

## **ACAAF Project Progress Report - 2005**

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**Project #: AB0001**

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**Submitted by: Dr. Michele Konschuh**

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### **Lutein content of yellow-fleshed potatoes grown in Alberta**

#### **1. Abstract**

Eating a diet rich in lutein has been linked to reduced rates of age-related macular degeneration and cataract formation as people age. In fact, many daily multivitamins are now enriched with lutein. This project built on a 2004 preliminary study finding that a serving of yellow-fleshed potatoes currently produced in Canada contain as much as 25 to 50% of the lutein added to multivitamins. To use the information from the 2004 study in a marketing strategy for potatoes, we required additional data to establish a reproducible quantity of lutein per serving. In this project, we grew ten varieties of yellow-fleshed potatoes in three Alberta locations. We harvested potatoes at three harvest dates and assessed composite samples from each location to determine the quantity of lutein present at different stages of development in each variety for each location. This information will enable us to determine a minimum amount of lutein contributed by an average potato serving and to determine the effect of growing location and time of harvest on lutein concentration. Armed with this information, we envision that partners from the potato industry (seed and table market sectors) will use the information to promote potatoes to health conscious consumers and retailers. While yellow-fleshed potatoes may not be the richest source of lutein, the knowledge that potatoes contain another health promoting compound may encourage potato consumption or provide good reasons to continue including potatoes in a balanced diet.

## 2. Introduction

Yukon Gold, a yellow-fleshed potato cultivar developed by Canadian potato breeders (Johnston & Rowberry 1981) found a niche in North America and has opened the door to acceptance of yellow-fleshed potatoes by consumers. The impetus to develop new, improved yellow-fleshed cultivars has now expanded beyond the goal of simply developing novelty types for specialty markets (Lu et al. 2001). The yellow color of the potato flesh is imparted by carotenoids and Lu et al. (2001) reported that both individual and total carotenoid contents were positively correlated to yellow-flesh intensity in potato tubers. Carotenoids may protect against a variety of chronic diseases including cardiovascular disease (Gaziano et al. 1995) and certain cancers (Colditz et al. 1985). Perhaps the clearest link between specific carotenoids and a health outcome is that for lutein and zeaxanthin with age-related macular degeneration (AMD), the leading cause of visual impairment and blindness in the U.S (Snodderly 1995). Lutein has been linked to reduced incidence of age-related macular degeneration and reduced cataract incidence. Lutein and zeaxanthin are two carotenoids that circulate in human blood plasma and are concentrated in the macula region of the eye. Consumption of foods rich in lutein and zeaxanthin is inversely related to AMD (Seddon et al. 1994) and short-term feeding of foods rich in lutein and zeaxanthin can substantially increase pigment density in the eyes of human subjects (Hammond et al. 1997). Conversely, people with macular degeneration have been found to have lower levels of zeaxanthin and lutein than people without, which supports the premise that these antioxidants provide some protection (Bliss 2003). Recent feeding studies have also shown that although spinach is rich in carotenoids, other foods may contain a more bio-available source of the compounds (Bliss 2003).

As of 2000, there was no dietary reference intake for lutein in the U.S. (Food and Nutrition Board / Institute of Medicine). It is widely believed, however, that health benefits would accrue as a result of increasing lutein consumption in the U.S. from the estimated average intake of 1.3 mg/day (Chug-Ahuja et al. 1993). Studies at the University of Florida looking at the effect of dietary lutein supplements of 2.4 mg/day showed an increase in serum lutein concentration and an increase in macular pigment density. Total carotenoid content in white-fleshed potato varieties ranges from 15 to 185  $\mu\text{g}/100\text{ g}$  fresh weight, while yellow fleshed varieties can exceed 500  $\mu\text{g}/100\text{g}$  fresh weight of lutein and up to 1.4 mg/100 g fresh weight of total carotenoids (Lu et al. 2001). A typical baked potato serving weighs approximately 170 g; so yellow-fleshed potatoes could supply a significant percentage of dietary lutein. Potato breeding programs in the US are selecting varieties of potato rich in lutein, however using existing varieties will allow us to generate interest by consumers and retailers years before the new varieties become available.

Screening of 20 yellow-fleshed potato varieties in 2004 established that some Alberta grown varieties contain up to 60 $\mu\text{g}/100\text{g}$  FW of lutein. Lutein is present in many leafy green vegetables and is included in vitamin pills for mature customers at rates of 225 to 600 $\mu\text{g}$ . As well, patients with early stages of AMD are encouraged to take lutein supplements (6 to 20 mg; 1000 $\mu\text{g}$  = 1mg) to increase their dietary intake of this potentially beneficial compound. An average potato (170g) could contribute up to 100 $\mu\text{g}$  of lutein. While there are better dietary sources of lutein available,

potatoes can be part of a healthful diet. Additional research in 2004 determined that lutein in potatoes is quite stable during storage and is not destroyed during cooking or processing. Presently, we know that lutein content varies by potato variety. We also have data to suggest that growing location and size of tubers at harvest has an impact on the lutein content. Nutritional information regulations will allow us to indicate that yellow-fleshed potatoes contain lutein, but we require information indicating the quantity provided in an average serving of potatoes. In order to quantify the amount of lutein in a serving of potato, we need at least two years of research showing the range of lutein concentrations and factors affecting lutein content.

This trial involved growing 10 yellow-fleshed potato varieties in three locations in Alberta and harvesting at three different times. Composite samples from each variety were analyzed for flesh color intensity, lutein concentration and concentration of total carotenoids. Information about the quantity of lutein provided by a serving of yellow-fleshed potatoes will allow the potato industry in Alberta to market products, fresh and processed, as functional foods to health-conscious consumers.

### 3. Objectives

- To determine lutein concentration in up to 10 potato varieties grown at three locations in Alberta;
- To determine what effect growing location and time of harvest have on lutein concentration in each variety;
- To determine the amount of lutein contributed in an average potato serving for use in marketing strategies.

