RESEARCH

# Effect of Cultivar and Environment on Carotenoid Profile of Pea and Chickpea

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## ABSTRACT

Increasing the carotenoid concentration of pulse crop seeds is part of a biofortification strategy. The objective of this research was to evaluate the concentration and distribution of carotenoids in the seeds of twelve pea (*Pisum sativum* L.) cultivars and eight chickpea (*Cicer arietinum* L.) cultivars grown at multiple locations during 2 yr in Saskatchewan, Canada using high performance liquid chromatography (HPLC) with a diode array detector. Lutein was the major carotenoid in both crops, with mean lutein concentration ranging from 7.2 μg $g^{-1}$ to 17.6 μg $g^{-1}$ and 6.3 μg $g^{-1}$ to 11.0 μg $g^{-1}$ in pea and chickpea, respectively. Violaxanthin, zeaxanthin, and β-carotene were also present in both crops. Green cotyledon pea cultivars had approximately twice as many total carotenoids (16–21 μg $g^{-1}$) than yellow cotyledon pea cultivars (7–12 μg $g^{-1}$). Cultivar had a greater effect than environment on carotenoid concentration in both crops. Location effects were significant for violaxanthin, lutein, and total carotenoid concentration for pea and for violaxanthin and zeaxanthin in chickpea. Year effect was significant for all carotenoids in pea and significant for β-carotene in chickpea. The cultivar × location interaction was significant for violaxanthin in pea and chickpea and for lutein in pea. Among the three seed tissues, carotenoid concentration was greatest in the cotyledon followed by the embryo axis and seed coat in both crops. The results of this investigation should be useful for improving nutritional quality in pulse crops.

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**Abbreviations:** BHT, butylated hydroxytoluene; C × E, cultivar by environment; CDC, Crop Development Centre, University of Saskatchewan; DCM, dichloromethane; HPLC, high performance liquid chromatography; MeOH, methanol.

Carotenoids are natural pigments synthesized by plants and some microorganisms. Humans and animals are not able to synthesize carotenoids de novo; thus they need to acquire them through their diet (Fraser and Bramley, 2004). More than 600 carotenoids have been identified in nature to date; around 40 of these are consumed in the typical human diet (Khachik et al., 1995). Carotenoids exhibit yellow, orange, and red color, but when they are bound to proteins, they acquire green, purple, or blue colors (Britton et al., 1997). β-Carotene is the most widely distributed carotenoid in plants and the one most efficiently converted to vitamin A. The fat-soluble vitamin A plays an important role in vision, bone growth, reproduction, cell division, and cell differentiation in mammals (Stephens et al., 1996). At least 3 million children in developing countries develop xerophthalmia (damage to the cornea of the eye), and 250,000 to 500,000 become blind each year because of vitamin A deficiency (Reifen, 2002).

Lutein and zeaxanthin do not have provitamin activity, but display biological activity in relation to human health. Lutein and its stereoisomer of zeaxanthin are the only carotenoids present in the macula region of the retina where they are effective

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against senile macular degeneration (Krinsky et al., 1990; Meydani et al., 1994; Olmedilla et al., 2001). Lutein may also help to protect skin from ultraviolet (UV) radiation-induced damage and reduce the risk of cardiovascular disease and cataracts (Alves-Rodrigues and Shao, 2004; Moeller et al., 2000). Carotenoids may also protect humans from skin disorders and several forms of cancer (Bramley, 2000; Snodderly, 1995). Carotenoids efficiently scavenge peroxyl radicals and play an important role in the protection of cellular membranes and lipoproteins against oxidative damage (Stahl et al., 2002).

Carotenoids are classified into two groups: the hydrocarbon carotenoids including α- and β-carotene and lycopene and the oxygenated carotenoids or xanthophylls (lutein, zeaxanthin, violaxanthin and β-cryptoxanthin). The first step in carotenoid biosynthesis is the condensation of two molecules of geranylgeranyl diphosphate by phytoene synthase to form phytoene, (Cunningham et al., 1994). A major branch point occurs after lycopene synthesis when cyclization mediated by the enzymes lycopene-β-cyclase and lycopene-epsilon-cyclase gives rise to α-carotene and β-carotene. α-Carotene is acted on by a β-ring hydroxylase to form zeinoxanthin, which is then hydroxylated by an ε-ring hydroxylase to produce lutein. β-Carotene can be hydroxylated in a two-step reaction to zeaxanthin, with β-cryptoxanthin as an intermediate product. In green tissues, zeaxanthin can be epoxidized to violaxanthin, which is the precursor of the plant hormone abscisic acid (Cunningham and Gantt, 1998).

Traditionally, pulse crops have been used for human consumption around the world. The nutritional value of pea and chickpea are important for human health in developing countries. Biofortification, enriching the nutritional contribution of staple crops through plant breeding, is one option that is now widely discussed internationally in the fields of nutrition and public health. Biofortification of staple food crops is a new approach to control deficiencies of carotenoids, Fe, and Zn in developing countries (Welch and Graham, 2005). Crop biofortification could also increase competitiveness through product differentiation, niche marketing, and value adding. Biofortification of staple food products is seen as one of the key strategies for alleviating micronutrient malnutrition afflicting poor communities, alongside the more traditional interventions of supplementation, fortification, and dietary diversification (White and Broadley, 2005). As pea and chickpea are significant food sources in many countries where malnutrition is prevalent, pea and chickpea with higher concentrations of carotenoids may contribute to solutions for human micronutrient malnutrition, especially for South Asian populations.

Many studies have been undertaken in recent years to provide data in relation to the carotenoid content of various crops, such as carrot [*Daucus carota* ssp. *sativus* (Hoffm.) Schübl. & Martens] (Chen et al., 1995), tomato (*Solanum lycopersicum* L.; Abushita et al., 2000; Fraser et al., 2001), wheat (*Triticum aestivum* L.; Hentschel et al., 2002; Hidalgo and Brandolini, 2008; Ramachandran et al., 2010), maize (*Zea mays* L.; Menkir and Maziya-Dixon, 2004), cassava (*Manihot esculenta* Crantz; Ssemakula et al., 2007), and potato (*Solanum tuberosum* L.; Haynes et al., 2010). Identification and quantification of individual carotenoids by HPLC is a highly sensitive, precise, and reproducible method. To date, only limited research on carotenoids has been reported in pea and chickpea seeds using HPLC analysis. Lutein and zeaxanthin were reported in chickpea seed (Abbo et al., 2005, 2010), and lutein, β-carotene, and violaxanthin were reported in cotyledons and seed coat of field pea (Holasová et al., 2009; McCallum et al., 1997). Lutein was reported as the major carotenoid in chickpea and pea (Abbo et al., 2005, 2010; Holasová et al., 2009; McCallum et al., 1997). Lutein concentration was reported to be greater in green cotyledon compared with yellow cotyledon pea (Holasová et al., 2009). The effect of cultivar and environment on carotenoid profile has not yet been reported in pea and only limited studies are available for chickpea (Abbo et al., 2010). Saskatchewan is a leading supplier of pea and chickpea in export markets; thus, characterizing the carotenoid profile in Saskatchewan-grown pulses is important. This research was conducted to assess the four individual carotenoids (violaxanthin, lutein, zeaxanthin, and β-carotene) and total carotenoids in pea and chickpea seeds. The information generated in this research will be used in developing nutritional marketing strategies for key export markets.

The objectives of this study were (i) to determine the significance of cultivar, environment, and the cultivar × environment (C × E) interaction on the concentration of carotenoids in seeds of pea and chickpea cultivars grown in Saskatchewan and identify the cultivars that contained higher levels of individual and total carotenoids and (ii) to determine the distribution of carotenoids in seed tissues (i.e., cotyledon, seed coat, and embryo axis) of contrasting pea and chickpea cultivars.

