased levels of anthocyanins in cold-stored tubers are likely associated with the conversion of starch to sugar (so called "cold sweetening") and subsequent conversion to anthocyanins. More anthocyanins were extracted from freeze-dried tuber samples than fresh samples. Both techniques exhibited a similar trend in that an increase in length of time in storage had a similar level of increase in anthocyanin concentration. Extraction of anthocyanins from freeze-dried tissue is more efficient and effective than using fresh tissue for evaluation of anthocyanin concentration in potato tubers.

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CHAPTER 1

Introduction

Potato (***Solanum tuberosum L.***) is the most important and popular vegetable crop in the United States and the world. Today, the United States produces approximately 208,700 metric tons annually on 0.5 million hectares (Greaser, 2001). Potatoes are considered nutritious and versatile, providing an energy source from carbohydrates, as well as protein and important vitamins B6 and C, as well as the minerals potassium, copper and manganese. When potatoes are consumed with their skin, they provide substantial fiber. The potato has no fat or cholesterol (Table 1) and contains 75-80% water (Rastovski, 1989).

The average American consumes about 57 Kg of potatoes per year. They are consumed in higher quantities than any other vegetable, making them one of the good sources of vitamin C in the American diet (Potatoes-Why, 2000).

Table1. Relative nutritional value of 100 gms of potato tubers.

Food Value	Relative % of TotalWt.	Minerals and Vitamins	Mg
Moisture	75.0	Calcium	10
Protein	2.0	Phosphorus	40
Fat	0.10	Iron	0.7
Minerals	0.60	Vitamin C Small amounts of Vitamin B Complex, Vitamin A and P	17
Fiber	0.40	Total phenolics	136-179
Carbohydrates	23.0		
	100		

(The source for Alterative medicines and Holistic health. 2000, Indian Gyan)

Types of Potatoes

The potato tuber varies in type, color and shape (Figure 2). Potatoes are categorized as waxy, starchy, and all-purpose cultivars (Nonnecke, 1989). Waxy types are better for boiling while the starchy types are best for baking. The all-purpose cultivars fall somewhere in between these two types.

Potatoes are also differentiated according to age, with new potatoes being sold soon after harvest. New potatoes generally have higher moisture and sugar content and cook quickly. They have a delicate, sweet flavor. Mature potatoes have thick skins, and may have been kept in cold storage for up to a year before being sold.

The most common potato cultivars grown in the United States are russets, long whites, round reds, and round whites (Greaser, 2001). 'Russet Burbank' is the primary baking potato. 'Russet Burbank' is the number one baking potato with sturdy brown skins with mesh-like netting on the surface, and starchy flesh. They are also good for the fresh market and for processing into frozen or fried products.

Long whites are most often the White Rose cultivar (Greaser, 2001). The newly harvested long whites are thin-skinned and waxy, and the mature ones are starchy.

Reds are smooth-skinned, usually 'Red LaSoda' or 'Red Pontiac' (Figure 1, 2). When more mature, they are waxy and good for boiling. Round whites are most often have a light tan skin.

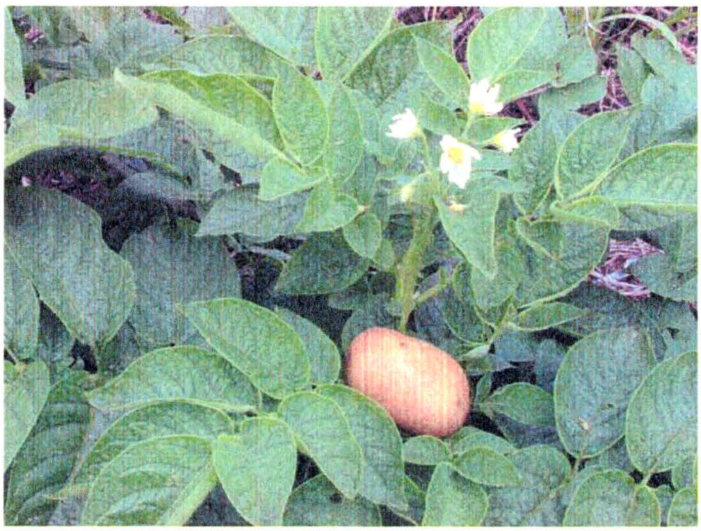


Figure 1. The red tuber is one type of colored potatoes.

The cultivar Yukon Gold has a light yellow flesh. It was developed at the University of Guelph, Ontario, Canada in 1989. Yukon Gold can be used for boiling, baking and for processing (Nonnecke, 1989).

Blue and purple potatoes (Figure 2) have a flesh that ranges from dark blue to light lavender. They are usually found in natural food stores (Brown, 2001).

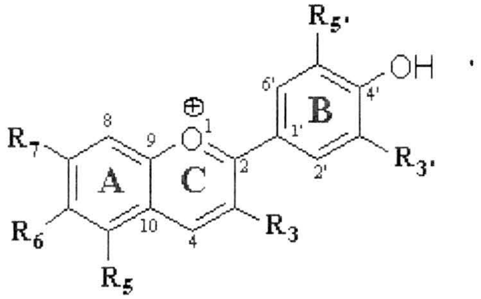
Figure 2. Potato tubers illustrating the various color types.

Anthocyanins

Anthocyanins are naturally occurring compounds that impart color to fruits, vegetables, and plants in general (Wrolstad, 2001). Anthocyanins are the pigments that give blueberries their blue color, as well as the red color in strawberries and red skinned potatoes. Anthocyanins play a major role in the high antioxidant activity levels found in red and blue fruits and vegetables (Wrolstad, 2001).

There are approximately 300 different anthocyanins that have been discovered to date. Anthocyanins belong to the group of plant compounds known as flavonoids (Wrolstad, 2001). They are being studied as possible anticancer agents. They are also being investigated for their ability to inhibit the development of low-density lipoproteins (LDL - bad cholesterol).

Each fruit and vegetable has its own anthocyanin profile; giving it a distinct 'fingerprint' (Anthocyanins, wholefood.com, 2003). Chmielewskab (1936) was the first one to do a detailed examination of anthocyanins in the cultivated potato. He studied the pigments in the purple-black cultivar called "Negresse". The first anthocyanin to be chemically defined in potato tubers was [Mv-3-(pcoumaroyl-rutinoside)-5-glucoside]. The six common anthocyanidins present in most plants (Figure 3) have been found in ***Solanum*** species. These six are pelargonidin (Pg), cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and malvidin (Mv) (Harborne, 1960).



anthocyanidin	R at 3,5,6,7,3',5'	color	Extinction Coefficient (E)	Reference
pelargonidin (Pg)	OH OH H OH H H	orange	1.78E+04	Schou(1927)
cyanidin (CY)	OH OH H OH OH H	orange-red	2.46 E+04	Schou(1927)
delphinidin (Dp)	OH OH H OH OH OH	blue-red	3.47 E+04	Schou(1927)
peonidin (Pn)	OH OH H OH OMe H	orange-red	1.13E+04	Somers(1966)
petunidin (Pt)	OH OH H OH OMe OH	blue-red	1.29E+04	Somers(1966)
malvidin (Mv)	OH OH H OH OMe OMe	blue	1.07E+04	Schou(1927)

Figure 3. Structure of anthocyanidins and the six common anthocyanins found in most plants (Lancashire, 1999).

Health Benefits of Anthocyanins

Anthocyanins are the active compound in many folk remedies. Bilberry (***Vaccinium myrtillus***) is an example, which was used for developing the night vision of British pilots in the World War II. Anthocyanins are probably the most important group of visible plant pigments with the exception of chlorophyll. They have been found in studies to have the strongest anti-oxidizing power of over 150 flavonoids tested (Elliot, 1992). In one study, the U.S. Department of Agriculture examined berries of different species and cultivars for their capacity to protect against oxidative damage. They found that different species have different amounts of anthocyanins. Blackberries had the highest antioxidative capacity against superoxide radicals and hydrogen peroxide (Wang et al., 2000). The relative amount of anthocyanins differed in different cultivars of the same species. The cultivars with the highest anti-oxidative capacity against superoxide radicals such as hydrogen peroxide and other oxidants are 'Hull' and 'Thornless' blackberries, 'Jewel' raspberry, 'Early Black' cranberry, and 'Elliot' blueberry (Wang et al., 2000).

Anthocyanin has been shown to have beneficial affect on human eyesight. In a French study, the anthocyanin in blueberries had a significant affect on eyesight for several hours after supplementation (Sterling, 2001). A recent Japanese study found that people taking 50 mg of black currant anthocyanins adapted better to the dark and had less eye fatigue than controls (Nakaish et al., 2000).

Research at the University of Connecticut found that table grapes protected the heart and blood vessels against oxidative tissue damage similar to red wine (Bernardino, 2002). This is likely because table grapes and red wine have many of the same polyphenols such as catechins and others that act in fighting diseases. The researchers tested the grapes in a model that mimics what happens during a heart attack - deprivation and re-introduction of blood to the heart and blood vessels that leads to inflammation, blood loss and permanent tissue damage. The damage is due partly to a dramatic rise in free radicals that overwhelm the heart's normal antioxidant mechanisms. The consumption of grapes leads to better blood flow and heart pumping capacity (Bernardino, 2002).

Strawberries are also high in antioxidants such as ellagic acid, and in anthocyanins, which give the fruit its red color. Compared with people who do not eat strawberries, those that do eat them have higher blood folate levels and lower blood homocysteine levels and lower blood pressure (Spiller, 2003).

At Oregon State University, researchers succeeded in producing a purple-fruited tomato that has high levels of anthocyanins (Savonen, 2004). Domestic varieties of tomatoes grown and consumed in the United States do not normally produce anthocyanins. The red color is derived from the carotenoid lycopene. This purple tomato resulted from crossing a domestic tomato plant with a genetic stock of tomato that included a gene incorporated from a wild relative with anthocyanin-containing fruit and the *Aft* gene. The result was a domestic-type tomato fruit containing the gene for the purple anthocyanin pigment.

Colored-fleshed potatoes may provide health benefits as well as a tasty flavor. They could increase the overall nutrition for consumers as well as serve as antioxidants in their diet. Brightly colored red, orange and purple-fleshed potatoes might one day provide health-promoting properties beyond those present in today's mostly white and cream-colored tubers (Brown, 2001).