### 4. Methods

Yellow-fleshed potatoes were grown in small plot in a cooperator's field near Lacombe, AB and in replicated plots at the Crop Diversification Centre South (CDCS) in Brooks, AB, and the Crop Diversification Centre North (CDCN) in Edmonton, AB. Ten different yellow-fleshed varieties were included in the trial (Table 1). The varieties were selected from preliminary research conducted with twenty yellow-fleshed varieties grown in 2004. Three companies agreed to provide seed for the trial. Early generation seed (E2 or E3) of each variety was used for this trial. Seed was cut, if necessary, to ensure seed pieces of no more than 70 to 85 g, suberized, and planted 30 cm apart in 6 m rows spaced 90 cm apart. Four replicates of each variety were grown in three randomized complete blocks (see plot plan), one for each harvest date.

**Table 1:** Yellow-fleshed potato varieties included in the 2005 lutein trial.

<i>Variety</i>	<i>Seed Supplied By</i>
Agata	Solanum International
Innovator	HZPC Americas
Island Sunshine	Parkland Seed Potatoes
Piccolo	Solanum International
Cecile (RZ94-83)	HZPC Americas
Sante	Parkland Seed Potatoes
Satina	Solanum International
Sinora	Parkland Seed Potatoes
Victoria	HZPC Americas
Yukon Gold	Check

At CDCS the plots were managed following the guidelines for the Western Canadian Potato Breeding Program. Potatoes were planted approximately 12 to 14 cm deep using a two-row wheel planter on May 13, 2005 at CDCS. The plots were hilled prior to emergence and were irrigated at CDCS to maintain soil moisture close to 70%. Eptam (2.0 L/ac) and Sencor (150 g/ac) were applied prior to planting (April 29) to control weeds. Foliar fungicides were applied approximately every 2 weeks during the growing season to prevent early blight and late blight from developing (Table 2). Insecticide was applied July 14 (Decis, 50 mL/ac) to control Colorado Potato Beetles. Potatoes in one block of treatments were hand topped and harvested August 2 (81 days after planting, DAP). A second block of potatoes was hand topped and harvested August 22 (101 DAP). The final block of potatoes was desiccated with Reglone (1 L/ac) August 29 and harvested mechanically September 20 (130 DAP).

**Table 2:** Foliar fungicides applied to the potato crop at CDCS to prevent early blight and late blight development.

<i>Date of Application</i>	<i>Fungicide</i>	<i>Rate</i>
July 8	Quadris	0.250 L/ac
July 20	Acrobat	1.0 kg/ac
August 12	Ridomil Gold/Bravo	8.83 L/10 ac
August 24	Pencozeb	0.90 kg/ac

At CDCN, plots were managed as rain-fed plots following the guidelines for the Western Canadian Potato Breeding Program. Potatoes were planted approximately 12 to 14 cm deep using a two-row wheel planter on May 11 and 12, 2005. Plots were hilled May 27, prior to emergence. Lorox (1.82 L/ac) was applied pre-emergent (June 1) to control weeds in plots for final harvest; other plots were hand weeded to control weeds. No fungicides were applied during the growing season. No insecticides were required. The first two sets of plots were harvested green August 2, 2005 (83 days after planting, DAP) and August 15, 2005 (96 DAP). Reglone (1.2 L/ac) was applied August 27 to desiccate the plots for the final harvest. The final set of plots was harvested at CDCN September 13 (127 DAP).

Tubers were weighed to obtain yield estimates and graded into small, medium, large and deformed categories. Medium tubers (48 to 88 mm in diameter) were weighed to obtain estimates of marketable yield. Yield estimates for each harvest date have been presented in ton/acre although small plot trials do not always accurately reflect commercial yield potential (Appendix). A sample of 25 marketable tubers was washed and used to determine specific gravity by the weight-in-air over weight-in-water method. Each of these tubers was then cut longitudinally to assess brown center, hollow heart and other internal defects. Also, a composite sample of marketable tubers of each variety from each location was submitted to the Food Science lab at CDCS for measurement of flesh color (chroma) and analysis of total carotenoids, lutein and zeaxanthin.

Field data were statistically analyzed using GLM and Duncan's Multiple Range Test ( $p \leq 0.05$ ; SAS). Lab analyses were based on composite samples, so no statistics were applied.

Flesh color was measured using a HunterLab ColorQuest color measurement instrument. Tubers were prepared for color analysis by dicing into 1 cm cubes and using approximately 250 mL for color measurement. Measurements were made in triplicate. Chroma was measured using the CIELCh color scale, D65 illuminant and a 10° observer angle. Chroma is a measure of color intensity. Previous unpublished work in our lab indicated that chroma is the best indicator of yellowness in white and yellow-fleshed potatoes.

The composite sample provided for carotenoid analysis was cut into approximately 1 cm cubes and freeze-dried. Carotenoid analysis was based on the method of Brown et al. (1993). Carotenoids were extracted from a 20 g sub-sample with 100 mL ethyl ether/hexane (1:1, v/v) and 10 g Celite 545 as a filter aid. Beta-apo-8'-carotenal was added as an internal standard. The mixture was blended for 1 minute and vacuum filtered through Whatman 1 filter paper. The filter cake and paper were extracted a second time with fresh extraction solvent and the filtrates combined. Most of

the yellow color was removed from the potatoes during the first extraction. The combined filtrate was evaporated on a rotary evaporator to remove the extraction solvents. The concentrated extract was transferred quantitatively with a small amount of methanol to a separatory funnel containing 50 mL saturated aqueous sodium chloride. The aqueous solution was extracted repeatedly with methylene chloride until all the yellow color was removed. Generally, two extractions were sufficient. The methylene chloride extract was dried over anhydrous sodium sulfate. The extract was then evaporated to dryness. The residue was re-dissolved in 1 mL of toluene and 3 mL of mobile phase. The extract was filtered through a 0.45 µm filter into an HPLC vial.

Analysis of carotenoids was performed on an Agilent 1100 liquid chromatography system using a Phenomenex Synergi Hydro RP column (4.6 x 250 mm) and diode-array detection at 450 nm. The mobile phase consisted of 58% acetonitrile, 35% methanol, and 7% tetrahydrofuran. Elution was isocratic at 0.8 mL/min. Linearity and retention times were verified with lutein and zeaxanthin standards. Carotenoid concentrations were calculated relative to the internal standard concentration.

