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Effects of Season and Postharvest Storage on the Carotenoid Content of *Solanum phureja* Potato Tubers

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The total carotenoid content was determined of tubers from 38 *Solanum phureja* lines grown in field plots over 3 years. The results indicated a significant difference between years, but the ranking was similar from year to year and the interaction between season and variety was small. Postharvest storage significantly reduced the carotenoid content of the tubers, and reducing the storage temperature further lowered the carotenoid content. Examination of the individual carotenoids revealed that lutein was the most stable and least likely to be reduced, while the levels of the carotenoids derived from β-carotene were significantly reduced during storage at either temperature. Exposure of the tubers to either mercury or sodium lights resulted in a significant increase in total carotenoid content, concomitant with elevated chlorophyll. Although both types of radiation produced a broadly similar increase in total carotenoid contents, differential effects on the individual carotenoid profile of the light-induced carotenoids were observed.

KEYWORDS: Potato; *Solanum phureja*; *Solanum tuberosum*; carotenoids; chlorophyll; storage; light

INTRODUCTION

The carotenoids are a diverse group of biologically active compounds biosynthetically derived from isopentenyl diphosphate (*1*, *2*). Over 700 carotenoids have been identified to date and are present in all major plant taxa (*3*). The highly unsaturated nature of carotenoids has been linked with their ability to act as light-harvesting pigments, which readily absorb photons in the green-red visible range of 450−570 nm (*4*), while their ability to act as antioxidants and free radical scavengers has also been associated with their possible health-promoting properties. Humans are unable to synthesize carotenoids and are thus dependent on their presence in the diet as a source of these micronutrients.

The major carotenoids identified in tubers derived from both diploid (*Solanum phureja* and *Solanum stenotomum*, also known as *Solanum tuberosum* Group Phureja and Group Stenotomum) and tetraploid potato (*S. tuberosum*) were the xanthophylls (oxygenated carotenoids) and, in particular, neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin (*5*−*7*). Lutein and zeaxanthin are the major pigments of the yellow spot in the retina of the human eye (*8*) where their ability to absorb blue light and to remove singlet-oxygen species protect the retina from free radical damage. Intervention studies have shown that high dietary intake of these two carotenoids can protect the consumer from age-related macular degeneration (*9*), which is a major cause of blindness in the elderly. Lutein is a common constituent of many leafy vegetables, but zeaxanthin is found in significant concentrations in far fewer dietary components. In view of these potential benefits associated with consuming higher concentrations of xanthophylls, considerable interest is currently being shown in the development of both transgenic and traditional breeding methods (*7*, *10*−*12*) to both increase total carotenoids and manipulate the relative concentrations of individual carotenoids in potatoes.

The levels of potential nutrients, antinutritional factors, and factors influencing processing quality in potatoes can be significantly affected by both seasonal differences in growing conditions and postharvest storage conditions. For example, storage at low temperature can result in the accumulation of reducing sugars (*13*), which may result in poor fry color during chip manufacture or fries production as well as to potentially higher levels of acrylamide (*14*) in products cooked at high temperatures (150−190 °C). Low-temperature storage can also influence glycoalkaloid content and result in increased accumulation of these potentially toxic compounds when tubers are subsequently exposed to light (*15*). As there is an increased trend toward displaying potatoes in commercial outlets under artificial lighting, the response of potato cultivars to such environments would appear to be of increased significance. The magnitude of all of these changes is generally cultivar-dependent, thus allowing breeding strategies to be developed to produce lines with optimum response to the desired storage conditions.

Little information in the public domain is available on the influence of pre- and postharvest environments on carotenoid content in potatoes. The main objective of this work was to ascertain the extent to which total carotenoid content was

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influenced by growing season in a collection of *S. phureja* potatoes adapted for Western European conditions and which differ greatly for flesh color. The effects of variations in storage temperature and exposure to light on total carotenoid content were also determined, and for a limited number of lines, the effect of storage conditions on each of the major constituent free carotenoids was investigated.