Storage Influence on Anthocyanins

One of the important factors that can effect anthocyanin concentration in all fruit and vegetables is length of storage and the temperature during storage. Studies have found that the anthocyanin concentration in colored potatoes increased at lower temperature (4 °C) but showed little or no change in storage at high temperatures (Lewis, 1999).

Storage conditions have been found to influence anthocyanins in other vegetables and some fruits. Fresh strawberries (***Fragaria x ananassa Duch.***), raspberries (***Rubus idaeus*** Michx.), highbush blueberries (***Vaccinium corymbosum* L.**) as well as lowbush blueberries (***Vaccinium angustifolium*** Aiton) were stored at 0, 10, 20 and 30 °C for up to eight days (Kalt et al., 1999). The storage temperature influenced whole fruit antioxidant capacity, total phenolics, anthocyanin and ascorbate content. An increase in antioxidant capacity of strawberries and raspberries occurred during storage at temperatures >0 °C, but at 0 °C there was less of an increase.

A study to investigate the effect of different storage temperatures on antioxidant levels and aroma compounds (volatiles), as well as the overall quality of strawberries, found that antioxidant levels, anthocyanins and total phenolic content, increased during storage at 0, 5 and 10 °C. At 0 °C storage the antioxidant capacity, anthocyanins, and total phenolic did not increase as much as the volatiles observed at 5 and 10 °C. Strawberries, stored at 10°C, had the greatest amount of both antioxidants and aroma compounds (Ayala-Zayala et al., 1999). However, the strawberries fruits stored at 0 °C maintained a better overall quality for the longest storage duration.

Carrots contain carotenes and phenolics, such as isocoumarins and anthocyanins, which influence flavor and color (Heredia, et al., 2001).These phenolic compounds increase the health and nutritional properties of human diets in which they are included. Research was done to find the effect of physiological stress such as application of ethylene, methyl jasmonate and storage temperature on the phenolic compound synthesis and/or accumulation in maroon carrots (Heredia, et al., 2001). They treated the maroon carrots with air as a control, 1000 ppm ethylene, and methyl jasmonate, and stored the carrots at 0,10, and 20 °C (transferring back and forth between 0 °C and 20 °C). Total carotenes, phenolics, anthocyanins and isocoumarins were evaluated spectrophotometrically. Titratable acidity, total soluble solids and pH were evaluated as quality parameters. In the ethylene treatment, they found an increase in carotenes at 0 °C while an increase in isocoumarins were found at all temperatures studied. They also found an increase in carotenes, anthocyanin and acidity with methyl jasmonate at alternating temperatures only. The treatments of ethylene, methyl jasmonate and higher temperatures increased the phytochemicals isocoumarins, carotenes and phenolics.

Purple or red–fleshed potatoes are a good source of anthocyanins, and a high accumulation of these pigments would increase the nutritional and commercial value of this product (Reyes et al., 2001). Four cultivars of purple and red potatoes were stored in 4 L jars for three weeks and subjected to 1000 ppm ethylene, methyl jasmonate and air as a control at temperatures of 2 °C or 20 °C to determine the effect of abiotic stresses on the accumulation of anthocyanin and phenolic compounds in these potatoes. Total anthocyanins and phenolics were determined spectrophotometrically. Results showed that the amount of anthocyanins in the genotypes RC2003-2, Penn Purple and NDC4089-

4 did not increase or decrease with methyl jasmonate or ethylene treatments when grown in Texas. However, "All Blue" grown in Texas increased by 34 - 53 % with ethylene treatment and 85- 96 % in the methyl jasmonate treatment. In addition, "All Blue" grown in Colorado and treated as before did not show a significant increase of anthocyanins when treated with ethylene. Texas samples had a low level of anthocyanin initially when compared to those grown in Colorado; this may be why they responded differently. Those grown in Colorado may have already reached their maximum potential for anthocyanin accumulation. However, the study demonstrated that pigmented cultivars of potatoes have a mechanism for synthesis of anthocyanins (Reyes et al., 2001) that appears to be environmentally influenced.

The objective of this study was to evaluate the influence of temperature storage and length of time in storage on the anthocyanin concentration in seven genotypes of pigmented potatoes Mountain Rose (CO 94183-1R/R), "All Blue", Purple Majesty (CO 94165-3P/P), VC1002-3W/Y, VC0967-5R/Y, VC97232-2R/Y and "Yukon Gold". In addition, a comparison of fresh weight vs. freeze-dried sample extraction procedures relative to anthocyanin content was evaluated.

CHAPTER 2

Materials and Methods

Potato tubers where grown in the San Luis Valley of Colorado under standard growing conditions and were harvested and maintained at 10-13 °C for approximately three weeks prior to initiation of this research. Seven genotypes (Table 1) were used in this study. The identity of specific anthocyanins in Mountain Rose (CO 94183-1R/R) and Purple Majesty (CO 94165-3P/P) that comprised the major pigments were determined by Liquid Chromatography Mass Spectrometry (LC/MS) in a laboratory of the New Zealand Institute for Crop and Food Research Limited New Zealand. This information was provided by C. Stushnoff (2005) from a collaborative research project with Paula Wilson CRI, NZ, confidential report No. 1377. Pigment composition of 'All Blue' was based upon reversed-phase High Performance Liquid Chromatography HPLC, Department of Plant Microbial (Lewis et al., 1998).

Table 2. The characteristics of the genotypes used on this study.

The specific anthocyanin characterization is based on Stushnoff et al., 2005 for Mountain Rose (CO 94183-1R/R) and Purple Majesty (CO 94165-3P/P) and Lewis et al., 1998 for 'All Blue'. *

Genotypes	Color of Skin	Color of Flesh	Kind of Anthocyanins *
1- CO 94183-1R/R	Red	Red	1- **Pn-3-(pC-rut)-5-glu** (Peonidin-3-rutinoside-5-glucoside+coumaric acid 2- **Pg-3-(pC-rut)-5-glu** (Pelargonidin-3-rutinoside-5-glucosid)+coumaric acid
2- 'All Blue'	Blue	Blue	1- **Pt-3-(pC-rut)-5-glu** (Petunidin-3-(p-coumaroyl-rutinoside)-5-glucoside) 2- **Mv-3-(pC-rut)-5-glu** (Malvidin-3-(p-coumaroyl-rutinoside)-5-glucoside)
3- CO 94165-3P/P	Purple	Purple	1- **Pt-3-(pC-rut)-5-glu** (Petunidin-3-rutinoside-5-glucoside+coumaric acid 2- **Mv-3-(pC-rut)-5-glu** (Malvidin-3-rutinoside-5-glucoside+coumaric acid
4- 'Yukon Gold'	Yellow	Yellow	
5- VC1002-3W/Y	White	Yellow	
6- VC0967-5R/Y	Red	Yellow	
7- CO97232-2R/	Red	Yellow	

Tubers were randomly separated into three tubers/sample and placed in paper bags and stored at 4 °C and 10 °C for varying intervals. The anthocyanin content was determined when tubers were initially placed in each storage temperature regime and after 4, 6, 8, 10, 12, 16, 20 and 24 weeks. Each tuber (three per treatment combination) was sampled fresh and freeze-dried. Samples of approximately 10 g fresh weights were freeze-dried in a Virtis cabinet freeze drier. Sliced samples were freeze-dried for five days. The shelf temperature of the freeze-drier was –25 °C and the coil temperature was –

65 °C. After freeze-drying, samples were processed in a similar matter as described for fresh tuber samples except that 2 g of the freeze-dried tissue was used.

Fresh samples of 10 g/tuber were homogenized with 38 ml of anthocyanin solvent (85:15 of 95% ETOH /1.5N HCl). They were covered for four hours at 3-4 °C and then centrifuged at 7000 rpm and 2 °C for 15 minutes in a Biofuge 17R; 2752 model. Two grams of the samples were then homogenized with 38 ml of anthocyanin solvent (85:15 95% ETHO 1.5N HCl) for the final solution. Note pigmentation of solution samples in Figures 4a and 4b.

The solvent was transferred into a 3.5 ml quartz cuvette and the absorbance measured at 350 & 750nm in a UV/Vis Molecular Devices Spectra Max plus384. Equipped with temperature regulated cuvette and microplate. The reading of the anthocyanin concentrations was done using SOFTmax pro version 3.1. Four read modes are possible using SOFTmax pro. Three may be performed either in a microplate (plate section) or in a cuvette (cuvette section). A single reading is made at one or more wavelengths (up to six separate wavelengths can be chosen using the spectra max instrument). Default values reported are optical density (OD).

The Beer-Lambert Law was used to calculate anthocyanin concentrations: C= A/LE where "C" is the concentration and "A" is the absorbance of the substance in solution, "L" is the length of light both through the solution (1cm) and cuvette while "E" is the extinction coefficient of the pigment at that particular absorbance wavelength (Bassey, 1993).

Example of the anthocyanin concentration calculation: CO94183-1(R/R) tubers, fresh sample, Initial, 4 °C): Anthocyanin concentration (C) = .20 / 1.13E+4M^{-1} cm^{-1} x 1cm=1.77E-05 Molar. Results were analyzed using two-way ANOVA with Bonferroni mean separation test and Multiple Regressions.

Figure 4a. The extract of line CO94183-1R/R, 'All Blue' and CO94165-3P/P respectively from the left.

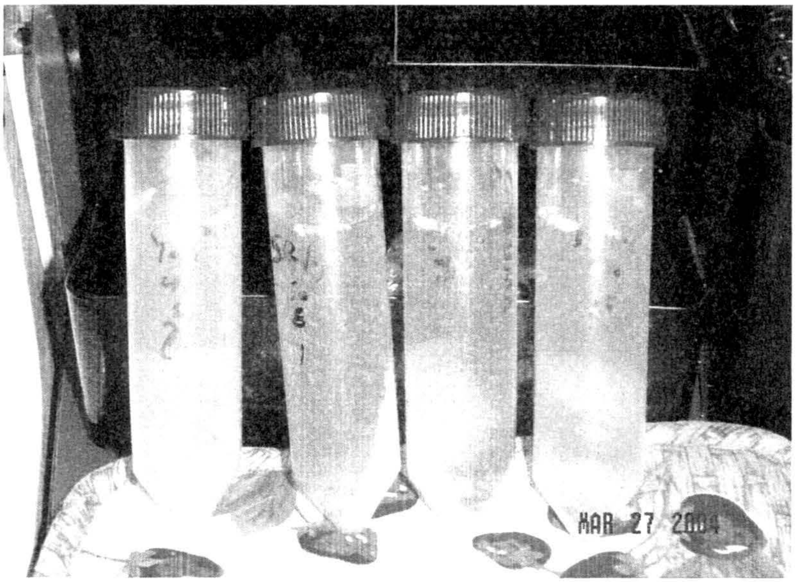


Figure 4b. The extract of 'Yukon Gold', VC0967-5R/Y, CO97232-2R/Y and VC1002-3W/Y respectively from the left.