## 5. Results

Composite samples of marketable potatoes from each location were used to determine the concentration of lutein and zeaxanthin per 100 g fresh weight of tuber. These samples were also used to determine flesh color intensity (chroma) of each potato variety at each location. The data for CDCS, CDCN, and Lacombe are reported in Table 3.

**Table 3:** Flesh color intensity (chroma) of yellow-fleshed potato tubers grown at three locations in Alberta, harvested approximately 80 days after planting (DAP), 100 DAP and 130 DAP.

Rank	Variety	CDCS (Brooks)			CDCN (Edmonton)			Lacombe		
		80 DAP	100 DAP	130 DAP	80 DAP	100 DAP	130 DAP	80 DAP	100 DAP	130 DAP
8	Agata	44.6	44.4	42.7	43.1	43.7	41.7	46.7	43.4	41.7
5	Cecile	46.2	45.0	47.1	46.7	48.2	48.9		47.6	45.5
10	Innovator	39.9	36.3	39.6	40.8	40.6	37.1	40.8	40.0	38.2
1	Island Sunshine	45.9	46.7	48.5	50.5	49.1	47.5	48.6	51.6	51.7
4	Piccolo	51.2	46.5	47.3	48.2	48.8	47.3	51.3	49.7	47.1
9	Sante	44.5	39.7	41.6	43.8	44.2	39.8	45.0	44.3	41.7
2	Satina	47.6	44.9	50.3	49.8	49.4	48.0	48.6	48.2	48.3
6	Sinora	46.9	43.0	46.0	47.0	47.3	46.1	48.3	46.3	46.0
3	Victoria	48.0	46.1	48.6	49.3	50.0	48.4	48.6	49.3	44.2
7	Yukon Gold	44.6	43.5	49.0	42.5	45.4	42.5	40.7	40.2	40.2

Chroma is the measurement of color most closely aligned with our perception of color. The chroma of tuber flesh ranged from as low as 36 in the least yellow samples (Innovator) to over 50 in several of the yellow-fleshed varieties; the higher the number, the more yellow the appearance of the flesh. Yellow flesh color is a heritable trait (Lu et al. 2001) and is characteristic of particular varieties. Chroma values for each variety were quite consistent at each location, but varied somewhat with harvest date and

between locations. An overall ranking of varieties (Table 3) indicates that in 2005, Island Sunshine has the deepest yellow flesh of the varieties looked at. Next in the overall ranking is Satina, followed by Victoria, Piccolo, Cecile, Sinora, Yukon Gold, Agata, Sante and Innovator. Days after planting were used to coordinate harvest between sites, but rates of growth and development differed in 2005 as can be seen in yield data from each site (Appendix). Perhaps a different measure, such as stage of crop development should be used in 2006 to compare locations. In general, chroma values appear to be variety dependent, however, growing conditions and size at the time of harvest appear to influence the chroma values as well.

In the literature, it is noted that individual and total carotenoid content are positively correlated with flesh color. In 2004, we found that flesh color was more closely correlated with total carotenoids ( $r^2=0.46$ ) than with lutein ( $r^2=0.30$ ), and even then, the correlation was positive but not all that strong. In 2005, flesh color appeared to be more independent of lutein concentration than in 2005, and even the correlation with total carotenoids was weaker. The correlations were stronger for samples from Lacombe and CDCN than from CDCS and may relate to tuber development. Certainly, carotenoids other than lutein and zeaxanthin contribute to yellow flesh color and compounds other than carotenoids also influence the chroma of potato tubers. The variation we see between harvest dates and growing locations may also be an indication of the variability in flesh color in general.

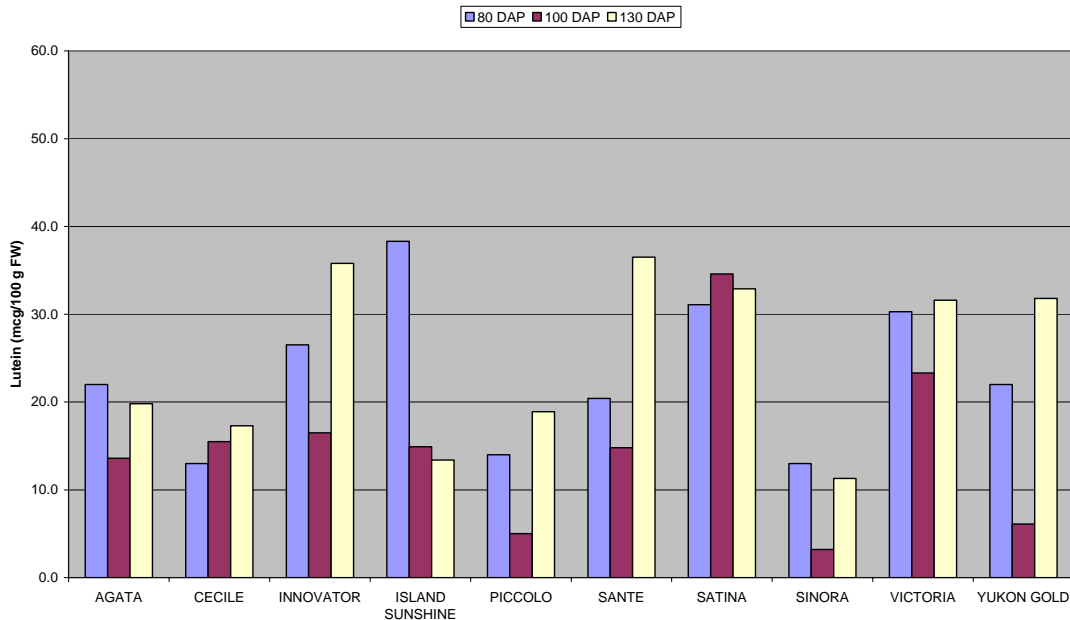
**Table 4:** Total carotenoid concentration of yellow-fleshed potato tubers grown at three locations in Alberta, harvested approximately 80 days after planting (DAP), 100 DAP and 130 DAP.