MATERIALS AND METHODS

Reagents and Apparatus. *Plant Material.* The potato lines and cultivars utilized in this study were grown in replicated field trials in 2001, 2002, and 2003 at Gourdie Farm (Dundee, United Kingdom) using normal agronomic practices. The tubers from each plot were harvested 2 weeks after foliage burn-down and stored at ambient temperatures (ca. 8 °C) and humidity for 3 weeks to allow for "curing" as per common agronomic practice. The tubers were then transferred to controlled environment stores set to a temperature of either 10 or 4 °C with relative humidity levels between 85 and 95%.

For the interseasonal comparison, samples were removed each year from the 4 °C controlled environment store after 12 weeks and representative samples were obtained from each replicate by selecting five average-sized tubers, which were then each manually cut into eighths. Two opposite eighths were taken from each tuber, bulked by replicate, diced, and immediately frozen by immersion in liquid nitrogen. The frozen samples were freeze-dried and, after being ground in a laboratory mill fitted with a 0.5 mm sieve, stored at −20 °C until required for analysis.

For the comparison of the effects of storage conditions undertaken in 2003, a subset of tubers were taken from each replicate of the 37 *S. phureja* lines immediately after the 3 week curing period and again after a further 12 weeks of storage at 4 and 10 °C. These were then sampled and freeze-dried as described above.

Light Exposure. The techniques used to investigate the effect of light exposure on the concentration of carotenoids and chlorophylls in potato tubers were similar to those previously described for the study of light on tuber glycoalkaloids (*15*−*17*). The tubers from 38 *S. phureja* lines were grown and harvested in 2001 and after curing as described above were stored at 4 °C and for 12 weeks. Two subsets consisting of five tubers from each replicate were removed from storage and cut longitudinally in half. One-half was placed cut-surface down on an absorbent paper-lined tray, which was then transferred to a controlled environment room set for 20 °C and ambient (85−95%) relative humidity. The tubers were illuminated with either sodium or mercury vapor lights adjusted with regard to height and position to produce an uniform photon flux density of 140 μmol m^{-2} s^{-1} at tray level. The paper lining the trays was watered twice a day to prevent any drying out of the tubers during the 7 day illumination period. The remaining half tubers were placed on identical trays, which were inserted into black polyethylene light-proof bags and stored in the environmental chamber for the duration of the experiment.

After 7 days, the trays were removed from the environmental chamber, each half tuber was manually quartered, and opposite quarters were bulked by replicate and treatment. After the samples were diced, the samples were frozen in liquid nitrogen, freeze-dried, milled, and stored at −20 °C until analyzed.

Chemical Analysis. Total carotenoid and chlorophyll contents were simultaneously determined by the spectrophotometric method of Hendry and Price (*18*), which has been previously used to analyze *S. tuberosum* tubers by Edwards et al. (*19*). The freeze-dried samples were extracted into ammoniacal acetone (acetone BDH "Analar" grade; VWR Ltd., Lutterworth, United Kingdom, and ammonium hydroxide; Sigma-Aldrich, Gillingham, United Kingdom), and after centrifugation, the absorbance was determined at 480, 645, 663, and 710 nm on a variable wavelength UV/vis spectrophotometer (Hitachi model U-3010, Hitachi Scientific Instruments, Nissei Sangyo Co. Ltd., Finchamstead, United Kingdom). The total carotenoid content was expressed in terms of micromoles per 100 g freeze-dried matter (μmol 100 g^{-1} FDM) and chlorophyll content as mg per 100 g freeze-dried matter (mg 100 g^{-1} FDM).