CHAPTER 3

Results

Only three of the seven lines tested [CO94183-1R/R, 'All Blue' and CO94165-3P/P] had anthocyanins that could be extracted by the technique used. Based on previous research by Stushnoff et al. (2005) and Lewis et al. (1998), red fleshed tubers have Pn-3-(pC-rut)-5-glu as well as Pg-3-(pC-rut)-5-glu, while 'All Blue' tubers and CO94165-3P/P have Pt-3-(pC-rut)-5-glu and Mv-3-(pC-rut)-5-glu. At a storage temperature of 4 °C, the anthocyanin concentration of fresh samples of CO94183-1R/R increased at each storage time interval evaluated (Table 3 and 13). At a storage temperature of 10 °C, there was also an increase in anthocyanin level with length of time in storage (Table 3 and 14). However, that increase difference was less (3.01E- 05m) at 10 °C as compared to (5.75E-05m) at 4 °C after 24 weeks in storage (Figures 6 and 7). In 'All Blue' tubers, both Pt-3-(pC-rut)-5-glu and Mv-3-(pC-rut)-5-glu increased in 4 °C storage over time. However, tubers stored at 10 °C did not have as great an increase in concentration (2.82E-05 and 3.40E-05m) as compared to 4 °C (6.82E-05 and 7.57E-05m) storage temperatures (Figure 8 and 9). The CO94165-3P/P genotype had similar results as 'All Blue', Table 3 and Figures 10 and 11.

Freeze-dried tissue samples of tubers were also analyzed for anthocyanin concentration. In this study, the concentration of the anthocyanin in CO94183-1R/R was high at 4 °C, 9.62E-05m, as compared to storage at 10 °C, 6.96E-05m, after 24 weeks in storage, see Table 4, 11 and 12 (Figures 12 and 13). In "All Blue" and CO94165-3P/P tubers, storage at 4 C also had apparently greater anthocyanin concentrations as compared to 10 °C for both cultivars, Table 4 and Figures 14, 15, 16 and 17.

Table 3. Relative anthocyanin concentration (M) as influenced by length of storage at 4 °C and 10 °C for fresh tubers.

Storage Temperature	Genotypes Designation	Pigments	Molar concentration after length of storage (Mx10-05) [1] (Weeks)						
			Initial	STDEV[3]	4	8	16	24 [2]	STDEV[3]
4 °C	CO 94183-1R/R	Pn-3-(pC-rut)-5-glu	1.74	4.17E-07	3.46	4.46	5.16	5.75	0
		Pg-3-(pC-rut)-5-glu	1.10	2.64E-07	2.19	2.83	3.28	3.65	0
	'All Blue'	Pt -3-(pC-rut)-5-glu	1.86	1.66E-06	3.02	4.20	5.66	6.30	7.38E-07
		Mv-3-(pC-rut)-5-glu	2.08	1.76E-06	3.64	4.91	6.82	7.57	1.32E-06
	CO 94165-3P/P	Pt -3-(pC-rut)-5-glu	3.00	3.65E-07	4.44	5.04	6.25	6.80	7.31E-07
		Mv-3-(pC-rut)-5-glu	3.61	4.40E-07	5.36	6.17	7.54	8.19	8.81E-07
10 °C	CO 94183-1R/R	Pn-3-(pC-rut)-5-glu	1.74	4.17E-07	1.74	2.12	2.64	3.01	1.27E-06
		Pg-3-(pC-rut)-5-glu	1.10	2.64E-07	1.10	1.31	1.69	1.91	7.94E-07
	'All Blue'	Pt -3-(pC-rut)-5-glu	1.86	1.66E-06	1.89	2.04	2.66	2.82	1.46E-06
		Mv-3-(pC-rut)-5-glu	2.08	1.76E-06	2.21	2.46	3.04	3.31	6.07E-07
	CO 94165-3P/P	Pt -3-(pC-rut)-5-glu	3.00	3.65E-07	3.00	3.13	3.67	4.15	1.44E-06
		Mv-3-(pC-rut)-5-glu	3.61	4.24E-07	3.58	3.77	4.42	5.08	2.66E-06

[1] Each data point represents an average of 3 samples per treatment combination.

[2] The anthocyanin concentrations at different storage times are in the in the appendix.

[3] Standard deviation for Initial and 24 weeks. Note that the standard deviations are in the appendix.

Table 4. Relative anthocyanin concentration (M) as influenced by length of storage at 4 °C and 10 °C for freeze-dried tubers samples.

Storage Temperature	Genotypes Designation	Pigments	Molar concentration after length of storage (Mx10-5) [1]						
			(Weeks)						
			Initial	STDEV[3]	4	8	16	24 [2]	STDEV[3]
4 °C									
	CO 94183-1R/R	Pn-3-(pC-rut)-5-glu	3.63	1.25E-06	6.75	8.02	8.59	9.66	1.11E-06
		Pg-3-(pC-rut)-5-glu	2.30	7.94E-07	4.33	5.09	5.45	6.10	1.06E-06
	'All Blue'	Pt-3-(pC-rut)-5- glu	3.49	1.67E-06	7.16	8.06	8.79	1.17	8.14E-06
		Mv-3-(pC-rut)-5-glu	4.35	1.01E-06	8.63	9.72	10.6	14.7	1.72E-06
	CO 94165-3P/P	Pt-3-(pC-rut)-5- glu	3.61	6.32E-07	6.45	7.58	8.55	10.7	1.67E-06
		Mv-3-(pC-rut)-5-glu	4.36	8..95E-07	7.82	9.10	10.3	12.9	2.16E-06
10 °C	CO 94183-1R/R	Pn-3-(pC-rut)-5-glu	3.63	1.25E-06	5.04	6.28	6.57	6.96	1.66E-06
		Pg-3-(pC-rut)-5-glu	2.30	7.94E-07	3.20	3.95	4.16	4.42	1.05E-06
	'All Blue'	Pt-3-(pC-rut)-5-glu	3.49	1.67E-06	4.19	5.74	6.95	7.70	7.30E-07
		Mv-3-(pC-rut)-5-glu	4.35	1.01E-06	4.69	6.92	8.26	9.19	1.93E-07
	CO 94165-3P/P	Pt-3-(pC-rut)-5-glu	3.61	6.32E-07	3.93	4.31	5.57	8.20	1.15E-06
		Mv-3-(pC-rut)-5-glu	4.36	8.95E-07	4.74	5.20	6.73	9.94	4.24E-07

[1] Each data point represents an average of 3 samples per treatment combination. Note that the standard deviations are in the appendix.
[2] The other anthocyanin concentrations in the other time of the storage are in the in the appendix.
[3] Standard deviation for Initial and 24 weeks. Note that the standard deviations are in the appendix.

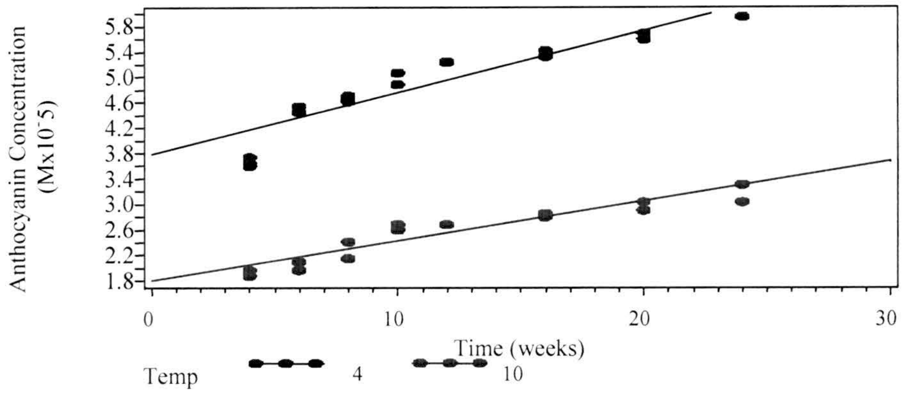


Figure 6. Relative amounts of **Pn-3-(pC-rut)-5-glu** in fresh tissue of CO94183-1R/R potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000036 + 9.725E-7*Time.

Anthocyanin concentration (Temp: 10) = 0.000016 + 6.207E-7*Time

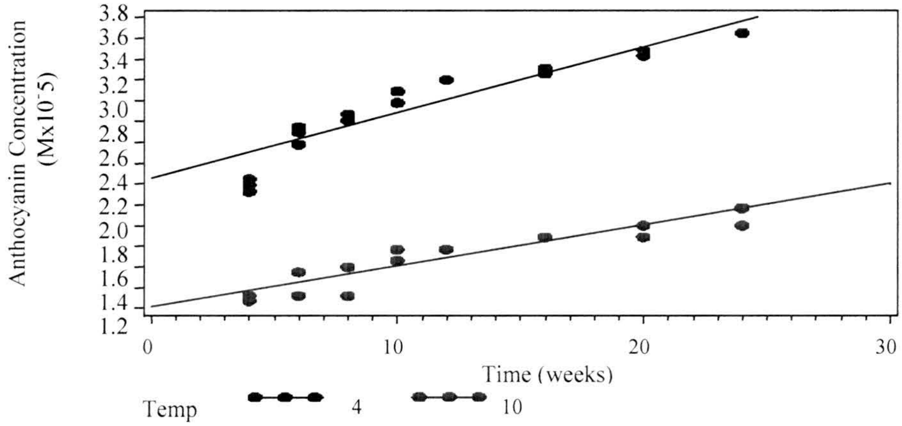


Figure 7. Relative amounts of **Pg-3-(pC-rut)-5-glu** in fresh tissue of CO94183-1R/R potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000023 + 6.268E-7*Time.

Anthocyanin concentration (Temp: 10) = 0.00001 + 3.944E-7*Time.