## MATERIALS AND METHODS

### Seed Samples and Field Trials

Seed samples of twelve pea cultivars were obtained from regional variety trials conducted in 2009 and 2010 by the Crop Development Centre (CDC), University of Saskatchewan. Trials were arranged as a randomized complete block design at each location (Saskatoon: 52°10'05.29" N, 106°30'18.96" W; Rosthern: 52°44'24.06" N, 106°14'08.80" W; Meath Park: 53°14'54.36" N, 105°21'10.90" W; and Scott: 52°22'36.73" N, 108°38'33.37" W) in Saskatchewan. The Saskatoon and Scott locations are located in the Dark Brown soil zone, Rosthern in the Thin Black soil zone, and Meath Park in the Black soil zone of the province. Seed subsamples of the twelve pea cultivars were taken at random from the entire harvested lot from two field plots at each location (i.e., two biological replicates).

**Table 1. Description of pea and chickpea cultivars evaluated.**

| Crop and cultivar | Cotyledon color | seed coat color | Breeder† | Pedigree |
|---|---|---|---|---|
| Pea | | | | |
| Agassiz | yellow | nonpigmented | AAFC, Canada | AC Tamor/Montana//Grande |
| CDC Bronco | yellow | nonpigmented | CDC, Canada | 93–7-39YG/Grande//CDC Winfield/Highlight |
| CDC Golden | yellow | nonpigmented | CDC, Canada | Local Syrian 1690/Alfetta//CDC Vienna/Express |
| CDC Meadow | yellow | nonpigmented | CDC, Canada | CDC Mozart/(Carneval/PI251051//Radley/CDC Vienna) |
| Cutlass | yellow | nonpigmented | CDC, Canada | Carneval///Miranda/Carrera//Montana/CDC-Winfield |
| Eclipse | yellow | nonpigmented | Limagrain, Netherlands | 87318–001/Cebeco1441 |
| Polstead | yellow | nonpigmented | Limagrain, Netherlands | Unknown |
| Thunderbird | yellow | nonpigmented | AAFC, Canada | Carneval//Montana/Miko |
| CDC Tetris | green | nonpigmented | CDC, Canada | 646–46/Espace |
| CDC Patrick | green | nonpigmented | CDC, Canada | SW Parade//203PMR-18G/4L25 |
| CDC Striker | green | nonpigmented | CDC, Canada | Majoret/P28RS-281 |
| Cooper | green | nonpigmented | Limagrain, Netherlands | Baccara/92585 |
| Chickpea—Kabuli varieties | | | | |
| Amit | yellow | nonpigmented | Terramax, Canada | Selection from a Bulgarian landrace |
| CDC Frontier | yellow | nonpigmented | CDC, Canada | FLIP91–22C/ICC14912 |
| CDC Luna | yellow | nonpigmented | CDC, Canada | FLIP 91–123C/FLIP84–79C//FLIP90- 127C |
| CDC Orion | yellow | nonpigmented | CDC, Canada | FLIP95–48C/93–120–63K |
| CDC Leader | yellow | nonpigmented | CDC, Canada | FLIP95–48C/CISN-SP-99PL21117 |
| Chickpea—Desi varieties | | | | |
| CDC Cabri | yellow | tan | CDC, Canada | ICCX-860027/ICCX-860047 |
| CDC Vanguard | yellow | tan | CDC, Canada | 92073–60D//92056–8/ICCV96029 |
| CDC Corinne | yellow | tan | CDC, Canada | Selection from landrace ICC12512 |

† AAFC, Agriculture and Agri-Food Canada; CDC, Crop Development Centre, University of Saskatchewan.

Seed samples of eight chickpea cultivars were obtained from regional variety trials conducted in 2009 and 2011 by the CDC. The 2010 trials were lost due to excess rainfall. Trials were arranged as a randomized complete block design at each location (Elrose: 51°16'17.68" N, 107°58'43.15" W; Pasqua: 50°20'48.74" N, 105°20'00.63" W; and Floral: 52°03'56.64" N, 106°26'03.45" W in 2009; Elrose, and Pasqua in 2011) in Saskatchewan. Elrose is located in the Brown soil zone, while Pasqua and Floral are in the Dark Brown soil zone. Chickpea seed subsamples were taken at random from the entire harvested lot from three replications at each location. Varietal descriptions of pea and chickpea cultivars used in study are presented in Table 1.

For the C × E interaction study, subsamples of 20 g of harvested pea and chickpea seeds were air dried to 14% moisture and stored at −20°C until milled. Samples were ground in a UDY mill equipped with a 0.5-mm screen. A vacuum was used to clean the mill between samples. Ground samples were stored at −20°C and 1 wk before extraction samples were kept at room temperature. One-gram samples were used for extraction.

For the tissue study, 400-mg seed samples of selected pea and chickpea cultivars were dehulled using a Satake Grain Testing Mill (Model TM05, Satake Corporation, Taito-ku, Tokyo, Japan) followed by air fractionation and hand sorting under 2× magnification to separate the cotyledon, seed coat, and embryo axis. These tissues were then ground using a ZM-200 Ultra centrifugal mill (Retsch, Haan, Germany) to pass a 0.5-mm sieve. Pea samples were derived from the Rosthern and Sutherland locations in 2011, while chickpea samples were derived from the Floral location in 2011.

## Standard Calibration

Standards of lutein and violaxanthin (ChromaDex, Irvine, CA), and zeaxanthin and β-carotene (95% purity; Sigma-Aldrich Canada, Oakville, ON) were used to construct linear standard curves by injecting 2 to 40 ng (violaxanthin) or 4 to 80 ng (others). Standard extraction solvents were initially premixed with 0.1% butylated hydroxytoluene (BHT) in dichloromethane (DCM) and methanol (MeOH), v/v (3:1) for β-carotene and v/v (1:1) for others. All the stock solutions of chemical references were stored at −80°C. Chromatographic peaks were identified by comparing retention times and absorbance spectra to those of standards. A peak was identified as a putative carotenoid if characteristic triple maxima were observed in the absorbance spectrum and retention time was 6.4, 10.6, 12.4, and 35.2 min for violaxanthin, lutein, zeaxanthin, and β-carotene, respectively. All carotenoids were detected at 450 nm, the maximum absorbance for lutein.

## Extraction and HPLC Sample Preparation

In preliminary studies, three solvent extraction methods [MeOH:DCM, MeOH:MTBE (methyl-*t*-butyl ether), and *n*-hexane] were evaluated for estimation of carotenoid concentration in selected cultivars of pea and chickpea. Methanol:dichloromethane had the lowest volatility and 100% recovery for total carotenoids and was therefore utilized for the entire study. To protect carotenoids from degradation, all standards and sample extractions were performed under subdued light conditions at room temperature of 20°C. Sample extraction solvents were initially premixed with 0.1% BHT in DCM and MeOH, v/v (1:1) and added at the rate of 5 mL $g^{-1}$ tissue in Pyrex tubes capped with Teflon liners (Kimble Glassware, VWR, Mississauga, ON, Canada). Samples were immediately

Table 2. Mean squares of combined ANOVA and coefficient of variation (CV) for carotenoids in pea and chickpea cultivars grown in Saskatchewan, Canada.

| | | | Mean squares | | | | |
|---|---|---|---|---|---|---|---|
| | Cultivar (C) | Year (Y) | Location (L) | C × Y | C × L | C × Y × L | CV |
| | | | | | | | % |
| Pea | | | | | | | |
| Violaxanthin | 4132.0*** | 438.3*** | 127.8*** | 44.1*** | 24.1*** | 10.5*** | 15.6 |
| Lutein | 1650.0*** | 144.7*** | 38.9*** | 14.7*** | 7.5*** | 6.5*** | 7.1 |
| Zeaxanthin | 39.3*** | 0.1 ns | 3.9ns | 7.2*** | 1.3ns | 2.3ns | 15.1 |
| β-carotene | 1475.4*** | 92.1*** | 1.1ns | 19.9*** | 0.7ns | 1.4ns | 14.6 |
| Total carotenoids | 2508.1*** | 126.9*** | 45.8*** | 13.7*** | 126.9*** | 5.7*** | 6.9 |
| Chickpea | | | | | | | |
| Violaxanthin | 361.3*** | 16.2 | 15.6*** | 2.5ns | 4.5*** | 1.5ns | 16.3 |
| Lutein | 42.6*** | 5.2ns | 2.0ns | 0.4ns | 0.8ns | 0.2ns | 11.2 |
| Zeaxanthin | 39.2*** | 2.1ns | 11.1*** | 0.7ns | 0.6ns | 0.1ns | 9.5 |
| β-carotene | 76.4*** | 102.7*** | 3.3ns | 5.4*** | 1.9ns | 3.0ns | 18.4 |
| Total carotenoids | 66.25*** | 1.3ns | 4.9ns | 0.3ns | 1.5ns | 0.1ns | 7.9 |

*** Significant at the 0.001 probability level.

vortexed followed by shaking at 200 rpm for 1 h in a Bigger Bill shaker (Thermolyne Corporation, Dubuque, IA). Extracts were treated with the diluent (100% acetonitrile) to remove proteins and some lipids, and centrifuged at 11,000 *g* for 5 min. For HPLC separation, the centrifuged supernatant was filtered (0.2 μm polyvinylidene fluoride), placed in 250-μL glass inserts (Agilent Technologies Canada Inc., Mississauga, ON, Canada) in 2-mL amber glass HPLC vials and analyzed by HPLC.