The identification and determination of the relative proportions of the major individual carotenoids, neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin were carried out using high-performance liquid chromatography (HPLC) as described by Morris et al. (*6*). The carotenoids were extracted by grinding the freeze-dried sample with sand mixed with anhydrous sodium sulfate and sodium bicarbonate in the presence of acetone (BDH Analar grade; VWR Ltd.). Separation was performed on a Gilson HPLC system (Anachem Ltd., Luton, United Kingdom) consisting of two high-pressure pumps and a UV variable wavelength detector set to 460 nm. All HPLC solvents used were of BDH "Hipersolv" grade (VWR Ltd.), and the carotenoids were eluted by a binary solvent gradient using acetonitrile−water (9:1) and ethyl acetate. The carotenoids neoxanthin, violaxanthin, and lutein were isolated from rocket (*Eruca sativa* Miller) by open column chromatography (*20*). Zeaxanthin was kindly provided by Professor Andrew Young, Liverpool John Moores University (United Kingdom) and antheraxanthin purchased from CaroteNature (www.Carotenature.com). Individual carotenoid concentrations were calculated in terms of micromol per 100 g freeze-dried matter (μmol 100 g^{-1} FDM).

Statistical Analysis. Regression analysis, correlations, and analyses of variance were carried out using Genstat for Windows, 8th edition (VSN Ltd., Oxford, United Kingdom).

RESULTS AND DISCUSSION

Interseasonal Variation. Total carotenoid levels were determined in 38 lines of *S. phureja* grown in replicated field trials in 2001 and 2002 and in 37 lines grown in 2003 [no sample of DB333(16) was available in this year] as well as in 15 *S. tuberosum* cultivars grown in the same field trials in 2002 and 2003. The values found for the *S. phureja* lines (**Table 1**) ranged from 0.6 to 7.5 μmol 100 g^{-1} FDM in 2001 as compared to 0.1 to 6.3 μmol 100 g^{-1} FDM in 2003. The higher values found were of the same order of magnitude as those reported for yellow-fleshed tubers [2.3−11.8 μmol 100 g^{-1} FDM, recalculated from Lu et al. (*7*)] from a *S. phureja* × *S. stenotonum* hybrid population, while the values for the *S. tuberosum* cultivars (**Table 3**) were within the range [0.1−2.0 μmol 100 g^{-1} FDM, recalculated from von Euler et al. (*21*), Cadwell et al. (*22*), and Lu et al. (*7*)] previously reported for commercial potato cultivars.

Statistical analysis revealed that there were highly significant differences both between years and cultivars ($P < 0.001$) for both the *S. phureja* lines and the *S. tuberosum* cultivars. In both species, the lowest values were found for the 2003 grown tubers with the total carotenoid content in 2003 being on average 32 and 56% lower as compared with 2002 grown material for the *S. phureja* lines and *S. tuberosum* cultivars, respectively. There was a statistically significant interaction between year and lines for the *S. phureja* material, but this accounted for only a small proportion of the total variance (**Table 2**); no interaction was observed for the *S. tuberosum* cultivars (**Table 3**). Generally, the ranking of lines and cultivars remained very similar from year to year with highly significant correlations being found between all years for the *S. phureja* lines (**Table 2**) and between the 2002 and 2003 *S. tuberosum* cultivars ($r = 0.841$, $P < 0.001$). These results clearly indicate that selection for high or low carotenoid tuber levels could be made on the basis of a single year's results, but valid comparisons cannot be made between data from different years despite the material being stored under similar environmental conditions. They also indicate that the nutritional quality of potatoes may vary significantly with season. Comparing the 2 years with high and low tuber carotenoid content, 2002 and 2003, it appears that the higher levels of solar irradiation (June−August 2003, 1864.9 MJ/m^2; June−August 2002, 1629.1 MJ/m^2) and drought conditions (June−August 2003, 80.4 mm; June−August 2002, 294.4 mm) may have been associated with about 33% less total tuber

Table 1. Total Carotenoid Content (μmol 100 g^{-1} FDM)[a] of 38 *S. phureja* Lines Grown in 2001, 2002, and 2003