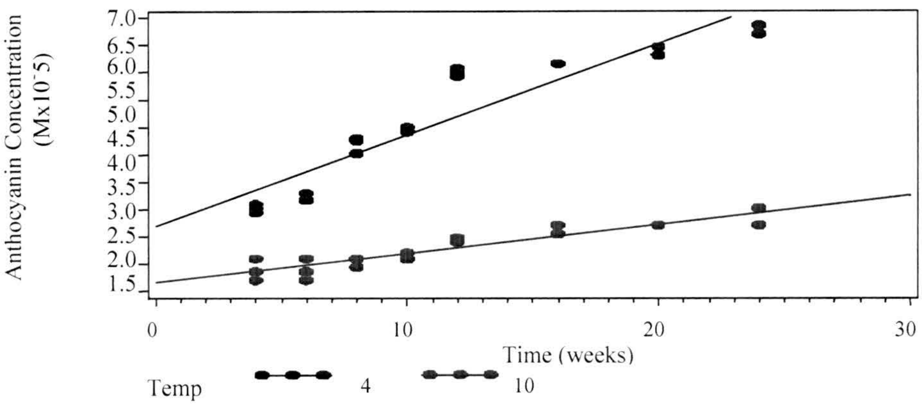


Figure 8. Relative amounts of **Pt-3-(pC-rut)-5-glu** in fresh tissue of 'All Blue' potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000027 + 1.665E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.000017 + 5.243E-7*Time.

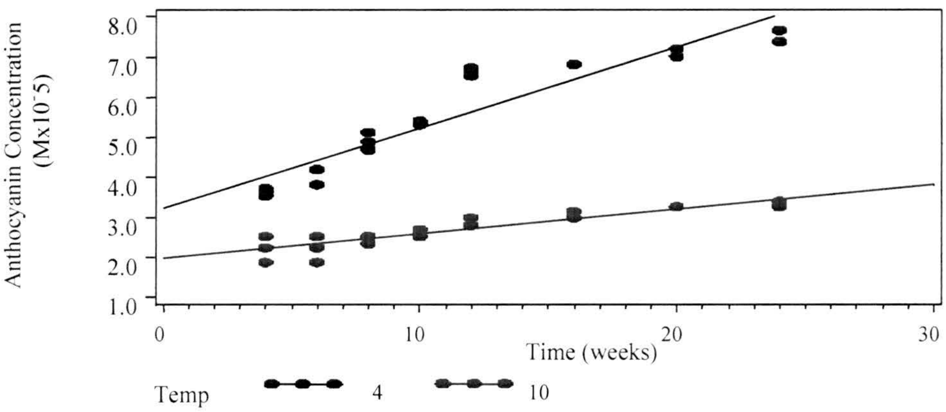


Figure 9. Relative amounts of **Mv-3-(pC-rut)-5-glu** in fresh tissue of 'All Blue' potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000032 + 2.001E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.00002 + 6.183E-7*Time.

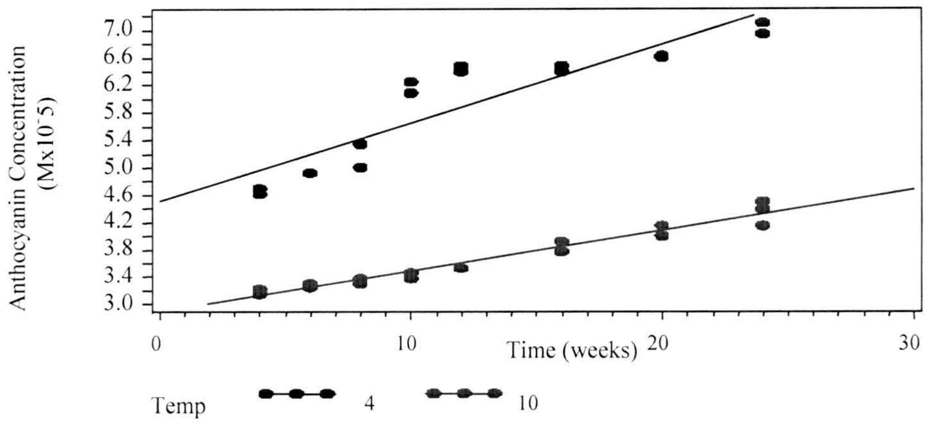


Figure 10. Relative amounts of **Pt-3-(pC-rut)-5-glu** in fresh tissue of CO94165-3P/P potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000043 + 1.139E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.000027 + 5.992E-7*Time.

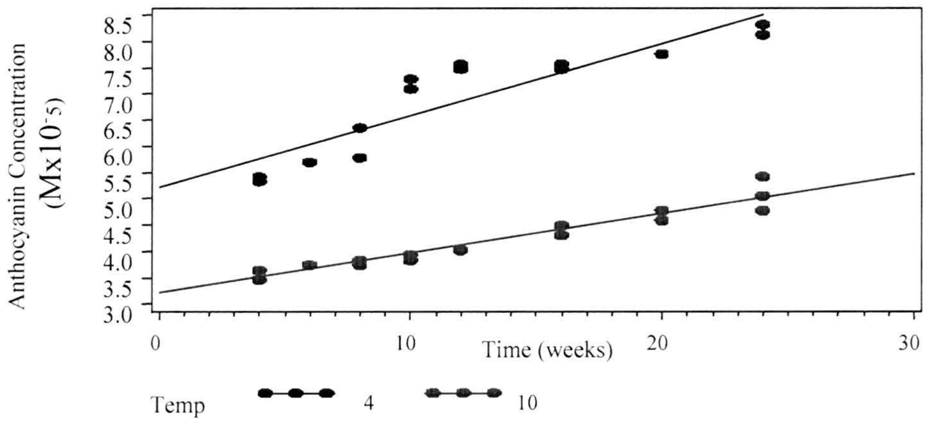


Figure 11. Relative amounts of Mv-3-(pC-rut)-5-glu in fresh tissue of CO94165-3P/P potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000052 + 1.367E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.000032 + 7.531E-7*Time.

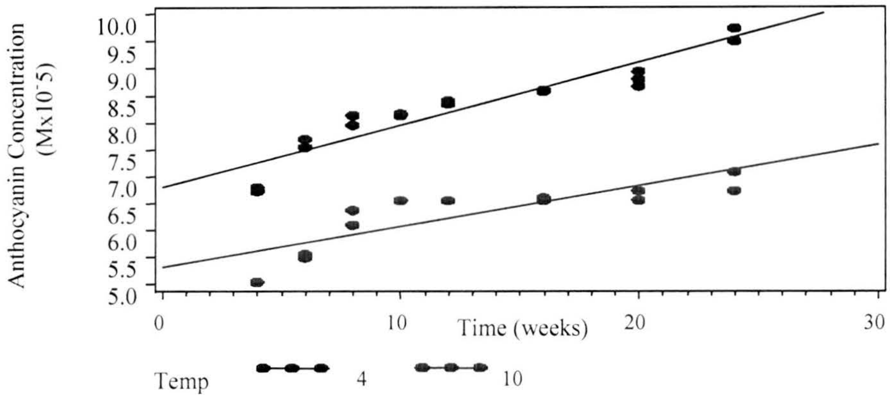


Figure 12. Relative amounts of **Pn-3-(pC-rut)-5-glu** in freeze-dried tissue of CO94183-1R/R potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000068 + 1.149E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.000053 + 7.623E-7*Time.

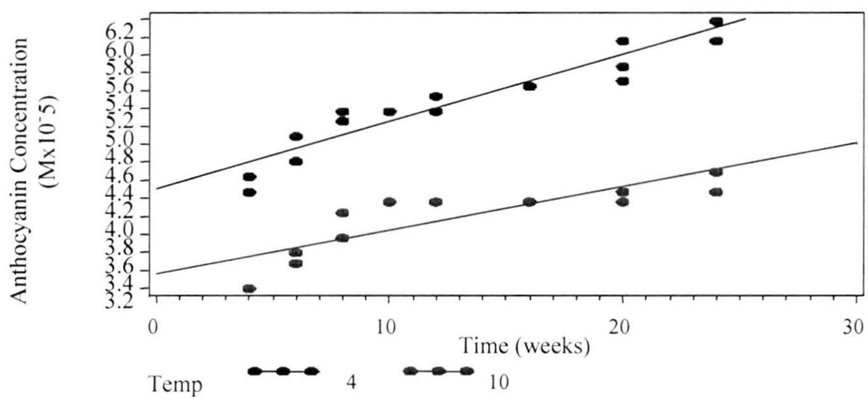


Figure 13. Relative amounts of **Pg-3-(pC-rut)-5-glu** in freeze-dried tissue of CO94183-1R/R potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000043 + 7.513E-7*Time.

Anthocyanin concentration (Temp: 10) = 0.000034 + 4.827E-7*Time.

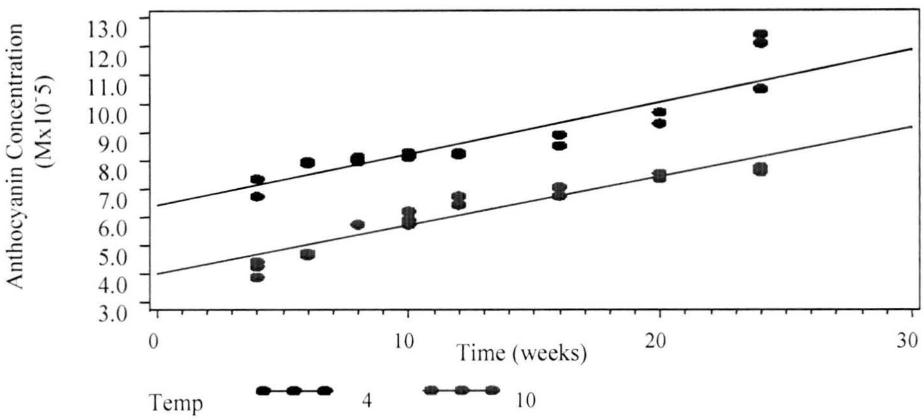


Figure 14. Relative amounts of **Pt-3-(pC-rut)-5-glu** in freeze-dried tissue of 'All Blue' potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000064 + 1.823E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.00004 + 1.706E-6*Time.