## HPLC Separation and Analysis

Chromatography was performed using the Agilent 1200 LC system with Chemstation software (Agilent Technologies, Santa Clara, CA) on a reverse-phase C30 column (3 μm, 4.6 by 250 mm), preceded by a 4 by 20 mm C30 guard column (Waters, YMC America, Newtown, PA) at 24°C with a diode array detector set at 190 to 600 nm. Isocratic elution (58:22:20, $CH_3CN$:$CH_3OH$:$CH_2Cl_2$) was used to separate compounds in the extracts for up to 40 min at flow rate of 0.8 mL $min^{-1}$, and injection volume was 20 μL per sample and carotenoids were detected at 450 nm. A column wash (40°C) with 50:49:1 (v/v/w) 2-propanol/water/acetic acid was performed after every 25 samples (Ramachandran et al., 2010). All eluent solvents were of HPLC grade (Fisher Scientific, Ottawa, ON, Canada or EMD Chemicals, Gibbstown, NJ). Carotenoid concentration was identified by UV-visible spectra analysis and by comparing their retention times with authentic standards. The concentration of carotenoids was calculated using the calibrated standard curve of each component, and each sample was extracted in two technical repeats and analyzed by HPLC.

## Statistical Analysis

Results of each carotenoid component were converted to μg $g^{-1}$. Technical repeats were averaged for each biological replicate. Analysis of variance was performed using the Mixed Linear Model procedure (PROC MLM) of SAS version 9.3 for Windows (SAS Institute, 2011). Cultivars were considered as fixed factor and environments and replications (blocks) within environment as random factors. Levene's test for homogeneity of variance and boxplot distributions were conducted for each location and year. Combined ANOVA was initially conducted across all locations and years; whenever there were significant interactions between cultivars, locations, and years, separate analyses were conducted for each location and year. Means were separated by LSD at the 0.05 level.

# RESULTS AND DISCUSSION

## Carotenoid Identification

Carotenoid concentration was evaluated for seeds of twelve pea cultivars grown at four locations for 2 yr and eight chickpea cultivars grown at three locations for 1 yr and two locations for 1 yr in Saskatchewan. Violaxanthin, lutein, zeaxanthin, and β-carotene were identified in the mature pea and chickpea seeds and were quantified using standard curves generated from primary standards of each carotenoid. Several unidentifiable peaks were also detected, but were not quantified. Total carotenoid concentration was calculated as the sum of mean values of the four individual carotenoids.

## Environmental Effects on Carotenoid Concentration in Pea and Chickpea Cultivars

Combined ANOVA indicated a significant effect of cultivar for all four carotenoids as well as for total carotenoid concentration in both pea and chickpea (Table 2). Year effect was significant for all carotenoids except zeaxanthin in pea and significant for β-carotene in chickpea. Location effect was significant for violaxanthin, lutein, and total carotenoids in pea and for violaxanthin and zeaxanthin in chickpea. The interaction between cultivar and location was significant for violaxanthin, lutein, and total carotenoids in pea and violaxanthin for chickpea, similar to reports in potato (Haynes et al., 2010), wheat (Matus-Cádiz et al., 2003; Ramachandran et al., 2010), maize (Menkir and Maziya-Dixon, 2004), and cassava (Ssemakula et al., 2007). The interaction between cultivar and year was significant for all carotenoids in pea and for β-carotene in

**Table 3. Mean Zeaxanthin and β-carotene concentrations in 12 pea cultivars grown at four locations in Saskatchewan, Canada in 2009 and 2010.**

| Cultivar | Zeaxanthin† | β-carotene† |
|---|---|---|
| | μg g$^{-1}$ | |
| Yellow cotyledon | | |
| Agassiz | 0.11d | 0.01c |
| CDC Bronco | 0.15b | 0.04c |
| CDC Golden | 0.18a | 0.03c |
| CDC Meadow | 0.18a | 0.03c |
| Cutlass | 0.15bc | 0.03c |
| Eclipse | 0.14bc | 0.03c |
| Polstead | 0.13c | 0.01c |
| Thunderbird | 0.13c | 0.02c |
| Green cotyledon | | |
| CDC Tetris | 0.18a | 1.52a |
| CDC Patrick | 0.20a | 1.48a |
| CDC Striker | 0.19a | 1.25b |
| Cooper | 0.19a | 1.25b |
| Overall | 0.16 | 0.47 |
| Location and year | | |
| Meath Park 2009 | 0.15a | 0.54a |
| Rosthern 2009 | 0.17a | 0.55a |
| Scott 2009 | 0.18a | 0.54a |
| Sutherland 2009 | 0.16a | 0.47a |
| Meath Park 2010 | 0.16a | 0.44a |
| Rosthern 2010 | 0.16a | 0.40a |
| Scott 2010 | 0.15a | 0.40a |
| Sutherland 2010 | 0.17a | 0.47a |
| SE‡ | 0.01 | 0.05 |

† Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

‡ SE, pooled standard error of mean calculated for each carotenoid (ug g$^{-1}$) from mean square of ANOVA for each location ($n$ = 192)

chickpea. The cultivar × year × location interactions were significant for violaxanthin, lutein and total carotenoids in pea, while their interaction effects were not significant for the carotenoid components in chickpea (Table 2).

## Carotenoid Concentration in Pea Cultivars

Mean zeaxanthin and β-carotene concentrations across locations and years in pea cultivars are shown in Table 3, while concentrations of violaxanthin, lutein, and total carotenoid are presented in Table 4. On average, pea cultivars were high in lutein (11.45 μg g$^{-1}$), followed by violaxanthin (0.52 μg g$^{-1}$), β-carotene (0.47 μg g$^{-1}$), and zeaxanthin (0.16 μg g$^{-1}$). Lutein was previously reported as the major source of carotenoids in pea seeds (Holasová et al., 2009; McCallum et al., 1997). Mean zeaxanthin concentration ranged from 0.11 μg g$^{-1}$ in Agassiz to 0.20 μg g$^{-1}$ in CDC Patrick (Table 3). Mean β-carotene concentration ranged substantially from 0.01 μg g$^{-1}$ in Agassiz and Polstead to 1.52 μg g$^{-1}$ in CDC Tetris. This result is similar to a range of 1 to 2 μg g$^{-1}$ of β-carotene in green cotyledon peas previously reported (Holasová et al., 2009). Field peas with yellow or orange cotyledons had β-carotene concentration 10 times lower than green cotyledon cultivars (Holasová et al., 2009).

Green cotyledon pea cultivars had greater β-carotene concentration (1.25–1.52 μg g$^{-1}$) than yellow cotyledon cultivars (0.01–0.04 μg g$^{-1}$), and green cotyledon cultivar CDC Tetris had the highest level of β-carotene (1.52 μg g$^{-1}$), which was similar to the β-carotene level (1.60 μg g$^{-1}$) observed in golden rice (*Oryza sativa* L.) endosperm (Beyer et al., 2002). Green cotyledon pea cultivars had approximately twice as many total carotenoids (16–21 μg g$^{-1}$) than found in yellow cotyledon pea cultivars (7–12 μg$^{-1}$), approximately 40% less total carotenoids than reported in golden rice 2 $T_2$ endosperm (8.8 to 36.7 μg g$^{-1}$), (Paine et al., 2005). However, α-carotene and β-cryptoxanthin were quantified in golden rice 2 which were not quantified in the present study.