S. phureja line	year 2001	year 2002	year 2003	line mean
DB 375(1)	7.5	8.6	4.8	7.0
851 T8	7.1	7.4	6.3	6.9
PHU 951 (901)	6.3	7.1	6.0	6.5
DB 375 (2)	6.9	8.0	4.4	6.4
80 CP 23	6.6	6.8	4.4	5.9
DB 354 (901)	6.3	6.0	4.2	5.5
DB 333 (16)	4.6	4.7	**	4.7
DB 358 (23)	4.8	4.5	4.2	4.5
DB 378 (1)	5.1	4.9	3.5	4.5
DB 170 (35)	4.3	4.6	4.1	4.4
DB 377 (4)	4.5	5.3	3.1	4.3
71 P 10	4.7	4.4	3.2	4.1
DB 358 (30)	3.9	4.3	3.6	3.9
DB 257(28)	3.8	4.3	3.3	3.8
PHU 950 (412)	3.6	4.3	3.3	3.8
DB 358 (24)	3.0	3.5	2.6	3.0
DB 441 (2)	3.0	3.8	1.9	2.9
DB 207 (35)	3.3	3.5	1.9	2.9
DB 337 (37)	2.8	3.5	1.6	2.7
HBX 77 (24)	2.6	3.2	2.1	2.6
DB 175 (5)	2.5	2.7	2.1	2.4
DB 323 (3)	2.4	2.5	1.3	2.1
DB 226 (70)	2.2	2.6	1.3	2.0
DB 168 (11)	2.1	2.8	1.0	2.0
842 P 75	2.1	1.6	2.0	1.9
71 T 6	1.9	2.1	1.7	1.9
HB 117 (55)	1.8	2.1	1.8	1.9
DB161 (10)	1.7	1.9	1.2	1.6
HB 165 (1)	1.9	1.9	0.8	1.5
81 S 66	1.5	1.7	1.2	1.5
DB 270 (43)	1.3	1.5	0.3	1.0
DB 271 (39)	1.2	1.3	0.5	1.0
DB 244 (37)	1.0	1.0	0.5	0.8
71 T 46	0.9	1.1	0.5	0.8
TC 43 (45)	1.0	1.0	0.4	0.8
DB 199 (10)	0.6	0.7	0.5	0.6
HB 171 (13)	0.6	0.4	0.3	0.4
DB 299 (39)	0.6	0.4	0.1	0.4
LSD ($P = 0.05$)[b]		0.67		
LSD ($P = 0.05$)[c]				0.39
year mean	3.2	3.5	2.4	
LSD ($P = 0.05$)[d]		0.11		

[a] Key: *μmol per 100 g freeze-dried matter, **no sample of DB 333 (*16*) was available from 2003 harvest. [b] Least significant difference line × year means. [c] Least significant difference between line means. [d] Least significant difference between year means.

carotenoid content. Clearly, further work utilizing plant material grown under controlled environmental conditions is required to confirm this hypothesis.

Storage Conditions. A subset of tubers from the 2003 harvest taken immediately after the curing period and again following a further 12 weeks of storage at either 10 or 4 °C were analyzed for total carotenoid content. When averaged, over all storage conditions the total carotenoid contents of the *S. phureja* lines ranged from 0.3 to 7.7 μmol 100 g^{-1} FDM (**Table 4**) and differed significantly between lines (**Table 5**). As with Vitamin C content, which on average was reduced by over 40% after 4 months at 4 °C (*23*), storage significantly reduced the total carotenoid content of the tubers when averaged over all lines with the magnitude of the decrease increasing as the temperature of storage was reduced from 10 to 4 °C. The majority of the lines studied generally reflected this overall trend, although some anomalous results were observed. This was reflected in the fact that a statistically significant interaction was seen between lines and storage environment. However, the interaction term accounted for only a small proportion of the overall variance indicating that the majority of the lines followed the general trend.

Table 2. Statistical Analysis of the Total Carotenoid (μmol 100 g^{-1} FDM) Data from 38 *S. phureja* Lines Grown in 2001, 2002, and 2003[a]

Two-Way Analysis of Variance

source of variation	degrees of freedom	variance ratio	statistical significance
line	37	195.81	<0.001
year	2	224.26	<0.001
line × year	73	3.99	<0.001

Correlation Matrix

year	correlation coefficient (r) 2001	2002	2003
2001			
2002	0.99		
2003	0.95	0.94	

[a] All r values statistically significant at $P < 0.001$ (***) level.