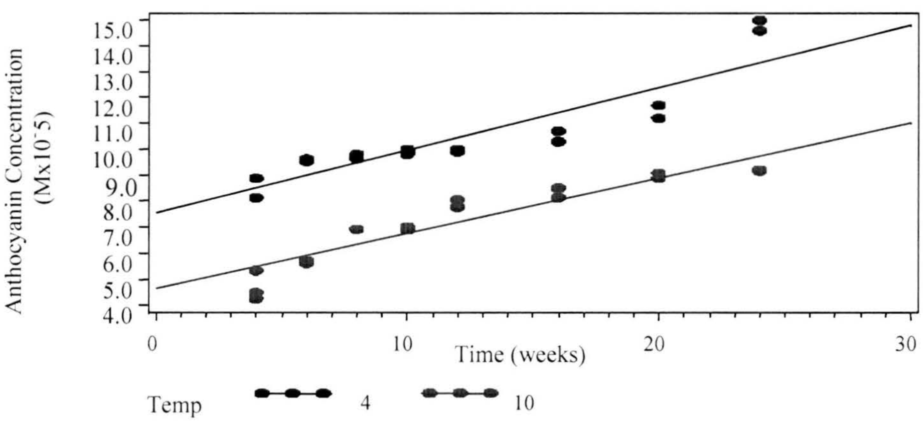


Figure 15. Relative amounts of **Mv-3-(pC-rut)-5-glu** in freeze-dried tissue of 'All Blue' potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000075 + 2.417E-6*Time.

Anthocyanin concentration (Temp: 10) = 0.000047 + 2.119E-6*Time.

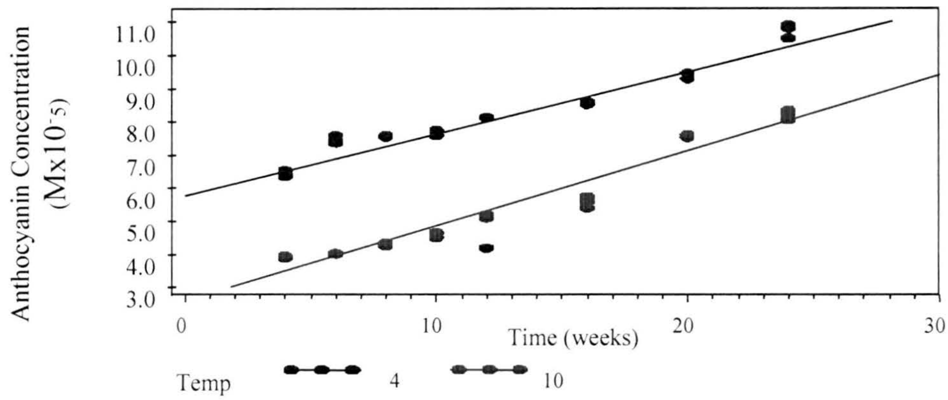


Figure16. Relative amounts of **Pt-3-(pC-rut)-5-glu** in freeze-dried tissue of CO94165-3P/P potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000058 + 1.865E-6*Time.
Anthocyanin concentration (Temp: 10) = 0.000026 + 2.265E-6*Time.

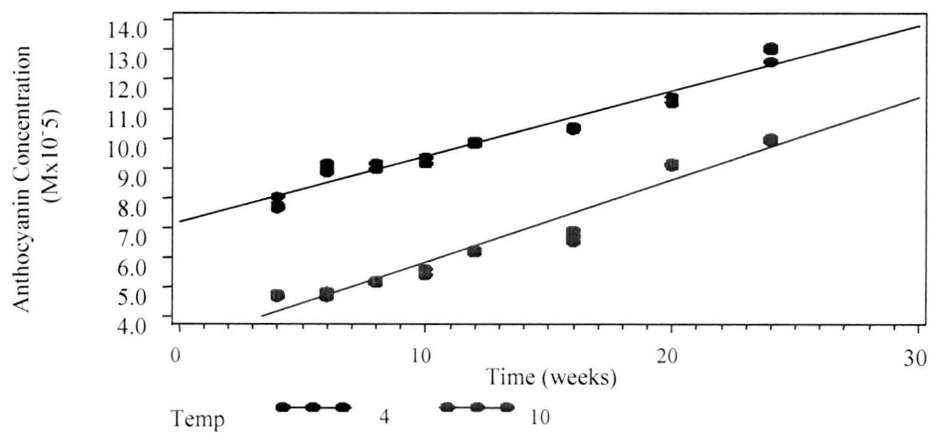


Figure17. Relative amounts of **Mv-3-(pC-rut)-5-glu** in freeze-dried tissue of CO94165- 3P/P potato tubers stored at 4°C and 10°C for different time periods.

Anthocyanin concentration (Temp: 4) = 0.000072 + 2.206E-6*Time.
Anthocyanin concentration (Temp: 10) = 0.000031 + 2.763E-6*Time.

Colored fleshed tubers held at 4 °C had an apparent increase in anthocyanin concentration as compared with the storage temperature of 10 °C. Both fresh and freeze-dried tissue samples of tubers were evaluated for relative anthocyanin concentration as influenced by storage temperature and length of time in storage. Although there were differences in anthocyanins levels in fresh or freeze-dried samples, the increase in anthocyanin level associated with both temperature and time in storage had similar tends. A cooler temperature and longer time in storage resulted in greater concentrations of anthocyanins (Table 5, 6, 7, 8, 9, and 10).

Table 5. A comparison of Pn-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of CO94183-1R/R potato tubers stored at 4 and 10 °C for different times.

Storage Temperature	Processed Types	Molar concentration after length of storage ($Mx10^{-5}$) (Weeks)				
		Initial	4	8	16	24
4 °C	Fresh	1.74	3.46	4.46	5.16	5.75
	Freeze-dried	3.63	6.75	8.02	8.59	9.66
10 °C	Fresh	1.74	1.74	2.12	2.64	3.01
	Freeze-dried	3.63	5.04	6.28	6.57	6.96

Table 6. A comparison of Pg-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of CO94183-1R/R potato tubers stored at 4 and 10 °C for different times.

Storage Temperature	processed Types	Molar concentration after length of storage ($M \times 10^{-5}$) (Weeks)				
		Initial	4	8	16	24
4 °C	Fresh	1.10	2.19	2.83	3.28	3.65
	Freeze-dried	2.30	4.33	5.09	5.45	6.10
10 °C	Fresh	1.10	1.10	1.31	1.69	1.91
	Freeze-dried	2.30	3.20	3.95	4.16	4.42

Table 7. A comparison of Pt-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of 'All Blue' potato tubers stored at 4 and 10 °C for different times.

Storage Temperature	processed Types	Molar concentration after length of storage (Mx10-5) (Weeks)				
		Initial	4	8	16	24
4 °C	Fresh	1.86	3.02	4.20	5.66	6.30
	Freeze-dried	3.49	7.16	8.06	8.79	11.7
10 °C	Fresh	1.86	1.89	2.04	2.66	2.82
	Freeze-dried	4.35	4.69	6.92	8.26	9.19

Table 8. A comparison of Mv-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of 'All Blue' potato tubers stored at 4 and 10 °C for different storage times.

Storage Temperature	processed Types	Molar concentration after length of storage (Mx10-5) (Weeks)				
		Initial	4	8	16	24
4 °C	Fresh	2.08	3.64	4.91	6.82	7.57
	Freeze-dried	4.35	8.63	9.72	10.6	14.7
10 °C	Fresh	2.08	2.21	2.46	3.04	3.31
	Freeze-dried	4.35	4.69	6.92	8.26	9.19

Table 9. A comparison of Pt-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of CO94165-3P/P potato tubers stored at 4 and 10 °C for different time periods.

Storage Temperature	processed Types	Molar concentration after length of storage ($M \times 10^{-5}$) (Weeks) Initial	4	8	16	24
4 °C	Fresh	3.00	4.44	5.04	6.25	6.80
	Freeze-dried	3.61	6.45	7.58	8.55	10.7
10 °C	Fresh	3.00	3.00	3.13	3.67	4.15
	Freeze-dried	3.61	3.93	4.31	5.57	8.20

Table 10. A comparison of Mv-3-(pC-rut)-5-glu concentration in fresh and freeze-dried tissue of CO94165-3P/P potato tubers stored at 4 and 10 °C for different time periods.

Storage Temperature	processed Types	Molar concentration after length of storage ($Mx10^{-5}$) (Weeks)				
		Initial	4	8	16	24
4 °C	Fresh	3.61	5.36	6.17	7.54	8.19
	Freeze-dried	4.36	7.82	9.10	10.3	12.9
10 °C	Fresh	3.61	3.58	3.89	4.42	5.08
	Freeze-dried	4.36	4.74	5.20	6.73	9.94

Discussion

'All Blue', CO94183-1R/R and CO94165-3P/P had significant amounts of anthocyanins. Significant amount of anthocyanin were not observed (Figure 4b) in the other genotypes (VC0967-5R/Y, CO97232-2R/Y, 'Yukon Gold' and VC1002-3W/Y). The yellow color in "Yukon Gold" is due to carotenoid pigments and not anthocyanins. VC0967-5R/Y and CO97232-2R/Y are red skinned and yellow fleshed. Although these genotypes contain anthocyanins in their skin, little pigment was extracted, (Fig 4a). This was because the bulk of the samples contained mostly flesh tissue and little epidermis tissue.

Research by Lewis et al. (1998 & 1999) reported that red-skinned and yellow-fleshed genotypes showed a change in anthocyanin concentration with storage. However, that work used peeled skin (tuber epidermis tissue) in a separate analysis for anthocyanins in tubers. Tuber flesh was shown to have minimal anthocyanins as compared to the skin (Lewis et al., 1998).

The increase in anthocyanin concentrations in cold storage (4 °C) was greater than that in warm storage (10 °C), (Table 11, 12, 13 and 14). This is likely due in large measure to the conversion of greater amounts of starch to sugar at cool temperatures as compared to warm temperatures. This is generally known as "cold sweetening" (Rastovski, 1989). The increase in sugar concentration during cold storage could in turn lead to an increase in anthocyanin synthesis. Sugar is an anthocyanin precursor. In addition, sugar more readily supplies energy for anthocyanin synthesis (Lewis et al. 1999).

Table 11. Comparison of initial pigment content to pigment in stored tubers (4, 6, 8, 10, 12, 16, 20, and 24 weeks) based on two way ANOVA and Bonferroni mean separation test for freeze-dried samples at 4°C. Initial pigment content was compared to content in samples from each storage time.