In general, the green cotyledon cultivars such as CDC Tetris and CDC Patrick also had higher violaxanthin concentration across locations in both years than the yellow cotyledon cultivars (Table 4). The range of violaxanthin concentrations reported here is similar to the levels reported (1.70–2.22 μg g$^{-1}$) in vegetable pea (Edelenbos et al., 2001) and from trace concentrations to 4.76 μg g$^{-1}$ of violaxanthin observed in bleached vs. unbleached field pea seeds (McCallum et al., 1997). The mean lutein concentration ranged from 6.65 μg g$^{-1}$ in Agassiz from Rosthern in 2009 to 19.40 μg g$^{-1}$ in CDC Tetris in Rosthern in 2010 (Table 4). These values are much greater than those previously reported (0.8–1.4 μg g$^{-1}$and 0.7 μg g$^{-1}$in green and yellow cotyledon pea seeds, respectively; Holasová et al., 2009).

Green cotyledon pea cultivars were richer in total carotenoids then yellow cotyledon cultivars. This could be due to greater expression of the lycopene cyclase gene in green cotyledon cultivars. Lycopene cyclase plays a major role in producing α- and β-carotene. α-Carotene is acted on by a β-ring hydroxylase to form zeinoxanthin, which is then hydroxylated by ε-ring hydroxylase to produce lutein, and in green tissues, zeaxanthin can be epoxidized to produce violaxanthin (Demmig-Adams and Adams, 2002). β-Carotene, and lutein levels are controlled by the transcriptional activity of the lycopene β-cyclase gene in kiwifruit (*Actinidia chinensis* Planch.; Khattak et al., 2008), and microalga *Dunaliella salina* (Zhu et al., 2008).

## Carotenoid Concentration in Chickpea Cultivars

Significant genotypic differences ($p < 0.05$) in mean carotenoid concentration in chickpea seeds were observed (Table 5). Overall mean showed chickpea cultivars were highest in lutein (7.70 μg g$^{-1}$) followed by zeaxanthin (5.76 μg g$^{-1}$), β-carotene (0.40 μg g$^{-1}$), and violaxanthin (0.05 μg g$^{-1}$). Lutein as the major source of carotenoids was reported in seeds of chickpea (Abbo et al., 2005, 2010) and wheat (Ramachandran et al., 2010).

Mean violaxanthin concentration in the desi cultivars was 0.10 μg g$^{-1}$and kabuli cultivars 0.02 μg g$^{-1}$. Mean zeaxanthin concentration ranged from 4.21 μg g$^{-1}$ in CDC

Table 4. Violaxanthin, lutein, and total carotenoid concentrations in 12 pea cultivars grown at four locations in Saskatchewan, Canada in 2009 and 2010.

| | Violaxanthin | | | | Lutein | | | | Total carotenoid† | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Meath Park | Rosthern | Scott | Sutherland | Meath Park | Rosthern | Scott | Sutherland | Meath Park | Rosthern | Scott | Sutherland |
| | μg g$^{-1}$ | | | | | | | | | | | |
| 2009 | | | | | | | | | | | | |
| Agassiz | 0.12 | 0.13 | 0.15 | 0.15 | 6.67 | 6.65 | 7.82 | 7.39 | 6.89 | 6.89 | 8.10 | 7.66 |
| CDC Bronco | 0.07 | 0.06 | 0.17 | 0.14 | 11.86 | 10.41 | 11.93 | 11.11 | 12.15 | 10.71 | 12.36 | 11.41 |
| CDC Golden | 0.12 | 0.10 | 0.13 | 0.13 | 11.99 | 11.80 | 11.48 | 10.69 | 12.42 | 12.22 | 11.86 | 11.00 |
| CDC Meadow | 0.10 | 0.12 | 0.22 | 0.15 | 10.70 | 11.30 | 11.85 | 10.89 | 11.08 | 11.71 | 12.35 | 11.20 |
| CDC Patrick | 1.09 | 1.01 | 1.34 | 1.51 | 16.80 | 16.23 | 17.15 | 16.15 | 19.74 | 19.12 | 20.43 | 19.25 |
| CDC Striker | 0.95 | 0.92 | 0.95 | 0.84 | 13.41 | 11.13 | 14.26 | 12.04 | 15.83 | 13.57 | 16.79 | 14.41 |
| CDC Tetris | 1.13 | 1.14 | 1.64 | 1.61 | 17.47 | 16.70 | 16.35 | 16.58 | 20.50 | 19.78 | 19.98 | 20.11 |
| Cooper | 0.92 | 0.95 | 1.15 | 1.18 | 14.19 | 13.51 | 13.61 | 13.30 | 16.53 | 15.85 | 16.22 | 15.86 |
| Cutlass | 0.11 | 0.12 | 0.14 | 0.19 | 7.62 | 8.42 | 9.02 | 8.97 | 7.99 | 8.78 | 9.34 | 9.31 |
| Eclipse | 0.11 | 0.11 | 0.18 | 0.25 | 7.90 | 8.01 | 9.11 | 7.92 | 8.20 | 8.40 | 9.46 | 8.30 |
| Polstead | 0.10 | 0.11 | 0.17 | 0.16 | 8.23 | 7.84 | 8.96 | 8.25 | 8.46 | 8.08 | 9.27 | 8.54 |
| Thunderbird | 0.06 | 0.06 | 0.19 | 0.11 | 7.39 | 7.36 | 9.57 | 7.78 | 7.61 | 7.61 | 9.98 | 8.04 |
| LSD 0.05 | 0.044 | 0.058 | 0.098 | 0.048 | 0.762 | 1.066 | 0.786 | 0.599 | 0.768 | 1.160 | 0.919 | 0.630 |
| 2010 | | | | | | | | | | | | |
| Agassiz | 0.18 | 0.18 | 0.12 | 0.19 | 7.25 | 7.09 | 6.80 | 8.07 | 7.60 | 7.39 | 7.03 | 8.39 |
| CDC Bronco | 0.15 | 0.17 | 0.09 | 0.20 | 11.46 | 11.10 | 10.57 | 14.25 | 11.76 | 11.44 | 10.77 | 14.64 |
| CDC Golden | 0.14 | 0.13 | 0.13 | 0.17 | 11.59 | 11.07 | 10.53 | 12.07 | 11.93 | 11.36 | 10.80 | 12.46 |
| CDC Meadow | 0.14 | 0.12 | 0.10 | 0.16 | 10.74 | 10.96 | 10.35 | 12.09 | 11.07 | 11.24 | 10.59 | 12.44 |
| CDC Patrick | 1.22 | 1.42 | 1.22 | 1.80 | 16.42 | 17.35 | 16.75 | 17.95 | 19.21 | 20.22 | 19.46 | 21.45 |
| CDC Striker | 1.08 | 1.09 | 0.90 | 1.19 | 15.92 | 13.06 | 14.14 | 14.74 | 18.37 | 15.46 | 16.37 | 17.28 |
| CDC Tetris | 1.82 | 1.60 | 1.72 | 1.81 | 17.96 | 19.40 | 17.90 | 18.55 | 21.29 | 22.39 | 20.98 | 21.99 |
| Cooper | 1.31 | 1.06 | 1.27 | 1.73 | 14.53 | 13.45 | 15.61 | 16.09 | 17.30 | 15.90 | 18.28 | 19.46 |
| Cutlass | 0.23 | 0.22 | 0.15 | 0.21 | 9.59 | 8.72 | 8.07 | 10.78 | 10.00 | 9.08 | 8.38 | 11.17 |
| Eclipse | 0.32 | 0.26 | 0.14 | 0.24 | 9.04 | 8.21 | 7.53 | 9.53 | 9.52 | 8.61 | 7.83 | 9.92 |
| Polstead | 0.18 | 0.16 | 0.12 | 0.20 | 8.34 | 8.04 | 7.50 | 9.72 | 8.67 | 8.35 | 7.78 | 10.12 |
| Thunderbird | 0.18 | 0.14 | 0.09 | 0.19 | 8.95 | 8.15 | 7.25 | 9.11 | 9.29 | 8.43 | 7.46 | 9.46 |
| LSD 0.05 | 0.143 | 0.061 | 0.047 | 0.057 | 0.749 | 0.732 | 0.765 | 0.317 | 0.850 | 0.784 | 0.791 | 0.334 |

† Total carotenoid concentration was calculated as the sum of four individual carotenoids.