Variety	CDCS (Brooks)			CDCN (Edmonton)			Lacombe		
	80 DAP	100 DAP	130 DAP	80 DAP	100 DAP	130 DAP	80 DAP	100 DAP	130 DAP
Agata	97.9	69.1	79.1	38.6	63.6	40.4	158.0	34.9	
Cecile	81.4	84.7	89.6	151.8	218.3	137.0		160.0	
Innovator	130.2	94.9	139.2	78.8	156.4	58.6	58.9	103.5	
Island Sunshine	196.2	77.9	64.3	102.8	86.7	58.2	132.6	90.9	107.5
Piccolo	84.7	34.4	107.6	112.3	116.2	85.2	283.3	126.3	
Sante	112.3	108.2	157.8	128.6	202.3	100.9	179.7	163.0	
Satina	160.9	192.5	140.1	186.1	257.4	173.9	187.2	192.4	
Sinora	64.7	17.1	53.1	65.9	78.7	46.1	47.4	23.1	
Victoria	186.1	179.7	177.3	202.8	242.5	136.8	220.6	240.9	85.0
Yukon Gold	113.7	38.3	124.3	69.1	136.8	80.1	116.8	95.9	

Total carotenoid concentration in yellow-fleshed potatoes ranged from 17 to 250  $\mu\text{g}$  / 100 g FW (Table 4). Total carotenoid was somewhat related between sites, but stronger correlations were observed for the 100 DAP harvest date than for the other two harvest dates.

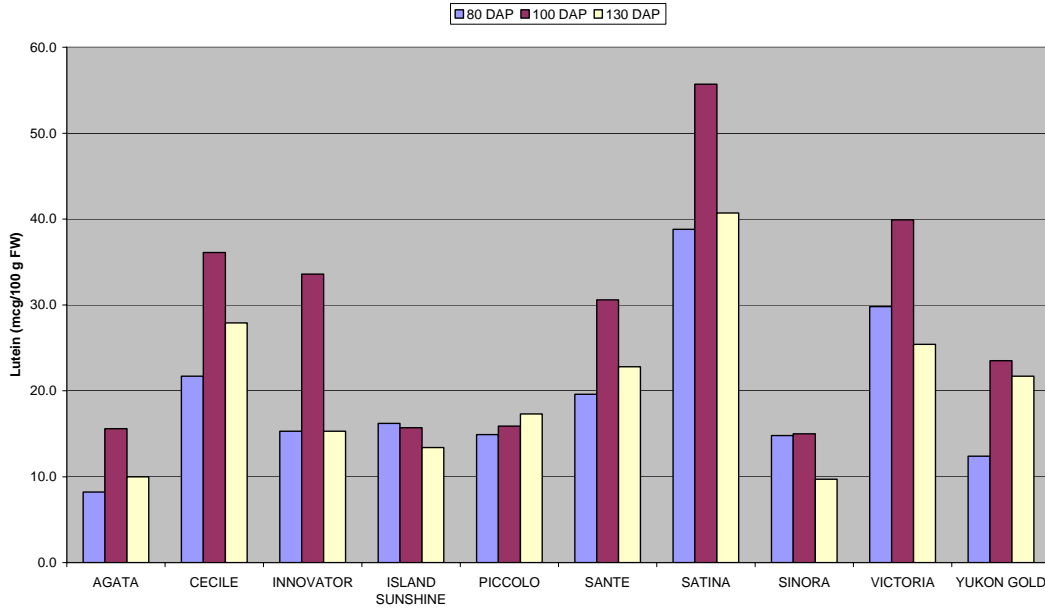
Lutein, zeaxanthin, and several other carotenoids have been reported to be present in yellow-fleshed tubers (Brown et al. 1993, Lu et al. 2001, Brethaupt and Bamedi 2002). Lutein was present in appreciable amounts in most of the varieties examined, even the check, Yukon Gold (Figures 1, 2 and 3). In most varieties, lutein made up 20 to 25% of the total carotenoid content at 130 DAP, and slightly less of the total carotenoid

concentration at 80 and 100 DAP. It was beyond the scope of this trial to identify other carotenoid compounds present in the potato tubers. Lutein concentration ranged from 3.2  $\mu\text{g}$  / 100 g FW in one sample of Sinora to over 50  $\mu\text{g}$  / 100 g FW in the variety Satina. Again, there was some correlation in lutein concentration between locations, especially with the 100 DAP harvest data. In 2004, we noted that growing location (environment, latitude, soil type, etc.) or the size and maturity of tubers at harvest might influence lutein concentration, but that variety was the greatest influencing factor. In 2005, we again saw that variety was the greatest factor affecting lutein concentration. The lutein concentration varied with harvest date and location, and there was no consistent trend observed with respect to harvest date or location in 2005. In general, varieties with the highest concentrations of lutein at one location also had higher concentrations of lutein at the other locations. The expensive nature of carotenoid extractions and analyses made composite samples more economical than replicated samples from each site. It is apparent, though, that we have no way of knowing whether differences observed are a function of limited sampling or variability within the variety. We require at least one additional year of data to be able to predict a minimum quantity of lutein per serving for many of these varieties. It is not clear whether the 10 varieties selected based on 2004 data actually represent the best varieties to market as functional foods. Additional variety screening would be recommended and replicated samples would be preferred to composite samples for this initiative.