	Length of time (Weeks)	Mean (Molar)	Kind of pigments					
			pn3r4rr[1]	pg3r4rr[1]	pt3r4ab[1]	mv3r4ab[1]	pt3r4pp[1]	mv3r4pp[1]
4°C	Initial	3.62						
	4	6.85	***	***	***	***	***	***
	6	7.57	***	***	***	***	***	***
	8	7.73	***	***	***	***	***	***
	10	7.92	***	***	***	***	***	***
	12	8.31	***	***	***	***	***	***
	16	8.71	***	***	***	***	***	***
	20	9.34	***	***	***	***	***	***
	24	10.87	***	***	***	***	***	***

*P<0.05, ** P<0.01, *** P<0.001, ns: not significant. [1] Pn= peonidin, Pg= pelargonidin, Pt =petunidin, Mv= malvidin, 3r= three replications, 4= storage temperature (4°C), rr = CO 94183-1R/R cultivar, ab= 'All Blue' cultivar, pp= CO 94165-3P/P cultivar.

Table 12. Comparison of initial pigment content to stored tubers (4, 6, 8, 10, 12, 16, 20, and 24 weeks) based on two way ANOVA and Bonferroni mean separation test for freeze-dried samples at 10°C. Initial pigment content was compared to content in samples from each storage time.

	Length of storage (Weeks)	Mean (Molar)	Kind of pigments					
			Pn3r10rr[1]	pg3r10rr[1]	pt3r10ab[1]	mv3r10ab[1]	pt3r10pp[1]	mv3r10pp[1]
10°C	Initial	3.62						
	4	4.29	*	ns	ns	ns	ns	ns
	6	4.69	***	ns	ns	*	ns	ns
	8	5.39	***	**	***	***	ns	ns
	10	5.62	***	***	***	***	ns	ns
	12	6.08	***	***	***	***	***	***
	16	6.37	***	***	***	***	***	***
	20	7.30	***	***	***	***	***	***
	24	7.73	***	***	***	***	***	***

*P<0.05, ** P<0.01, *** P<0.001, ns: not significant. [1] Pn= peonidin. Pg= pelargonidin. Pt =petunidin. Mv= malvidin. 3r= three replications. 10= storage temperature (10°C). rr = CO 94183-1R/R cultivar. ab= 'All Blue' cultivar. pp= CO 94165-3P/P cultivar.

Table 13. Comparison of initial pigment content to stored tubers (4, 6, 8, 10, 12, 16, 20, and 24 weeks) based on two way ANOVA and Bonferroni mean separation test for fresh samples at 4°C. Initial pigment content was compared to content in samples from each storage time.

	Length of time (Weeks)	Mean (Molar)	Kind of pigments Pn3r4rr[1]	pg3r4rr[1]	pt3r4ab[1]	mv3r4ab[1]	pt3r4pp[1]	mv3r4pp[1]
4°C	Initial	2.232						
	4	3.688	***	***	***	***	***	***
	6	4.094	***	***	***	***	***	***
	8	4.601	***	***	***	***	***	***
	10	5.118	***	***	***	***	***	***
	12	5.696	***	***	***	***	***	***
	16	5.785	***	***	***	***	***	***
	20	6.007	***	***	***	***	***	***
	24	6.377	***	***	***	***	***	***

*P<0.05, ** P<0.01, *** P<0.001, ns: not significant. [1] Pn= peonidin. Pg= pelargonidin. Pt =petunidin. Mv= malvidin. 3r= three replications. 4= storage temperature (4°C). rr = CO 94183-1R/R cultivar. ab= 'All Blue' cultivar. pp= CO 94165-3P/P cultivar.

Table 14. Comparison of initial pigment content to stored tubers (4, 6, 8, 10, 12, 16, 20, and 24 weeks) based on two way ANOVA and Bonferroni mean separation test for fresh samples at 10°C. Initial pigment content was compared to content in samples from each storage time.

	Length of storage (Weeks)	Mean (Molar)	Kind of pigments Pn3r10rr[1]	pg3r10rr[1]	pt3r10ab[1]	mv3r10ab[1]	pt3r10pp[1]	mv3r10pp[1]
10°C	Initial	2.23						
	4	2.83	ns	ns	ns	ns	ns	ns
	6	3.05	ns	ns	ns	ns	ns	ns
	8	3.23	ns	ns	ns	ns	ns	ns
	10	3.37	**	ns	ns	ns	ns	ns
	12	3.38	**	ns	*	***	**	ns
	16	3.83	***	ns	**	***	***	***
	20	4.09	***	*	**	***	***	***
	24	4.47	***	**	***	***	***	***

*P<0.05, ** P<0.01, *** P<0.001, ns: not significant. [1] Pn= peonidin. Pg= pelargonidin. Pt =petunidin. Mv= malvidin. 3r= three replications. 10= storage temperature (10°C). rr = CO 94183-1R/R cultivar. ab= 'All Blue' cultivar. pp= CO 94165-3P/P cultivar.

There are two other reasons for increased concentrations of anthocyanins in potatoes while in storage. This relates to the loss of moisture while in storage as well as the loss of starch (sugars) due to general respiration. If the anthocyanins did not degrade with storage, one would see a relative increase in their amounts on a per weight basis as water was lost and starch was used in respiration. There were greater levels of anthocyanins in 4 °C stored tubers compared to tubers at stored 10 °C. Although moisture loss and respiration may lead to increase anthocyanins on a per weight, it is likely that the there would be reduced respiration and moisture loss at 4 °C. Thus, the observation in the potatoes stored at 4 °C demonstrated a higher level of anthocyanin synthesis.

The freeze-dried and fresh samples both exhibited similar trends in that with greater storage time there was an increase in anthocyanin content ($p<0.0001$), (Tables 15 and 16). Because of ease of processing large numbers of samples, freeze-drying would be most useful in future studies. Large number of tubers can be easily freeze-dried and then held for anthocyanin extraction as time allows. It would be difficult to process these samples from fresh tissue all at the same time.

There differences in anthocyanin levels when comparing fresh and freeze-dried (demonstrated greater amounts) were likely associated with incomplete extraction of tissue from a blender used in fresh samples and varying levels of moisture in tubers.

It is evident from this research that higher levels of anthocyanins occur when tubers are stored at 4 °C. Thus, cold storage of colored tubers would not only preserve their anthocyanins but seems to actually increase their levels.

Table 15. Summary 2 way-ANOVA for anthocyanin pigments and temperatures for fresh samples.

Source of Variation	Df	% total variation	Mean square	F	P-value
Interaction	88	9.73	2.136	7.903	$p<0.0001$
Fresh/temp	11	47.56	83.57	236.6	$p<0.0001$
Time	8	39.59	95.65	353.9	$p<0.0001$

Table 16. Summary 2 way-ANOVA for anthocyanin pigments and temperatures for freeze-dried samples.

Source of Variation	Df	% total variation	Mean square	F	P-value
Interaction	88	8.05	1.334	21.65	p<0.0001
Freeze-dried/temp	11	72.06	95.48	1649	p<0.0001
Time	8	18.98	34.59	561.4	p<0.0001

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Appendix

Table 17. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 4°C of CO94183-1R/R fresh and freeze-dried tubers (Pn-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**1.77E-05**	**3.40E-05**	**4.25E-05**	**4.51E-05**	**4.87E-05**	**5.04E-05**	**5.13E-05**	**5.49E-05**	**5.75E-05**
Rep2	**1.77E-05**	**3.54E-05**	**4.34E-05**	**4.45E-05**	**4.87E-05**	**5.04E-05**	**5.22E-05**	**5.49E-05**	**5.75E-05**
Rep3	**1.68E-05**	**3.45E-05**	**4.25E-05**	**4.42E-05**	**4.69E-05**	**5.05E-05**	**5.13E-05**	**5.40E-05**	**5.75E-05**
Average	1.74E-05	3.46E-05	4.28E-05	4.46E-05	4.81E-05	5.05E-05	5.16E-05	5.46E-05	5.75E-05
Standard deviation	4.17E-07	5.77E-07	4.12E-07	3.72E-07	8.34E-07	2.71E-08	4.17E-07	4.17E-07	0
Freeze-dried									
Rep1	**3.72E-05**	**6.80E-05**	**7.55E-05**	**8.14E-05**	**8.14E-05**	**8.41E-05**	**8.58E-05**	**8.94E-05**	**9.50E-05**
Rep2	**3.72E-05**	**6.73E-05**	**7.70E-05**	**7.96E-05**	**8.17E-05**	**8.35E-05**	**8.58E-05**	**8.67E-05**	**9.73E-05**
Rep3	**3.45E-05**	**6.73E-05**	**7.70E-05**	**7.96E-05**	**8.14E-05**	**8.41E-05**	**8.60E-05**	**8.80E-05**	**9.73E-05**
Average	3.63E-05	6.75E-05	7.65E-05	8.02E-05	8.15E-05	8.39E-05	8.59E-05	8.80E-05	9.66E-05
Standard deviation	1.25E-06	3.50E-07	7.02E-07	8.34E-07	1.33E-07	2.69E-07	7.50E-08	1.08E-06	1.11E-06

Table 18. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of CO94183-1R/R fresh and freeze-dried tubers (Pn-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**1.77E-05**	**1.77E-05**	**1.77E-05**	**1.95E-05**	**2.40E-05**	**2.48E-05**	**2.65E-05**	**2.83E-05**	**3.10E-05**
Rep2	**1.77E-05**	**1.77E-05**	**1.90E-05**	**2.21E-05**	**2.48E-05**	**2.48E-05**	**2.60E-05**	**2.70E-05**	**2.83E-05**
Rep3	**1.68E-05**	**1.68E-05**	**1.77E-05**	**2.21E-05**	**2.48E-05**	**2.48E-05**	**2.65E-05**	**2.83E-05**	**3.10E-05**
Average	1.74E-05	1.74E-05	1.81E-05	2.12E-05	2.45E-05	2.48E-05	2.64E-05	2.79E-05	3.01E-05
Standard deviation	4.17E-07	4.17E-07	6.13E-07	1.23E-06	3.67E-07	0	2.58E-07	6.12E-07	1.27E-06
Freeze-dried									
Rep1	**3.72E-05**	**5.04E-05**	**5.55E-05**	**6.10E-05**	**6.55E-05**	**6.55E-05**	**6.55E-05**	**6.55E-05**	**6.73E-05**
Rep2	**3.72E-05**	**5.04E-05**	**5.49E-05**	**6.37E-05**	**6.55E-05**	**6.55E-05**	**6.55E-05**	**6.55E-05**	**7.08E-05**
Rep3	**3.45E-05**	**5.04E-05**	**5.49E-05**	**6.37E-05**	**6.55E-05**	**6.55E-05**	**6.60E-05**	**6.73E-05**	**7.08E-05**
Average	3.63E-05	5.04E-05	5.51E-05	6.28E-05	6.55E-05	6.55E-05	6.57E-05	6.61E-05	6.96E-05
Standard deviation	1.25E-06	0	2.98E-07	1.28E-06	0	0	2.42E-07	8.34E-07	1.66E-06