Orion to 7.46 μg g$^{-1}$ in CDC Corinne. Mean lutein concentration ranged from 6.28 μg g$^{-1}$ in CDC Orion to 10.96 μg g$^{-1}$ in CDC Corinne, and these are higher than the levels (2.75–6.22 μg g$^{-1}$) previously reported in chickpea (Abbo et al., 2010). Mean β-carotene concentration ranged from 0.24 μg g$^{-1}$ in Amit to 0.56 μg g$^{-1}$ in CDC Corinne. Overall mean β-carotene concentration was 0.40 μg g$^{-1}$, which was equal to the concentration of β-carotene reported previously (Khattak et al., 2008).

Desi chickpea cultivars had higher average total carotenoid concentration (16.80 μg g$^{-1}$) than kabuli cultivars (12.33 μg g$^{-1}$; Table 4). Chickpea seeds had a greater concentration of zeaxanthin than that found in pea seeds. In the carotenoid biosynthetic pathway, lycopene β-cyclase produces β-carotene, which is hydroxylated in a two-step reaction to produce zeaxanthin (Demmig-Adams and Adams, 2002), and accordingly, greater expression of the lycopene β-cyclase gene may increase the levels of carotenoids in chickpea seeds.

In chickpea, growing environment significantly affected violaxanthin and zeaxanthin concentration (Table 2 and 5). Lutein concentration ranged from 7.04 μg g$^{-1}$ at Elrose 2011 to 8.28 μg g$^{-1}$at Floral 2009. Total carotenoid concentration ranged from 13.42 μg g$^{-1}$ at Elrose 2011 to 14.42 μg g$^{-1}$ at Elrose 2009 (Table 5).

## Trait Correlation in Pea and Chickpea

Concentrations of the four individual carotenoids were positively correlated with each other in both pea and chickpea ($P < 0.0001$; Table 6). Positive correlations were previously reported between lutein and chlorophyll concentration in pea ($r = 0.77$, $P < 0.01$; Holasová et al., 2009), and between lutein and zeaxanthin concentration in chickpea ($r = 0.66$, $P < 0.05$; Abbo et al., 2005).

## Environmental Effects on Carotenoid Concentration in Pea Seed Tissues

In many countries, peas and desi chickpeas are consumed as cotyledons after splitting and dehulling, while the seed coat and embryo axis tissues enter animal feed markets. Therefore, the second part of the study was conducted to determine the distribution of carotenoids in each of three seed tissues (cotyledon, seed coat, and embryo axis) in selected cultivars from two market classes of field pea (green and yellow cotyledons) and chickpea (kabuli and desi). CDC Patrick (green cotyledon) and CDC Meadow

**Table 5. Carotenoid concentration in eight chickpea cultivars grown at three locations in 2009 and two locations in 2011 in Saskatchewan, Canada.**

| Cultivars | Mean carotenoid concentrations† | | | | |
|---|---|---|---|---|---|
| | Violaxanthin | Lutein | Zeaxanthin | β-carotene | Total carotenoids‡ |
| | μg g$^{-1}$ | | | | |
| Kabuli cultivars | | | | | |
| Amit | 0.02c | 7.13c | 5.89c | 0.24d | 13.30d |
| CDC Frontier | 0.02c | 6.48cd | 5.16e | 0.31c | 11.99e |
| CDC Luna | 0.02c | 6.89cd | 5.54cde | 0.38b | 12.84d |
| CDC Orion | 0.02c | 6.28d | 4.21f | 0.29cd | 10.81f |
| CDC Leader | 0.02c | 7.01c | 5.40de | 0.30c | 12.74ed |
| Desi cultivars | | | | | |
| CDC Cabri | 0.10a | 8.52b | 5.82cd | 0.57a | 15.01c |
| CDC Vanguard | 0.10a | 9.00b | 6.58b | 0.55a | 16.25b |
| CDC Corinne | 0.09b | 10.96a | 7.46a | 0.56a | 19.08a |
| Overall | 0.05 | 7.70 | 5.76 | 0.40 | 14.00 |
| Location and year | | | | | |
| Elrose 2009 | 0.06a | 8.11a | 5.88ab | 0.37a | 14.42a |
| Pasqua 2009 | 0.05ab | 7.63a | 5.44b | 0.33a | 13.46a |
| Floral 2009 | 0.05ab | 8.28a | 6.17a | 0.33a | 14.85a |
| Elrose 2011 | 0.06a | 7.04a | 5.88ab | 0.49a | 13.42a |
| Pasqua 2011 | 0.04b | 7.85a | 5.44b | 0.47a | 13.88a |
| SE§ | 0.01 | 0.76 | 0.30 | 0.01 | 1.24 |

† Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

‡ Total carotenoid concentration was calculated as the sum of four individual carotenoids.

§ SE, pooled standard error of mean calculated for each carotenoid (ug g$^{-1}$) from mean square of ANOVA for each location ($n$ = 120).

**Table 6. Correlation coefficients among violaxanthin, lutein, zeaxanthin, β-carotene, and total carotenoid concentration of pea and chickpea evaluated across environments in Saskatchewan, Canada.**

| | Violaxanthin | Lutein | Zeaxanthin | β-carotene | Total carotenoids |
|---|---|---|---|---|---|
| Pea | | | | | |
| Violaxanthin | 1 | 0.87*** | 0.56*** | 0.93*** | 0.92*** |
| Lutein | | 1 | 0.68*** | 0.85*** | 0.99*** |
| Zeaxanthin | | | 1 | 0.52*** | 0.67*** |
| β-carotene | | | | 1 | 0.90*** |
| Total carotenoids | | | | | 1 |
| Chickpea | | | | | |
| Violaxanthin | 1 | 0.69*** | 0.58*** | 0.73*** | 0.73*** |
| Lutein | | 1 | 0.70*** | 0.54*** | 0.95*** |
| Zeaxanthin | | | 1 | 0.42*** | 0.87*** |
| β-carotene | | | | 1 | 0.59*** |
| Total carotenoids | | | | | 1 |

*** Significant at the 0.001 probability level.

(yellow cotyledon) are commercially produced in Saskatchewan. These two cultivars were grown at two locations (Rosthern and Sutherland) in 2011. Results of this research may contribute to value-added crop utilization strategies for field pea growers.

Combined ANOVA showed significant variation ($p < 0.0001$) in all four individual carotenoids and total carotenoids in pea seed tissues (Table 7). Tissues and cultivars differed significantly for each carotenoid, while the effect of location and the cultivar × location interaction were not significant (Table 7). Similarly, the C × E interaction was not significant for lutein, zeaxanthin, and β-carotene in wheat (Matus-Cádiz et al., 2003; Ramachandran et al., 2010).