Table 3. Total Carotenoid Content (μmol 100 g^{-1} FDM)[a] of 15 *S. tuberosum* Cultivars Grown in 2002 and 2003

S. tuberosum cultivar	year 2002	year 2003	cultivar mean
Record	1.8	1.1	1.4
Brodick	1.3	0.7	1.0
Desiree	1.4	0.6	0.8
Estima	1.1	0.4	0.8
Cara	1.1	0.3	0.7
Wilja	0.9	0.5	0.7
Montrose	0.8	0.5	0.7
Nadine	0.9	0.3	0.6
12601 AB 1	0.8	0.3	0.6
Saxon	0.7	0.4	0.6
Ailsa	0.7	0.4	0.5
Maris Piper	0.6	0.3	0.5
Stirling	0.6	0.3	0.4
Home Guard	0.6	0.2	0.4
Pentland Dell	0.5	0.2	0.4
LSD ($P = 0.05$)[b]	0.33		
LSD ($P = 0.05$)[c]			0.23
year mean	0.9	0.4	
LSD ($P = 0.05$)[d]	0.09		

Two-Way Analysis of Variance

source of variation	degrees of freedom	variance ratio	statistical significance
cultivar	14	12.37	<0.001
year	1	143.30	<0.001
cultivar × year	14	1.83	NS[e]

[a] Key: μ = μmol per 100 g freeze-dried matter. [b] Least significant difference cultivar × year means. [c] Least significant difference between line means. [d] Least significant difference between year means. [e] NS, not significant.

Statistical analysis of the data (**Table 5**) indicated significant ($P < 0.001$) correlation coefficients between the results for the samples taken at harvest and after storage again suggesting that in the majority of the lines storage at either temperature had a similar effect on all lines.

The relative concentration (% total carotenoids) of the five major nonesterified carotenoids, neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin, present in potato tubers was determined in a selection of lines that covered the range in total carotenoid content in the *S. phureja* selections. The results (**Table 6**) expressed as a percentage of total carotenoids revealed

Table 4. Total Carotenoid Content (μmol 100 g^{-1} FDM)[a] of *S. phureja* Lines at Harvest and after Storage at Either 10 or 4 °C for 12 Weeks[a]

	storage conditions			
S. phureja line	harvest (1)	10 °C (2)	4 °C (3)	line mean
DB 375(1)	9.3	9.1	4.8	7.7
DB 375(2)	8.0	7.5	4.4	6.6
851 T8	7.1	6.0	6.3	6.5
DB 354 (901)	7.1	8.1	4.2	6.5
80 CP 23	7.4	6.9	4.4	6.2
PHU 951 (901)	5.0	5.1	6.0	5.4
71 P 46	6.4	5.3	3.2	5.0
DB 170 (35)	4.8	5.0	4.1	4.7
DB 358 (23)	4.8	4.5	4.2	4.5
DB 377 (4)	5.3	4.0	3.1	4.1
DB 378(1)	5.2	3.5	3.5	4.1
PHU 950 (412)	4.4	3.7	3.3	3.8
DB 358 (30)	4.0	2.9	3.6	3.5
DB 257 (28)	3.6	2.5	3.3	3.2
DB 358 (24)	3.5	1.9	2.6	2.7
DB 441 (2)	3.1	2.8	1.9	2.6
HBX 77 (24)	3.3	2.1	2.1	2.5
DB 337 (37)	3.4	2.2	1.6	2.4
DB 207 (35)	2.7	2.6	1.9	2.4
DB 175 (5)	2.7	2.3	2.1	2.3
DB 323 (3)	2.7	1.7	1.3	1.9
842 P 75	1.7	1.8	2.0	1.9
HB 117 (55)	2.1	1.5	1.8	1.8
DB 226 (70)	2.1	1.9	1.3	1.8
DB 168 (11)	1.7	2.2	1.0	1.7
DB161 (10)	1.8	1.5	1.2	1.5
71 T 6	1.5	1.2	1.7	1.4
81 S 66	1.6	0.9	1.2	1.2
HB 165 (1)	1.5	0.7	0.8	1.0
DB 271 (39)	1.1	0.7	0.5	0.8
71 T 10	0.9	0.8	0.5	0.7
DB 270 (43)	0.9	0.7	0.3	0.7
TC 43 (45)	1.1	0.4	0.4	0.6
DB 244 (37)	0.9	0.4	0.5	0.6
HB 171 (13)	0.7	0.3	0.3	0.4
DB 199 (10)	0.6	0.2	0.5	0.4
DB 299 (39)	0.4	0.4	0.1	0.3
LSD ($P = 0.05$)[b]		0.90		
LSD ($P = 0.05$)[c]				0.52
storage mean	3.4	2.8	2.3	
LSD ($P = 0.05$)[d]		0.15		