Table 19. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 4°C of CO94183-1R/R fresh and freeze-dried tubers (Pg-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**1.12E-05**	**2.13E-05**	**2.58E-05**	**2.87E-05**	**3.09E-05**	**3.20E-05**	**3.26E-05**	**3.48E-05**	**3.65E-05**
Rep2	**1.12E-05**	**2.25E-05**	**2.75E-05**	**2.81E-05**	**3.09E-05**	**3.20E-05**	**3.31E-05**	**3.48E-05**	**3.65E-05**
Rep3	**1.07E-05**	**2.19E-05**	**2.70E-05**	**2.81E-05**	**2.98E-05**	**3.20E-05**	**3.26E-05**	**3.43E-05**	**3.65E-05**
Average	1.10E-05	2.19E-05	2.68E-05	2.83E-05	3.05E-05	3.20E-05	3.28E-05	3.46E-05	3.65E-05
Standard deviation	2.64E-07	4.58E-07	7.00E-07	2.64E-07	5.29E-07	0	2.64E-07	2.64E-07	0
Freeze-dried									
Rep1	**2.36E-05**	**4.44E-05**	**4.61E-05**	**5.17E-05**	**5.17E-05**	**5.34E-05**	**5.45E-05**	**5.67E-05**	**5.96E-05**
Rep2	**2.36E-05**	**4.27E-05**	**4.89E-05**	**5.06E-05**	**5.17E-05**	**5.17E-05**	**5.45E-05**	**5.51E-05**	**6.18E-05**
Rep3	**2.19E-05**	**4.27E-05**	**4.89E-05**	**5.06E-05**	**5.17E-05**	**5.34E-05**	**5.45E-05**	**5.96E-05**	**6.18E-05**
Average	2.30E-05	4.33E-05	4.79E-05	5.09E-05	5.17E-05	5.28E-05	5.45E-05	5.71E-05	6.10E-05
Standard deviation	7.94E-07	7.94E-07	1.32E-06	5.29E-07	0	7.95E-07	0	1.85E-06	1.06E-06

Table 20. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of CO94183-1R/R fresh and freeze-dried tubers (Pg-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**1.12E-05**	**1.12E-05**	**1.12E-05**	**1.12E-05**	**1.46E-05**	**1.57E-05**	**1.69E-05**	**1.80E-05**	**1.97E-05**
Rep2	**1.12E-05**	**1.12E-05**	**1.35E-05**	**1.40E-05**	**1.57E-05**	**1.57E-05**	**1.69E-05**	**1.69E-05**	**1.80E-05**
Rep3	**1.07E-05**	**1.07E-05**	**1.12E-05**	**1.40E-05**	**1.57E-05**	**1.57E-05**	**1.69E-05**	**1.80E-05**	**1.97E-05**
Average	1.10E-05	1.10E-05	1.20E-05	1.31E-05	1.54E-05	1.57E-05	1.69E-05	1.76E-05	1.91E-05
Standard deviation	2.64E-07	2.64E-07	1.05E-06	1.32E-06	5.29E-07	0	0	5.29E-07	7.94E-07
Freeze-dried									
Rep1	**2.36E-05**	**3.20E-05**	**3.60E-05**	**3.76E-05**	**4.16E-05**	**4.16E-05**	**4.16E-05**	**4.16E-05**	**4.27E-05**
Rep2	**2.36E-05**	**3.20E-05**	**3.48E-05**	**4.04E-05**	**4.16E-05**	**4.16E-05**	**4.16E-05**	**4.16E-05**	**4.49E-05**
Rep3	**2.19E-05**	**3.20E-05**	**3.48E-05**	**4.04E-05**	**4.16E-05**	**4.16E-05**	**4.16E-05**	**4.27E-05**	**4.49E-05**
Average	2.30E-05	3.20E-05	3.52E-05	3.95E-05	4.16E-05	4.16E-05	4.16E-05	4.19E-05	4.42E-05
Standard deviation	7.94E-07	0	5.29E-07	1.32E-06	0	0	0	5.29E-07	1.05E-06

Table 21. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 4°C of 'All Blue' fresh and freeze-dried tubers (Pt-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.09E-05**	**3.10E-05**	**3.30E-05**	**4.03E-05**	**4.42E-05**	**5.50E-05**	**5.66E-05**	**5.81E-05**	**6.36E-05**
Rep2	**1.70E-05**	**2.95E-05**	**3.18E-05**	**4.26E-05**	**4.42E-05**	**5.58E-05**	**5.66E-05**	**5.81E-05**	**6.20E-05**
Rep3	**1.80E-05**	**3.02E-05**	**3.18E-05**	**4.30E-05**	**4.50E-05**	**5.43E-05**	**5.66E-05**	**5.97E-05**	**6.36E-05**
Average	1.86E-05	3.02E-05	3.22E-05	4.20E-05	4.44E-05	5.50E-05	5.66E-05	5.87E-05	6.30E-05
Standard deviation	1.66E-06	6.32E-07	5.73E-07	1.19E-06	3.65E-07	6.33E-07	0	7.30E-07	7.38E-07
Freeze-dried									
Rep1	**3.72E-05**	**6.74E-05**	**7.91E-05**	**8.06E-05**	**8.29E-05**	**8.29E-05**	**8.53E-05**	**9.30E-05**	**1.05E-04**
Rep2	**3.33E-05**	**7.36E-05**	**7.98E-05**	**7.98E-05**	**8.14E-05**	**8.22E-05**	**8.91E-05**	**9.69E-05**	**1.21E-04**
Rep3	**3.41E-05**	**7.36E-05**	**7.91E-05**	**8.14E-05**	**8.22E-05**	**8.22E-05**	**8.91E-05**	**9.30E-05**	**1.24E-04**
Average	3.49E-05	7.16E-05	7.93E-05	8.06E-05	8.22E-05	8.24E-05	8.79E-05	9.43E-05	1.17E-04
Standard deviation	1.67E-06	2.92E-06	3.65E-07	6.32E-07	6.32E-07	3.65E-07	1.82E-06	1.825E-06	8.14E-06

Table 22. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of 'All Blue' fresh and freeze-dried tubers (Pt-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.09E-05**	**1.70E-05**	**1.70E-05**	**2.09E-05**	**2.09E-05**	**2.40E-05**	**2.71E-05**	**2.71E-05**	**2.71E-05**
Rep2	**1.70E-05**	**2.10E-05**	**2.10E-05**	**2.10E-05**	**2.10E-05**	**2.48E-05**	**2.71E-05**	**2.71E-05**	**2.71E-05**
Rep3	**1.80E-05**	**1.86E-05**	**1.86E-05**	**1.94E-05**	**2.20E-05**	**2.48E-05**	**2.55E-05**	**2.71E-05**	**3.02E-05**
Average	1.86E-05	1.89E-05	1.89E-05	2.04E-05	2.13E-05	2.45E-05	2.66E-05	2.71E-05	2.82E-05
Standard deviation	1.66E-06	1.64E-06	1.64E-06	7.47E-07	4.88E-07	3.8E-07	7.69E-07	3.03E-13	1.46E-06
Freeze-dried									
Rep1	**3.72E-05**	**4.26E-05**	**4.65E-05**	**5.74E-05**	**6.20E-05**	**6.43E-05**	**7.05E-05**	**7.36E-05**	**7.60E-05**
Rep2	**3.33E-05**	**3.88E-05**	**4.73E-05**	**5.74E-05**	**5.89E-05**	**6.43E-05**	**6.74E-05**	**7.52E-05**	**7.75E-05**
Rep3	**3.41E-05**	**4.42E-05**	**4.73E-05**	**5.74E-05**	**5.74E-05**	**6.74E-05**	**7.05E-05**	**7.36E-05**	**7.75E-05**
Average	3.49E-05	4.19E-05	4.70E-05	5.74E-05	5.94E-05	6.54E-05	6.95E-05	7.42E-05	7.70E-05
Standard deviation	1.67E-06	2.28E-06	3.65E-07	0	1.93E-06	1.46E-06	1.46E-06	7.30E-07	7.30E-07

Table 23. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 4°C of 'All Blue' fresh and freeze-dried tubers (Mv-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.30E-05**	**3.74E-05**	**4.21E-05**	**4.90E-05**	**5.33E-05**	**6.64E-05**	**6.82E-05**	**7.01E-05**	**7.66E-05**
Rep2	**1.87E-05**	**3.55E-05**	**3.83E-05**	**5.14E-05**	**5.33E-05**	**6.73E-05**	**6.82E-05**	**7.01E-05**	**7.38E-05**
Rep3	**2.06E-05**	**3.64E-05**	**3.83E-05**	**4.70E-05**	**5.42E-05**	**6.54E-05**	**6.82E-05**	**7.20E-05**	**7.66E-05**
Average	2.08E-05	3.64E-05	3.96E-05	4.91E-05	5.36E-05	6.64E-05	6.82E-05	7.07E-05	7.57E-05
Standard deviation	1.76E-06	7.63E-07	1.76E-06	1.79E-06	4.40E-07	7.63E-07	8.57E-13	8.81E-07	1.32E-06
Freeze-dried									
Rep1	**4.49E-05**	**8.13E-05**	**9.53E-05**	**9.72E-05**	**1.00E-04**	**1.00E-04**	**1.03E-04**	**1.12E-04**	**1.46E-04**
Rep2	**4.25E-05**	**8.88E-05**	**9.63E-05**	**9.63E-05**	**9.81E-05**	**9.91E-05**	**1.07E-04**	**1.17E-04**	**1.46E-04**
Rep3	**4.30E-05**	**8.88E-05**	**9.53E-05**	**9.81E-05**	**9.91E-05**	**9.91E-05**	**1.07E-04**	**1.12E-04**	**1.50E-04**
Average	4.35E-05	8.63E-05	9.56E-05	9.72E-05	9.91E-05	9.94E-05	1.06E-04	1.14E-04	1.47E-04
Standard deviation	1.01E-06	3.52E-06	4.40E-07	7.63E-07	7.63E-07	4.41E-07	2.20E-06	2.20E-06	1.72E-06