## Carotenoids Distribution in Pea Seed Tissues

Among the three seed tissues, carotenoid concentration was greatest in cotyledon, followed by embryo axis and seed coat in both cultivars. CDC Patrick had significantly greater concentration of all carotenoids than CDC Meadow in all three seed tissues (Table 8). CDC Patrick possessed 11 times greater violaxanthin and 2 times greater lutein concentration than CDC Meadow in both whole seed and cotyledon tissues. Seed coats of green cotyledon field pea cultivars (CDC Patrick and Cooper) had greater lutein concentration than those of yellow cotyledon pea cultivars (Marles et al., 2013) supporting our results. In cotyledons, CDC Patrick had a substantial concentration of β-carotene (1.45 ug g$^{-1}$), while only a trace amount (0.02 ug g$^{-1}$) was detected in CDC Meadow. Typical sample chromatograms

**Table 7. Mean squares of combined ANOVA for distribution of carotenoids in different tissues (whole seed, cotyledon, seed coat, and embryo axis) of pea cultivars grown at Rosthern and Sutherland, SK, Canada in 2011 ($n$ = 48).**

| | Mean squares | | | | |
|---|---|---|---|---|---|
| | Tissue | Cultivar (C) | Location (L) | C × L | CV |
| | | | | | % |
| Violaxanthin | 1.08*** | 6.40*** | 0.03ns | 0.06ns | 12.3 |
| Lutein | 638.43*** | 451.72*** | 12.25ns | 4.04ns | 2.9 |
| Zeaxanthin | 0.56*** | 1.02*** | 0.01ns | 0.01ns | 16.1 |
| β-carotene | 1.74*** | 7.31*** | 0.02ns | 0.01ns | 14.1 |
| Total carotenoids | 768.80*** | 756.52*** | 13.6ns | 3.17ns | 2.8 |

*** Significant at the 0.001 probability level.

**Table 8. Distribution of carotenoids in seeds tissues of cultivars CDC Meadow and CDC Patrick.**

| | | | Mean carotenoids concentration‡ | | | | |
|---|---|---|---|---|---|---|---|
| Cultivar† | Market class | Tissue type | Violaxanthin | Lutein | Zeaxanthin | β-carotene | Total carotenoids§ |
| | | | μg g$^{-1}$ | | | | |
| CDC Patrick | green | whole seed | 1.18a | 20.10a | 0.31a | 1.41a | 23.00a |
| CDC Meadow | yellow | whole seed | 0.10b | 11.34b | 0.24b | 0.04b | 11.73b |
| CDC Patrick | green | cotyledon | 1.19a | 20.31a | 0.29a | 1.45a | 23.26a |
| CDC Meadow | yellow | cotyledon | 0.10b | 11.14b | 0.17b | 0.02b | 11.42b |
| CDC Patrick | green | Seed coat | 0.04a | 1.10a | 0.04a | 0.00a | 1.18a |
| CDC Meadow | yellow | Seed coat | 0.00b | 0.36b | 0.00b | 0.00b | 0.36b |
| CDC Patrick | green | embryo axis | 0.90a | 17.00a | 0.42a | 1.00a | 19.32a |
| CDC Meadow | yellow | embryo axis | 0.19b | 11.01b | 0.08b | 0.00b | 11.28b |
| SE | | | 0.10 | 0.90 | 0.14 | 0.04 | 1.20 |

† Cultivars were grown at Rosthern and Sutherland, SK, Canada in 2011 ($n$ = 48).

‡ Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

§ Total carotenoid concentration was calculated as the sum of four individual carotenoids.

of carotenoid profile of CDC Meadow and CDC Patrick cotyledons are presented (Fig. 1A and B).

Lutein was detected in seed coats of CDC Patrick (1.10 ug g$^{-1}$) and CDC Meadow (0.36 ug g$^{-1}$), while the other carotenoids were presented in trace amounts in both cultivars. CDC Patrick had approximately 2 times (23.3 ug g$^{-1}$) greater total carotenoid concentration than CDC Meadow (11.4 ug g$^{-1}$) in cotyledons, and these are almost equal to the whole seed concentrations in both cultivars. In contrast, mungbean [*Vigna radiata* (L.) R. Wilczek] had a high concentration of carotenoids in the seed coat of green cotyledon cultivars leading to a greater concentration of total carotenoids in green cultivars compared with yellow cultivars (Harina and Ramirez, 1978).

In both pea cultivars, total carotenoid concentration was greater in cotyledons than in embryo axes, which were greater than seed coats. The cotyledon comprised 89.8 and 90.9% of the mass (dry weight basis) of the whole seed of CDC Patrick and CDC Meadow, respectively, while the seed coat comprised 9.2 and 8.2%, respectively, and the embryo axis comprised 1.0 and 0.9%, respectively. Thus, the cotyledon is by far the major source of carotenoids in pea, and removal of the seed coat and embryo axis in processing would not result in a substantial loss of carotenoids for human diets.

## Carotenoids Distribution in Chickpea Seed Tissues

Carotenoid distributions in chickpea seed tissues were analyzed for seven cultivars from different market classes, among them five desi cultivars and two kabuli cultivars (Table 9). Among the three seed tissues, total carotenoid concentration was greatest in the cotyledon, followed by the embryo axis and seed coat in kabuli cultivars. However, in desi cultivars, individual and total carotenoid concentrations were greater in the seed coat, followed by the cotyledon and embryo axis (Table 9). As expected, extracts of desi seed coats had greater pigmentation than found in kabuli seed coats. Pigmentation intensity corresponded with carotenoid accumulation in transgenic *Arabidopsis thaliana* (L.) Heynh. seeds (Lindgren et al., 2003); these researchers also reported that seed specific expression of phytoene synthase increased the concentrations of carotenoids, xanthophylls, and abscisic acid.

Overall mean total carotenoids were greatest in the cotyledon, followed by the seed coat and embryo axis in chickpea cultivars. The cotyledon comprised 89.9 and 95.2% of the mass (dry weight basis) of the whole seed of CDC Corinne (desi) and CDC Orion (kabuli), respectively, while the seed coat comprised 9.2 and 4.0%, respectively, and the embryo axis comprised 0.9 and 0.7%, respectively. Greatest carotenoid concentration was associated with the seed coat of CDC Jade (38.06 ug g$^{-1}$) and