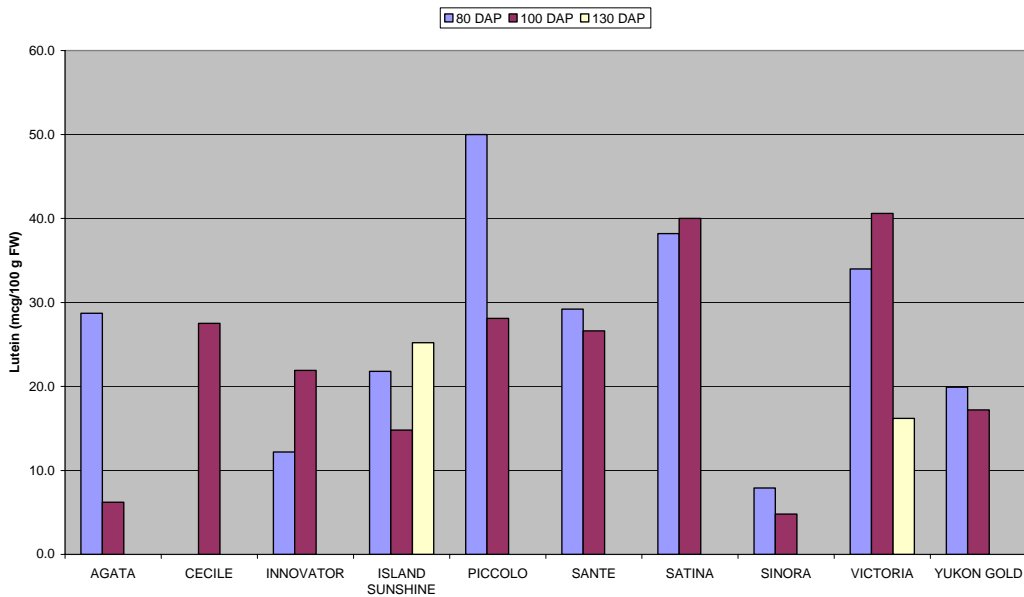


**Figure 1:** Concentration ( $\mu\text{g}$  / 100g FW) of lutein extracted from yellow-fleshed potatoes harvested at 80, 100 and 130 days after planting (DAP) at CDCS in Brooks, AB.





**Figure 2:** Concentration ( $\mu\text{g} / 100\text{g FW}$ ) of lutein extracted from yellow-fleshed potatoes harvested at 80, 100 and 130 days after planting (DAP) at CDCN in Edmonton, AB.



**Figure 3:** Concentration ( $\mu\text{g} / 100\text{g FW}$ ) of lutein extracted from yellow-fleshed potatoes harvested at 80, 100 and 130 days after planting (DAP) at Lacombe, AB.

## 6. Conclusions

Total carotenoid content in the yellow-fleshed potatoes studied ranged from 17 to 250 µg per 100 g FW and was influenced by variety, the growing location, and the time of harvest. The concentration of lutein in yellow-fleshed potatoes depended on the variety, the growing location and time of harvest, and ranged from 3.2 µg per 100 g FW to over 50 µg per 100 g FW in the varieties studied. Lutein accounted for approximately 25 % of the total carotenoid concentration depending on the time of harvest. Data gathered so far indicates that an average size yellow-fleshed potato (170 g) can contribute up to 100 µg of lutein, depending on the variety. Although lutein concentration can be influenced by growing location and time of harvest, chroma, total carotenoid and lutein are determined most through genetics. Satina and Victoria had consistently higher lutein than other varieties in 2005.

## 7. Presentation to Industry

The lab analyses from the 2005 growing season began in January. Some data is still not available as a result of some technical difficulties with the HPLC system. This report will be provided to all of our industry sponsors once it is approved. A poster version of the results will be prepared for the Potato Growers of Alberta Annual Meeting in November. Other presentations of data will be arranged as requested by sponsors and stakeholders.

## 8. Industry Reaction

The reaction from industry so far has been positive. Potato production has remained static in Alberta while acres of potatoes are being reduced in other growing areas. This project is being perceived as an effort to add value to fresh potato sales, and to provide a marketing angle to processors and packers alike. We have industry funds from several interested parties. The information generated in the preliminary project (2004) and the two-year Ag & Food Council project (2005 – 2007) will give us a firm base of scientific information from which to initiate a marketing study. It is not clear how industry intends to use the information to advantage. A collective effort between industry competitors is unlikely. Individual industry members may capitalize on this information differently.