[a] Key: μ, μmol per 100 g freeze-dried matter; 1, immediately postcuring; 2, stored for 12 weeks postskin set at 10 °C; and 3, stored for 12 weeks postskin set at 4 °C. [b] Least significant difference line × storage means. [c] Least significant difference between line means. [d] Least significant difference between storage means.

that, despite major differences in the relative proportions of the individual carotenoids between lines, in all lines storage at either temperature altered the overall carotenoid composition. When averaged over all lines, storage at 10 °C significantly reduced the proportion of violaxanthin, antheraxanthin, and zeaxanthin. Similar effects were noted at 4 °C for both antheraxanthin and zeaxanthin, but the decrease noted in the percentage proportion of violaxanthin was not statistically significant. The relative proportion of neoxanthin was unaffected, relative to the values obtained at harvest, after storage at 4 °C. The results after storage at 10 °C for neoxanthin were inconsistent on an individual line basis, but averaged over all lines, the relative proportion of this carotenoid was significantly higher than the value at harvest. In contrast, the relative concentration of lutein, a carotenoid derived from α-carotene, increased with storage at both temperatures. A previous study (*6*) also showed a decrease in total carotenoid content in a *S. phureja* line and in the *S. tuberosum* cultivar, Desiree, after 9 months of storage at 4 °C. Similar changes in carotenoid composition were also reported (*6*) with for example the levels of antheraxanthin and zeaxanthin

Table 5. Statistical Analysis of the Total Carotenoid (μmol 100 g^{-1} FDM) Data from 37 *S. phureja* Lines Harvested and Stored in 2003[a]

Two-Way Analysis of Variance

source of variation	degrees of freedom	variance ratio	statistical significance
line	36	124.31	<0.001
storage environment	2	94.89	<0.001
line × storage	72	4.87	<0.001

Correlation Matrix

	correlation coefficient (r)		
storage	at harvest	10 °C storage	4 °C storage
at harvest	1.000		
10 °C storage	0.972	1.000	
4 °C storage	0.896	0.876	1.000

[a] All r values statistically significant at $P < 0.001$ (***) level.

Table 6. Effect of Storage on the Concentration (μmol 100 g^{-1} FDM) and Relative Amounts (% Total Carotenoids) of Individual Carotenoids in *S. phureja* Tubers