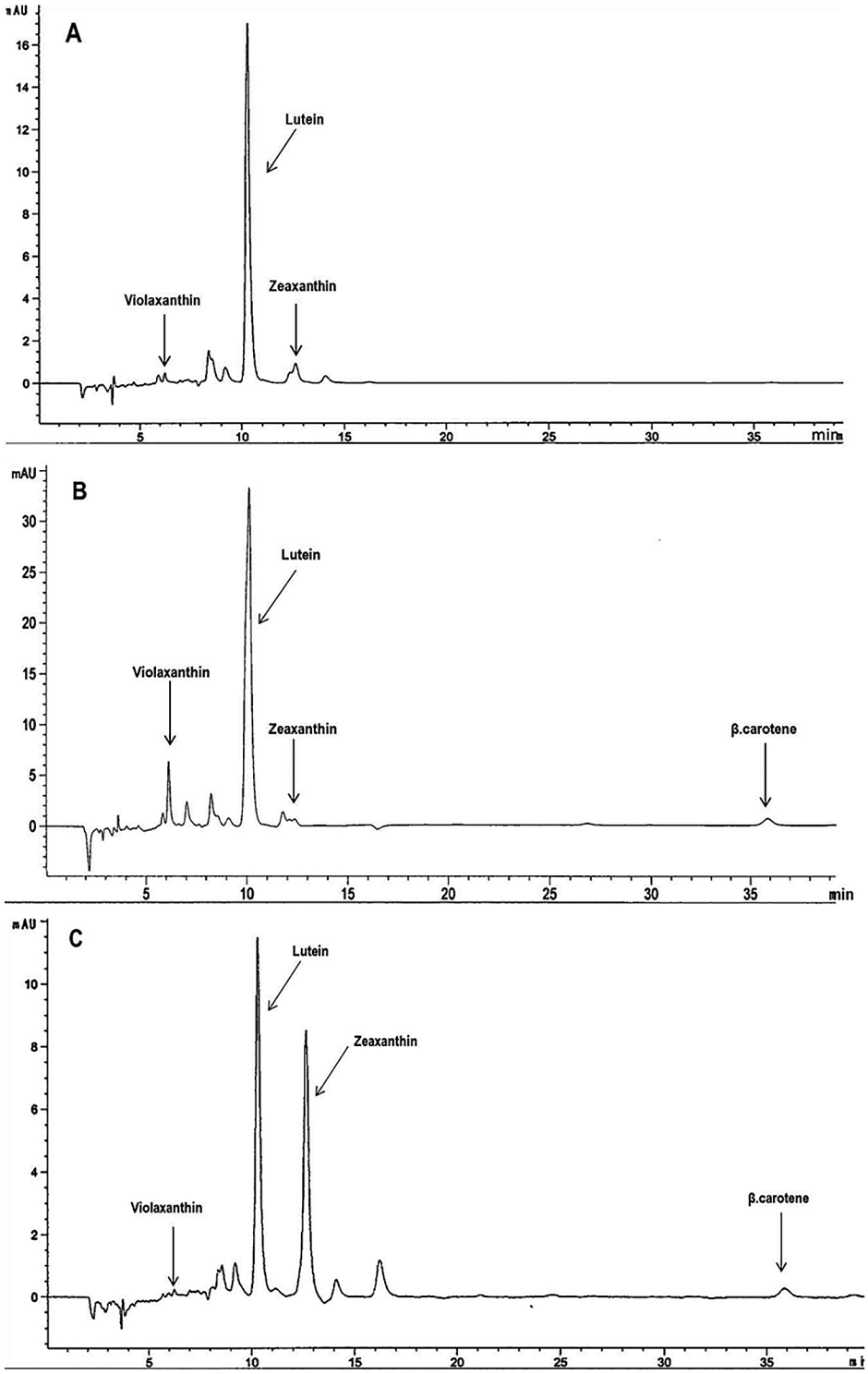


Figure 1. Typical sample chromatogram of carotenoid profile of (A) yellow cotyledon pea cultivar CDC Meadow, (B) green cotyledon pea cultivar CDC Patrick, and (C) desi chickpea cultivar CDC Corinne.

CDC Ebony (36.0 ug $g^{-1}$), and lowest carotenoid concentration in the seed coat of the non-pigmented kabuli cultivars (1.02–2.09 ug $g^{-1}$). Similarly in mungbean, greater concentration of carotenoids was found in green seed coat cultivars compared with yellow seed coat cultivars (Harina and Ramirez, 1978).

In chickpea, lutein was the major carotenoid, followed by zeaxanthin in whole seed for all cultivars (Table 9), and this was also the case in mature wheat grains (Ramachandran et al., 2010). Typical sample chromatogram of carotenoid profile of CDC Corinne whole seed was presented (Fig. 1C). Among the seven chickpea cultivars, CDC Jade and CDC Ebony had the greatest carotenoid concentrations in all three seed tissues. Seed coats of these two cultivars were particularly rich in lutein and violaxanthin. A substantial amount of β-carotene was

Table 9. Distribution of carotenoids in seeds tissues of contrasting cultivars of chickpea.

| Cultivars | Seed coat color | Tissue type | Mean carotenoids concentration† | | | | |
|---|---|---|---|---|---|---|---|
| | | | Violaxanthin | Lutein | Zeaxanthin | β-carotene | Total carotenoids ‡ |
| | | | μg g$^{-1}$ | | | | |
| CDC Ebony | black | whole seed | 0.22b | 14.49b | 8.60a | 1.01b | 24.31b |
| CDC Jade | green | whole seed | 2.16a | 22.37a | 5.36d | 2.40a | 32.20a |
| 551–1 | tan | whole seed | 0.05d | 6.86e | 5.18e | 0.55cd | 12.65e |
| 603–3 | tan | whole seed | 0.03de | 7.26d | 5.65c | 0.47de | 13.36d |
| CDC Corrine | tan | whole seed | 0.08c | 10.55c | 7.70b | 0.63c | 18.95c |
| CDC Leader | nonpigmented | whole seed | 0.03de | 6.28f | 4.12f | 0.44de | 10.87f |
| CDC Orion | nonpigmented | whole seed | 0.02e | 5.71g | 3.57f | 0.42e | 10.15g |
| CDC Ebony | black | cotyledon | 0.04b | 12.01b | 8.23b | 0.68b | 21.00b |
| CDC Jade | green | cotyledon | 1.40a | 21.93a | 6.03b | 2.74a | 32.10a |
| 551–1 | tan | cotyledon | 0.03bc | 8.16d | 6.51b | 0.77b | 15.47c |
| 603–3 | tan | cotyledon | 0.04b | 7.25e | 6.39b | 0.53bc | 14.21c |
| CDC Corinne | tan | cotyledon | 0.04b | 10.51c | 8.76a | 0.73b | 20.00b |
| CDC Leader | nonpigmented | cotyledon | 0.03bc | 7.43e | 4.40c | 0.54bc | 12.40d |
| CDC Orion | nonpigmented | cotyledon | 0.01c | 5.85f | 4.11c | 0.36c | 10.33e |
| CDC Ebony | black | seed coat | 7.67a | 24.06b | 2.06c | 2.21a | 36.00a |
| CDC Jade | green | seed coat | 5.52b | 26.70a | 4.17b | 1.67b | 38.06a |
| 551–1 | tan | seed coat | 0.62c | 15.10c | 14.55a | 0.08c | 30.34b |
| 603–3 | tan | seed coat | 0.25d | 4.99d | 4.30c | 0.00c | 9.52c |
| CDC Corrine | tan | seed coat | 0.72c | 15.33c | 14.44a | 0.00c | 30.50b |
| CDC Leader | nonpigmented | seed coat | 0.00e | 0.68e | 0.34d | 0.00c | 2.09d |
| CDC Orion | nonpigmented | seed coat | 0.03ed | 1.38e | 0.69d | 0.00c | 1.02d |
| CDC Ebony | black | embryo axis | 0.22bc | 11.71b | 7.55a | 0.56bc | 20.05b |
| CDC Jade | green | embryo axis | 1.03a | 19.86a | 7.16b | 2.16a | 30.22a |
| 551–1 | tan | embryo axis | 0.17c | 7.20b | 5.84d | 0.43bc | 13.65c |
| 603–3 | tan | embryo axis | 0.16c | 10.00b | 6.10c | 0.52bc | 16.80bc |
| CDC Corrine | tan | embryo axis | 0.23bc | 12.85b | 6.11c | 0.74b | 19.92b |
| CDC Leader | nonpigmented | embryo axis | 0.26b | 8.25b | 4.50e | 0.34c | 15.05bc |
| CDC Orion | nonpigmented | embryo axis | 0.17c | 8.19b | 4.10f | 0.42bc | 14.83bc |
| SE§ | | all tissues | 0.01 | 1.74 | 0.05 | 0.01 | 1.92 |

† Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

‡ Total carotenoid concentration was calculated as the sum of four individual carotenoids. Chickpea cultivars were grown in Saskatoon, SK, Canada in 2011.

§ Standard error ($n$ = 56).

accumulated only in black seed coat cultivar CDC Ebony (2.21 ug g$^{-1}$) and green seed coat cultivar CDC Jade (1.67 ug g$^{-1}$), with a trace amount in 551–1 (0.08 ug g$^{-1}$), while other cultivars had no β-carotene.

In developing countries where fruits and vegetables are expensive in off seasons, pea and chickpea would be a good source of dietary carotenoids. Saskatchewan-grown pea and chickpea are rich in individual and total carotenoids, greater than reported in rice, wheat, cassava, and potato. Hence, the consumption of pea and chickpea could address the problem of vitamin A and age-related macular degeneration deficiencies in developing countries. Future research will involve the evaluation of the carotenoid profile of a wider diversity of pea and chickpea germplasm to potentially identify material with even greater potential for biofortification.

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## References

Abbo, S., D.J. Bonfil, Z. Berkovitch, and R. Reifen. 2010. Towards enhancing lutein concentration in chickpea, cultivar and management effects. Plant Breed. 129:407–411 10.1111/j.1439-0523.2010.01767.x.

Abbo, S., C. Molina, R. Jungmann, M.A. Grusak, Z. Berkovitch, R. Reifen, G. Kahl, P. Winter, and R. Reifen. 2005. QTL governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor. Appl. Genet. 111:185–195. doi:10.1007/s00122-005-1930-y

Abushita, A.A., H.G. Daood, and P.A. Biacs. 2000. Changes in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. J. Agric. Food Chem. 48:2075–2081. doi:10.1021/jf990715p

Alves-Rodrigues, A., and A. Shao. 2004. The science behind lutein. Toxicol. Lett. 150:57–83. doi:10.1016/j.toxlet.2003.10.031

Beyer, P., S. Al-Babili, X. Ye, P. Lucca, P. Schaub, R. Welsch, and I. Potrykus. 2002. Golden rice: Introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat Vitamin A deficiency. J. Nutr. 132:506S–510S.