## 9. References

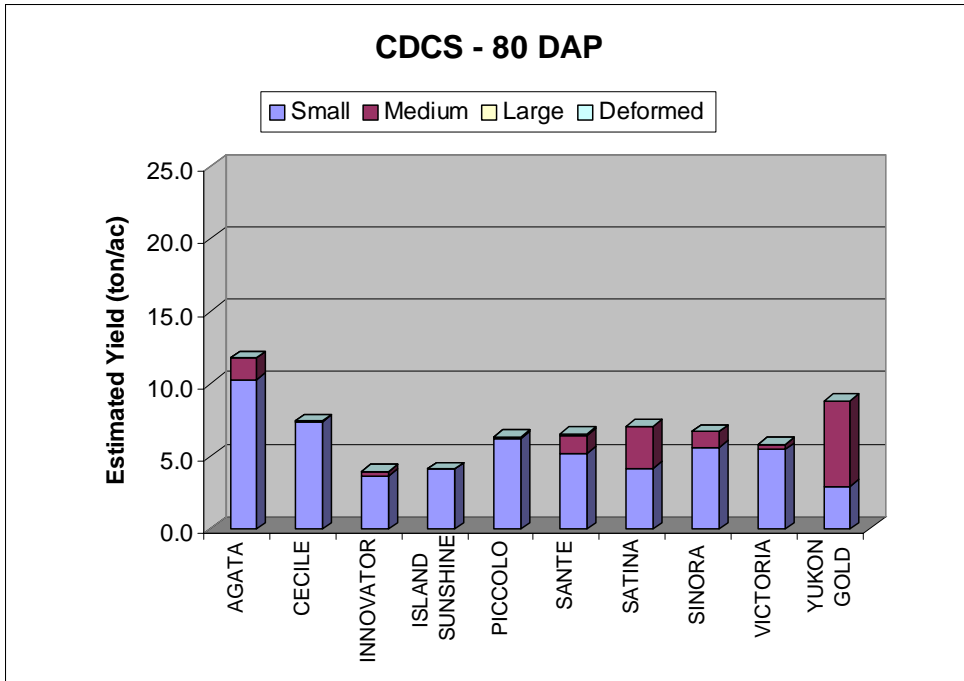
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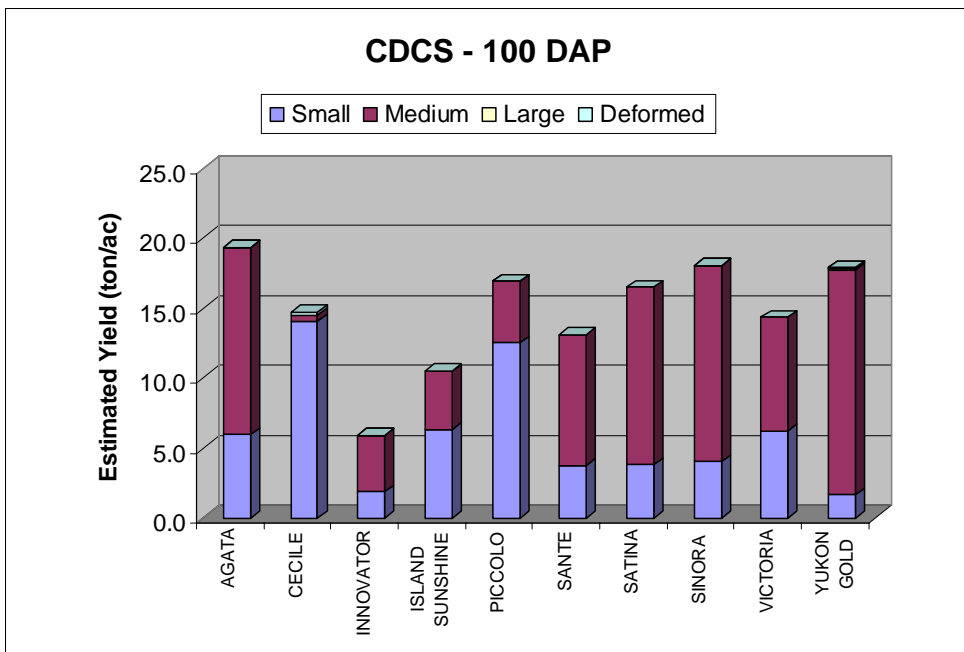
## 10. Summary

The color of yellow-fleshed potatoes is imparted by carotenoids. Carotenoids are anti-oxidant compounds that may protect against a variety of chronic diseases and certain cancers. Lutein is a specific carotenoid compound associated with a reduced incidence of age-related macular degeneration and cataract formation. This project involved growing ten yellow-fleshed potato varieties in three Alberta locations, harvesting at three different times and analyzing them for tuber flesh color intensity, total carotenoid content and lutein concentration. Total carotenoid content ranged from 17 to 250  $\mu\text{g}$  per 100 g FW and was positively correlated with tuber flesh color intensity, especially when tubers were harvested at 100 days after planting. Lutein accounted for approximately 25% of the total carotenoid content in many varieties and ranged from 3.2  $\mu\text{g}$  per 100 g FW in one variety (Sinora) check to over 50  $\mu\text{g}$  per 100 g FW in the variety Satina. Lutein concentration was determined most by variety, but varied with time of harvest and between locations. Satina and Victoria had consistently higher concentrations of lutein than most of the varieties studied. An average serving of Satina potatoes would provide approximately 100  $\mu\text{g}$  of dietary lutein. Potato varieties with significant concentrations of lutein may be marketed in the future as functional foods.