Table 24. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of "All Blue" fresh and freeze-dried tubers (Mv-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.30E-05**	**1.87E-05**	**1.87E-05**	**2.52E-05**	**2.52E-05**	**2.80E-05**	**2.99E-05**	**3.27E-05**	**3.27E-05**
Rep2	**1.87E-05**	**2.52E-05**	**2.52E-05**	**2.52E-05**	**2.52E-05**	**2.99E-05**	**3.15E-05**	**3.27E-05**	**3.27E-05**
Rep3	**2.06E-05**	**2.24E-05**	**2.24E-05**	**2.34E-05**	**2.70E-05**	**2.99E-05**	**2.99E-05**	**3.27E-05**	**3.40E-05**
Average	2.08E-05	2.21E-05	2.21E-05	2.46E-05	2.58E-05	2.93E-05	3.04E-05	3.27E-05	3.31E-05
Standard deviation	1.76E-06	2.66E-06	2.66E-06	8.81E-07	8.32E-07	8.81E-07	7.51E-07	0	6.07E-07
Freeze-dried									
Rep1	**4.49E-05**	**4.25E-05**	**5.61E-05**	**6.92E-05**	**7.01E-05**	**7.76E-05**	**8.50E-05**	**8.88E-05**	**9.16E-05**
Rep2	**4.25E-05**	**4.49E-05**	**5.70E-05**	**6.92E-05**	**6.90E-05**	**7.76E-05**	**8.13E-05**	**9.07E-05**	**9.20E-05**
Rep3	**4.30E-05**	**5.33E-05**	**5.70E-05**	**6.92E-05**	**6.92E-05**	**8.05E-05**	**8.13E-05**	**8.88E-05**	**9.20E-05**
Average	4.35E-05	4.69E-05	5.67E-05	6.92E-05	6.94E-05	7.85E-05	8.26E-05	8.94E-05	9.19E-05
Standard deviation	1.01E-06	4.61E-06	4.40E-07	8.57E-13	4.85E-07	1.38E-06	1.76E-06	8.81E-07	1.93E-07

Table 25. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 4°C of CO94165-3P/P fresh and freeze-dried tubers (Pt-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.95E-05**	**4.42E-05**	**4.73E-05**	**4.81E-05**	**6.05E-05**	**6.20E-05**	**6.28E-05**	**6.43E-05**	**6.74E-05**
Rep2	**3.02E-05**	**4.50E-05**	**4.73E-05**	**5.15E-05**	**5.89E-05**	**6.28E-05**	**6.28E-05**	**6.40E-05**	**6.74E-05**
Rep3	**3.02E-05**	**4.42E-05**	**4.73E-05**	**5.15E-05**	**5.89E-05**	**6.28E-05**	**6.20E-05**	**6.43E-05**	**6.90E-05**
Average	3.00E-05	4.44E-05	4.73E-05	5.04E-05	5.94E-05	6.25E-05	6.25E-05	6.42E-05	6.80E-05
Standard deviation	3.65E-07	3.65E-07	0	1.62E-06	7.30E-07	3.65E-07	3.65E-07	1.60E-07	7.31E-07
Freeze-dried									
Rep1	**3.70E-05**	**6.36E-05**	**7.60E-05**	**7.60E-05**	**7.60E-05**	**8.14E-05**	**8.60E-05**	**9.30E-05**	**1.05E-04**
Rep2	**3.57E-05**	**6.43E-05**	**7.37E-05**	**7.55E-05**	**7.75E-05**	**4.20E-05**	**8.53E-05**	**9.46E-05**	**1.09E-04**
Rep3	**3.57E-05**	**6.55E-05**	**7.44E-05**	**7.60E-05**	**7.75E-05**	**8.14E-05**	**8.53E-05**	**9.46E-05**	**1.08E-04**
Average	3.61E-05	6.45E-05	7.47E-05	7.58E-05	7.70E-05	6.83E-05	8.55E-05	9.41E-05	1.07E-04
Standard deviation	6.32E-07	7.94E-07	9.46E-07	2.21E-07	7.30E-07	1.86E-05	3.65E-07	7.30E-07	1.67E-06

Table 26. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of CO94165-3P/P fresh and freeze-dried tubers (Pt-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**2.95E-05**	**3.02E-05**	**3.05E-05**	**3.10E-05**	**3.26E-05**	**3.33E-05**	**3.72E-05**	**3.80E-05**	**4.30E-05**
Rep2	**3.02E-05**	**3.02E-05**	**3.08E-05**	**3.10E-05**	**3.26E-05**	**3.33E-05**	**3.57E-05**	**3.95E-05**	**3.95E-05**
Rep3	**3.02E-05**	**2.95E-05**	**3.10E-05**	**3.18E-05**	**3.18E-05**	**3.33E-05**	**3.72E-05**	**3.95E-05**	**4.19E-05**
Average	3.00E-05	3.00E-05	3.08E-05	3.13E-05	3.23E-05	3.33E-05	3.67E-05	3.90E-05	4.15E-05
Standard deviation	3.65E-07	3.45E-07	2.08E-07	3.65E-07	3.65E-07	4.29E-13	7.30E-07	7.30E-07	1.44E-06
Freeze-dried									
Rep1	**3.70E-05**	**3.88E-05**	**4.03E-05**	**4.34E-05**	**4.65E-05**	**5.19E-05**	**5.58E-05**	**7.60E-05**	**8.05E-05**
Rep2	**3.57E-05**	**3.95E-05**	**4.01E-05**	**4.34E-05**	**4.65E-05**	**5.19E-05**	**5.40E-05**	**7.52E-05**	**8.22E-05**
Rep3	**3.57E-05**	**3.95E-05**	**4.00E-05**	**4.25E-05**	**4.50E-05**	**5.10E-05**	**5.74E-05**	**7.52E-05**	**8.33E-05**
Average	3.61E-05	3.93E-05	4.01E-05	4.31E-05	4.60E-05	5.16E-05	5.57E-05	7.55E-05	8.20E-05
Standard deviation	6.32E-07	3.65E-07	1.29E-07	4.29E-07	7.12E-07	4.42E-07	1.37E-06	3.65E-07	1.15E-06

Table 27. Increase in anthocyanin concentration with replications and standard deviation with length of storage (weeks) at 4°C of CO94165-3P/P fresh and freeze-dried tubers (MV-3-(pC-rut)-5-glu).

Fresh									
Replication	Initial	4	6	8	10	12	16	20	24
Rep1	**3.55E-05**	**5.33E-05**	**5.70E-05**	**5.79E-05**	**7.29E-05**	**7.48E-05**	**7.57E-05**	**7.76E-05**	**8.13E-05**
Rep2	**3.64E-05**	**5.42E-05**	**5.70E-05**	**6.36E-05**	**7.10E-05**	**7.57E-05**	**7.57E-05**	**7.76E-05**	**8.13E-05**
Rep3	**3.64E-05**	**5.33E-05**	**5.70E-05**	**6.36E-05**	**7.10E-05**	**7.57E-05**	**7.48E-05**	**7.76E-05**	**8.32E-05**
Average	3.61E-05	5.36E-05	5.70E-05	6.17E-05	7.17E-05	7.54E-05	7.54E-05	7.76E-05	8.19E-05
Standard deviation	4.40E-07	4.40E-07	1.05E-12	2.64E-06	8.81E-07	4.40E-07	4.24E-07	0	8.81E-07
Freeze-dried									
Rep1	**4.49E-05**	**7.66E-05**	**9.16E-05**	**9.16E-05**	**9.16E-05**	**9.81E-05**	**1.04E-04**	**1.12E-04**	**1.26E-04**
Rep2	**4.30E-05**	**7.76E-05**	**8.88E-05**	**8.97E-05**	**9.35E-05**	**9.91E-05**	**1.03E-04**	**1.14E-04**	**1.31E-04**
Rep3	**4.30E-05**	**8.04E-05**	**8.97E-05**	**9.16E-05**	**9.35E-05**	**9.81E-05**	**1.03E-04**	**1.14E-04**	**1.30E-04**
Average	4.36E-05	7.82E-05	9.00E-05	9.10E-05	9.28E-05	9.84E-05	1.03E-04	1.13E-04	1.29E-04
Standard deviation	8..95E-07	1.60E-06	1.16E-06	8..95E-07	8..95E-07	4.71E-07	4.715E-07	9.42E-07	2.16E-06

Table 28. Increase in anthocyanin concentration with replications and standard deviations with length of storage (weeks) at 10°C of CO94165-3P/P fresh and freeze-dried tubers (MV-3-(pC-rut)-5-glu).

Replication	Initial	4	6	8	10	12	16	20	24
Fresh									
Rep1	**3.55E-05**	**3.64E-05**	**3.74E-05**	**3.74E-05**	**3.93E-05**	**4.02E-05**	**4.49E-05**	**4.58E-05**	**5.42E-05**
Rep2	**3.64E-05**	**3.64E-05**	**3.74E-05**	**3.74E-05**	**3.93E-05**	**4.02E-05**	**4.30E-05**	**4.77E-05**	**4.77E-05**
Rep3	**3.64E-05**	**3.46E-05**	**3.74E-05**	**3.83E-05**	**3.83E-05**	**4.02E-05**	**4.49E-05**	**4.77E-05**	**5.05E-05**
Average	3.61E-05	3.58E-05	3.74E-05	3.77E-05	3.89E-05	4.02E-05	4.42E-05	4.70E-05	5.08E-05
Standard deviation	4.24E-07	8.48E-07	0	4.24E-07	4.71E-07	0	8.81E-07	8.81E-07	2.66E-06
Freeze-dried									
Rep1	**4.49E-05**	**4.67E-05**	**4.86E-05**	**5.23E-05**	**5.61E-05**	**6.26E-05**	**6.73E-05**	**9.16E-05**	**9.91E-05**
Rep2	**4.30E-05**	**4.77E-05**	**4.77E-05**	**5.23E-05**	**5.61E-05**	**6.26E-05**	**6.54E-05**	**9.07E-05**	**9.91E-05**
Rep3	**4.30E-05**	**4.77E-05**	**4.67E-05**	**5.14E-05**	**5.42E-05**	**6.17E-05**	**6.92E-05**	**9.07E-05**	**1.00E-04**
Average	4.36E-05	4.74E-05	4.77E-05	5.20E-05	5.55E-05	6.23E-05	6.73E-05	9.10E-05	9.94E-05
Standard deviation	8.95E-07	4.71E-07	7.76E-07	4.24E-07	8.95E-07	4.24E-07	1.55E-06	4.24E-07	4.24E-07