Bramley, P.M. 2000. Is lycopene beneficial to human health? Phytochemistry 54:233–236. doi:10.1016/S0031-9422(00)00103-5

Britton, G., R. Weesie, D. Askin, J.D. Warburton, L. Gallardo-Guerrero, F.J. Jansen, H.J.M. de Groot, J. Lugtenburg, J.P. Cornard, and J.C. Merlin. 1997. Carotenoid blues: Structural studies on carotenoproteins. Pure Appl. Chem. 69:2075–2084. doi:10.1351/pac199769102075

Chen, B.H., H.Y. Peng, and H.E. Chen. 1995. Changes of carotenoids, color and vitamin A content during processing of carrot juice. J. Agric. Food Chem. 43:1912–1918. doi:10.1021/jf00055a029

Cunningham, F.X., and E. Gantt. 1998. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:557–583. doi:10.1146/annurev.arplant.49.1.557

Cunningham, F.X., Z. Sun, D. Chamovitz, J. Hirschberg, and E. Gantt. 1994. Molecular structure and enzymatic function of lycopene cyclase from the *cyanobacterium Synechococcus* sp. strain PCC7942. Plant Cell 6:1107–1121.

Demmig-Adams, B., and W.W. Adams. 2002. Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153. doi:10.1126/science.1078002

Edelenbos, M., L.P. Christensen, and K. Grevsen. 2001. HPLC Determination of Chlorophyll and Carotenoid Pigments in Processed Green Pea Cultivars (*Pisum sativum* L.). J. Agric. Food Chem. 49:4768–4774. doi:10.1021/jf010569z

Fraser, P.D., and P.M. Bramley. 2004. The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res. 43:228–265. doi:10.1016/j.plipres.2003.10.002

Fraser, P.D., P. Bramley, and G.B. Seymour. 2001. Effect of Cnr mutation on carotenoid formation during tomato fruit ripening. Phytochem. 58:75–79. doi:10.1016/S0031-9422(01)00175-3

Harina, T.H., and D.A. Ramirez. 1978. The amount and distribution of carotenoinds in the mungbean seed (*Vigna radiata*, Wilczek). Philipp. J. Crop Sci. 3:65–70.

Haynes, K.G., B.A. Clevidence, D.D. Rao, B.T. Vinyard, and J.M. White. 2010. Genotype × environment interactions for potato tuber carotenoid content. J. Am. Soc. Hortic. Sci. 135:250–258.

Hentschel, V., K. Kranl, J. Hollmann, M.G. Lindhauer, V. Bohm, and R. Bitsch. 2002. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. J. Agric. Food Chem. 50:6663–6668. doi:10.1021/jf025701p

Hidalgo, A., and A. Brandolini. 2008. Kinetics of carotenoids degradation during the storage of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and bread wheat (*Triticum aestivum* L. ssp. *aestivum*) flours. J. Agric. Food Chem. 56:11300–11305. doi:10.1021/jf802448t

Holasová, M., R. Dostálova, V. Fieldlerová, and J. Horáček. 2009. Variability of lutein content in peas (*Pisum sativum* L.) in relation to the variety, season and chlorophyll content. Czech J. Food Sci. 27:S188–S191.

Khachik, F., G.R. Beecher, and J.C. Smith. 1995. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J. Cell. Biochem. 22:236–246. doi:10.1002/jcb.240590830

Khattak, A.B., A. Zeb, and N. Bibi. 2008. Impact of germination time and type of illumination on carotenoid content, protein solubility and in vitro protein digestibility of chickpea (*Cicer arietinum* L.) sprouts. Food Chem. 109:797–801. doi:10.1016/j.foodchem.2008.01.046

Krinsky, N.I., M.D. Russett, G.J. Handelman, and D.M. Snodderly. 1990. Structural and geometric isomers of carotenoids in human plasma. J. Nutr. 120:1654–1662.

Lindgren, O.L., K.G. Stålberg, and A.S. Höglund. 2003. Seed-specific over expression of an endogenous arabidopsis phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and Abscisic acid. Plant Physiol. 132:779–785. doi:10.1104/pp.102.017053

Marles, M.A.S., T.D. Warkentin, and K.E. Bett. 2013. Field pea (*Pisum sativum* L.) hulls as a rich source of dietary carotenoids. J. Sci. Food Agric. 93:363–370. doi:10.1002/jsfa.5782

Matus-Cádiz, M.A., P. Hucl, C.E. Perron, and R.T. Tyler. 2003. Genotype × environment interaction for grain color in hard white spring wheat. Crop Sci. 43:219–226. doi:10.2135/cropsci2003.0219

McCallum, J., G. Timmerman-Vaughan, T. Frew, and A. Russel. 1997. Biochemical and genetic linkage analysis of green seed color in field pea. J. Am. Soc. Hortic. Sci. 122:218–225.

Menkir, A., and B. Maziya-Dixon. 2004. Influence of genotype and environment on β-carotene content in tropical yellow-endosperm maize genotypes. Maydica 49:313–318.

Meydani, M., A. Martin, J.D. Ribaya Mercado, J. Gong, J.B. Blumberrg, and R.M. Russel. 1994. Beta-carotene supplementation increases antioxidant capacity of plasma in older women. J. Nutr. 124:2397–2403.

Moeller, S.M., P.F. Jacques, and J.B. Blumberg. 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J. Am. Coll. Nutr. 19:522S–527S. doi:10.1080/07315724.2000.10718975

Olmedilla, B., F. Granado, I. Blanco, M. Vaquero, and C. Cajigal. 2001. Lutein in patients with cataracts and age-related macular degeneration: A long-term supplementation study. J. Sci. Food Agric. 81:904–909. doi:10.1002/jsfa.905

Paine, J.A., C.A. Shipton, S. Chaggar, R.M. Howells, M.J. Kennedy, G. Vernon, S.Y. Wright, E. Hinchliffe, J.L. Adams, A.L. Silverstone, and R. Drake. 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat. Biotechnol. 23:482–487. doi:10.1038/nbt1082

Ramachandran, A., C.J. Pozniak, J.M. Clarke, and A.K. Singh. 2010. Carotenoid accumulation during grain development in durum wheat. J. Cereal Sci. 52:30–38. doi:10.1016/j.jcs.2010.02.014

Reifen, R. 2002. Vitamin A as an anti-inflammatory agent. Proc. Nutr. Soc. 3:397–400. doi:10.1079/PNS2002172

SAS Institute. 2011. The system for windows. Version 9.3. SAS Inst., Cary, NC.

Snodderly, D.M. 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am. J. Clin. Nutr. 62:1448S–1461S.

Ssemakula, G., A.G.O. Dixon, and B. Maziya-Dixon. 2007. Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (*Manihot esculenta* Crantz). J. Tropical Agric. 45:14–20.

Stahl, W., N. Ale-Agha, and M.C. Polidori. 2002. Non-antioxidant properties of carotenoids. Biol. Chem. 383:553–558. doi:10.1515/BC.2002.056

Stephens, D., P.L. Jackson, and Y. Gutierrez. 1996. Subclinical vitamin A deficiency: A potentially unrecognized problem in the United States. Pediatr. Nurs. 22:377–389.

Welch, R.M., and R.D. Graham. 2005. Agriculture: The real nexus for enhancing bioavailable micronutrients in food crops. J. Trace Elem. Med. Biol. 18:299–307. doi:10.1016/j.jtemb.2005.03.001

White, P.J., and M.R. Broadley. 2005. Biofortifying crops with essential mineral elements. Trends Plant Sci. 10:586–593. doi:10.1016/j.tplants.2005.10.001

Zhu, Y.H., J.G. Jiang, and Q. Chen. 2008. Characterization of cDNA of lycopene β-cyclase responsible for a high level of β-carotene accumulation in *Dunaliella salina*. Biochem. Cell Biol. 86:285–292. doi:10.1139/O08-012