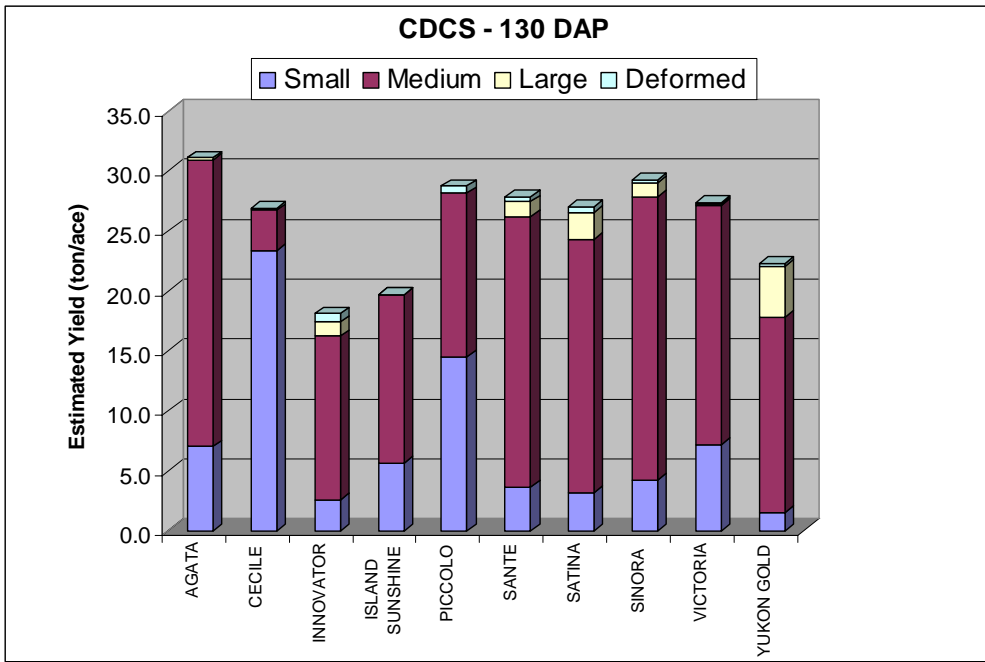
Appendix



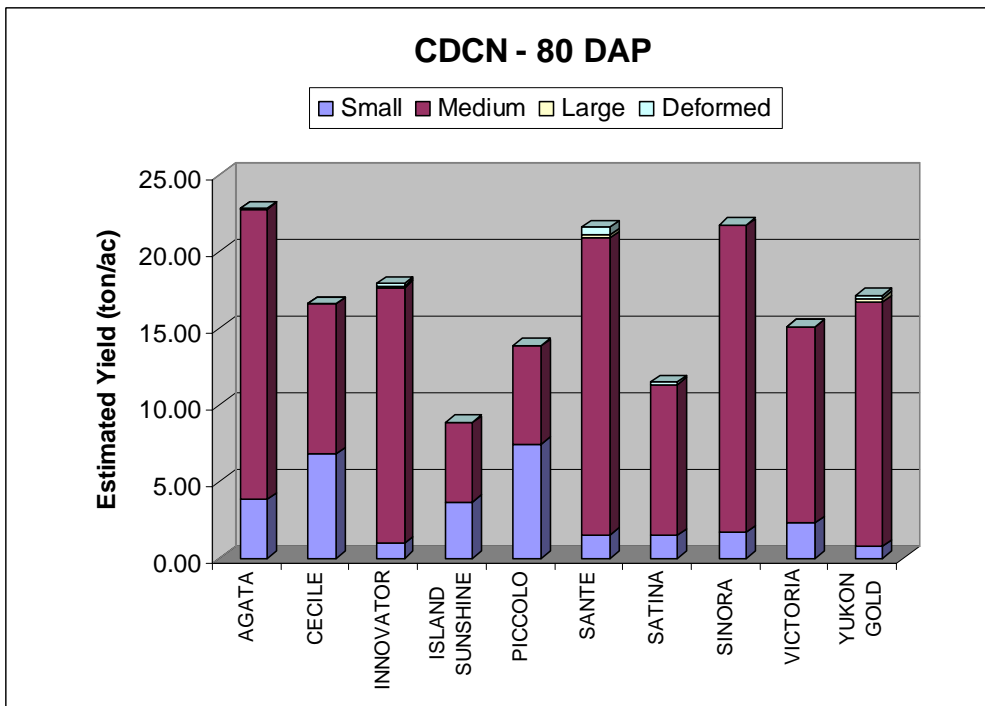
**Figure A1:** Estimated yield and grade (ton/ac) 80 days after planting (DAP) of each variety of yellow-fleshed potatoes grown at CDCS in Brooks in 2005.



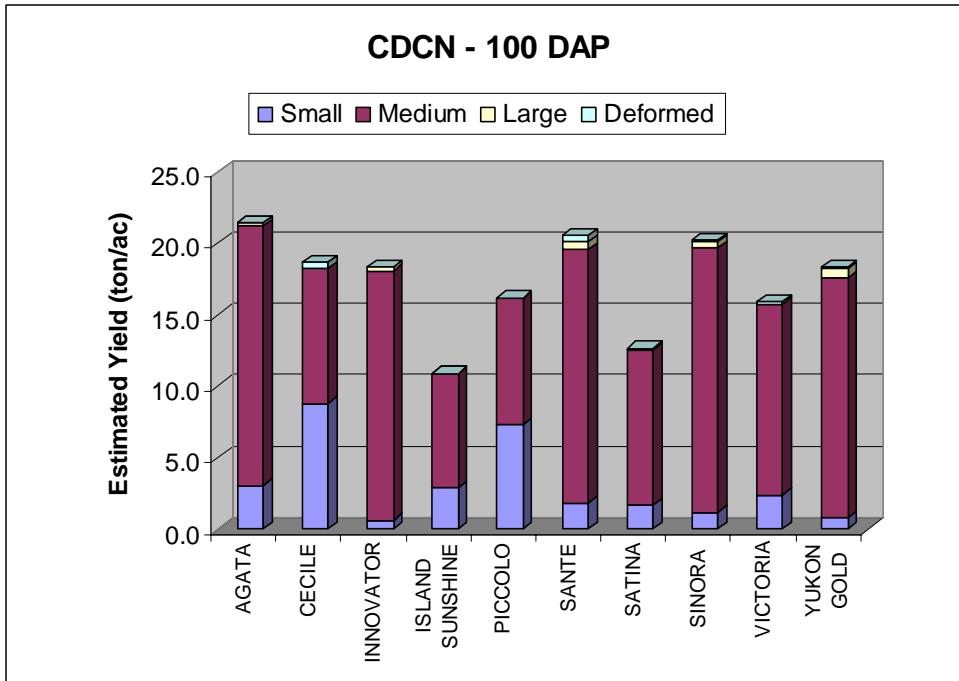
**Figure A2:** Estimated yield and grade (ton/ac) 100 days after planting of each variety of yellow-fleshed potatoes grown at CDCS in Brooks in 2005.



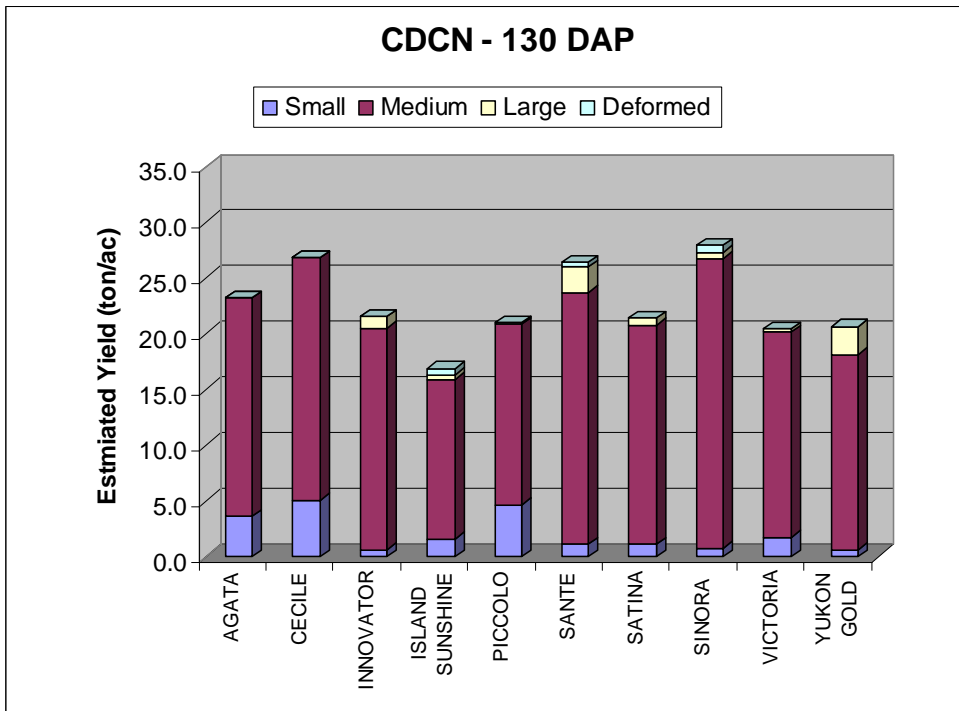
**Figure A3:** Estimated yield and grade (ton/ac) 130 days after planting of each variety of yellow-fleshed potatoes grown at CDCS in Brooks in 2005.



**Figure A4:** Estimated yield and grade (ton/acre) 80 days after planting of each variety of yellow-fleshed potatoes grown at CDCN in Edmonton in 2005.



**Figure A5:** Estimated yield and grade (ton/acre) 100 days after planting of each variety of yellow-fleshed potatoes grown at CDCN in Edmonton in 2005.



**Figure A6:** Estimated yield and grade (ton/acre) 130 days after planting of each variety of yellow-fleshed potatoes grown at CDCN in Edmonton in 2005.

**Table A1:** Specific gravity of yellow-fleshed potato varieties grown at CDCS (Brooks) and CDCN (Edmonton) in 2005 and harvested at different times after planting.

<i>Variety</i>	<i>CDCS</i>			<i>CDCN</i>		
	<i>80 Day</i>	<i>95 Day</i>	<i>Final</i>	<i>80 Day</i>	<i>95 Day</i>	<i>Final</i>
Agata	1.070	1.061	1.063	1.054	1.072	1.072
Innovator	1.089	1.071	1.078	1.074	1.100	1.094
Island Sunshine	1.077	1.072	1.087	1.069	1.098	1.099
Piccolo	1.080	1.075	1.081	1.065	1.088	1.088
Cecile (RZ94-83)	1.067	1.063	1.068	1.069	1.087	1.084
Sante	1.070	1.067	1.080	1.068	1.093	1.090
Satina	1.054	1.060	1.073	1.055	1.080	1.088
Sinora	1.083	1.074	1.081	1.087	1.094	1.096
Victoria	1.064	1.065	1.077	1.068	1.083	1.093
Yukon Gold	1.087	1.085	1.091	1.083	1.100	1.098



Lutein 2005  
20/bag

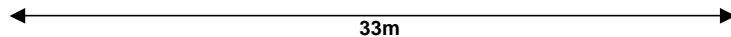


80 day harvest

95 day harvest

1	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>
2	1001 2 Cecile	2001 6 Sante	3001 4 Island Sunshine	4001 4 Island Sunshine
3	1002 1 Agata	2002 2 Cecile	3002 2 Cecile	4002 6 Sante
4	1003 9 Victoria	2003 9 Victoria	3003 9 Victoria	4003 1 Agata
5	1004 4 Island Sunshine	2004 7 Satina	3004 5 Piccolo	4004 3 Innovator
6	1005 8 Sinora	2005 5 Piccolo	3005 3 Innovator	4005 8 Sinora
7	1006 10 Yukon Gold	2006 1 Agata	3006 7 Satina	4006 9 Victoria
8	1007 7 Satina	2007 3 Innovator	3007 6 Sante	4007 10 Yukon Gold
9	1008 6 Sante	2008 4 Island Sunshine	3008 10 Yukon Gold	4008 5 Piccolo
10	1009 5 Piccolo	2009 8 Sinora	3009 1 Agata	4009 2 Cecile
11	1010 3 Innovator	2010 10 Yukon Gold	3010 8 Sinora	4010 7 Satina
12	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>

1	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>
2	5001 5 Piccolo	6001 10 Yukon Gold	7001 8 Sinora	8001 8 Sinora
3	5002 6 Sante	6002 7 Satina	7002 3 Innovator	8002 9 Victoria
4	5003 3 Innovator	6003 6 Sante	7003 1 Agata	8003 4 Island Sunshine
5	5004 9 Victoria	6004 5 Piccolo	7004 10 Yukon Gold	8004 7 Satina
6	5005 4 Island Sunshine	6005 9 Victoria	7005 5 Piccolo	8005 2 Cecile
7	5006 7 Satina	6006 1 Agata	7006 9 Victoria	8006 5 Piccolo
8	5007 1 Agata	6007 4 Island Sunshine	7007 2 Cecile	8007 1 Agata
9	5008 8 Sinora	6008 3 Innovator	7008 7 Satina	8008 3 Innovator
10	5009 10 Yukon Gold	6009 8 Sinora	7009 6 Sante	8009 6 Sante
11	5010 2 Cecile	6010 2 Cecile	7010 4 Island Sunshine	8010 10 Yukon Gold
12	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>



Final Harvest

1	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>
2	9001 9 Victoria	1101 7 Satina	1201 6 Sante	1301 5 Piccolo
3	9002 2 Cecile	1102 3 Innovator	1202 3 Innovator	1302 6 Sante
4	9003 5 Piccolo	1103 8 Sinora	1203 10 Yukon Gold	1303 2 Cecile
5	9004 10 Yukon Gold	1104 4 Island Sunshine	1204 4 Island Sunshine	1304 1 Agata
6	9005 3 Innovator	1105 6 Sante	1205 2 Cecile	1305 9 Victoria
7	9006 4 Island Sunshine	1106 2 Cecile	1206 9 Victoria	1306 10 Yukon Gold
8	9007 7 Satina	1107 9 Victoria	1207 8 Sinora	1307 3 Innovator
9	9008 1 Agata	1108 1 Agata	1208 1 Agata	1308 7 Satina
10	9009 8 Sinora	1109 5 Piccolo	1209 7 Satina	1309 8 Sinora
11	9010 6 Sante	1110 10 Yukon Gold	1210 5 Piccolo	1310 4 Island Sunshine
12	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>	<b>Guard</b>

6m