	μmol 100 g^{-1} FDM (% total carotenoids)					storage mean
	DB 375	851 T8	PHU 950	DB 207	DB299	
			neoxanthin			
harvest	0.3 (3%)	0.5 (7%)	0.6 (14%)	0.3 (12%)	0.07 (15%)	0.4 (10%)
10 °C	0.5 (6%)	1.0 (16%)	0.6 (16%)	0.4 (17%)	0.05 (13%)	0.5 (14%)
4 °C	0.2 (4%)	0.6 (10%)	0.3 (10%)	0.2 (11%)	0.01 (13%)	0.3 (10%)
LSD ($P = 0.05$)[a]						0.11 (1.6)
cultivar mean	0.3 (4%)	0.7 (11%)	0.5 (13%)	0.3 (13%)	0.04 (14%)	
LSD ($P = 0.05$)[b]			0.08 (2.1)			
			violaxanthin			
harvest	1.2 (13%)	2.7 (38%)	1.9 (42%)	1.1 (41%)	0.07 (17%)	1.4 (30%)
10 °C	1.3 (15%)	2.1 (35%)	1.1 (30%)	0.4 (16%)	0.04 (11%)	1.0 (21%)
4 °C	1.1 (22%)	2.4 (39%)	1.3 (39%)	0.5 (31%)	0.02 (18%)	1.1 (29%)
LSD ($P = 0.05$)[a]						0.19 (4.8)
cultivar mean	1.2 (17%)	2.4 (37%)	1.4 (37%)	0.7 (29%)	0.04 (14%)	
LSD ($P = 0.05$)[b]			0.24 (6.2)			
			antheraxanthin			
harvest	3.0 (32%)	1.8 (25%)	0.3 (7%)	0.3 (10%)	0.05 (12%)	1.1 (17%)
10 °C	2.4 (27%)	0.9 (15%)	0.3 (8%)	0.4 (16%)	0.02 (6%)	0.8 (14%)
4 °C	1.0 (20%)	0.4 (6%)	0.2 (6%)	0.1 (6%)	0.00 (4%)	0.3 (8%)
LSD ($P = 0.05$)[a]						0.27 (2.8)
cultivar mean	2.1 (29%)	1.0 (16%)	0.3 (7%)	0.3 (11%)	0.03 (7%)	
LSD ($P = 0.05$)[b]			0.34 (3.7)			
			lutein			
harvest	0.7 (8%)	1.1 (15%)	1.3 (29%)	0.7 (26%)	0.2 (37%)	0.8 (23%)
10 °C	1.0 (11%)	1.6 (27%)	1.4 (39%)	1.1 (41%)	0.2 (62%)	1.1 (36%)
4 °C	0.6 (13%)	2.5 (39%)	1.3 (41%)	0.8 (44%)	0.1 (60%)	1.1 (39%)
LSD ($P = 0.05$)[a]						NS (6.5)
cultivar mean	0.8 (10%)	1.7 (27%)	1.4 (36%)	0.9 (37%)	0.2 (53%)	
LSD ($P = 0.05$)[b]			0.32 (8.4)			
			zeaxanthin			
harvest	4.2 (46%)	1.1 (15%)	0.4 (8%)	0.3 (13%)	0.08 (19%)	1.2 (20%)
10 °C	3.8 (41%)	0.6 (11%)	0.3 (9%)	0.3 (10%)	0.03 (9%)	1.0 (16%)
4 °C	2.0 (43%)	0.4 (6%)	0.2 (6%)	0.2 (8%)	0.01 (10%)	0.6 (15%)
LSD ($P = 0.05$)[a]						0.40 (3.4)
cultivar mean	3.4 (43%)	0.7 (11%)	0.3 (8%)	0.2 (10%)	0.04 (13%)	
LSD ($P = 0.05$)[b]			0.52 (4.4)			

[a] Least significant difference between treatments averaged over all lines. [b] Least significant difference between lines averaged over all treatments.

decreasing in the stored *S. phureja* line while the proportion of violaxanthin in the stored sample of Desiree was also reduced from 51 to 23% of the total nonesterified carotenoids.

Presenting our data with respect to μmol 100 g^{-1} FDM (**Table 6**) revealed that during storage violaxanthin, antheraxanthin, and zeaxanthin levels, averaged over all lines, were reduced significantly during storage at both temperatures while the concentration of lutein was almost unchanged at the end of the

storage period. This may be explained by a number of possibilities: (i) any lutein chemically destroyed by, for example, reaction with potentially damaging oxidants was replaced at a faster rate than the other major carotenoids, (ii) the activity of enzymes that catabolize the β-carotene-derived carotenoids zeaxanthin, antheraxanthin, and violaxanthin was greater in the *S. phureja* potato tubers than those utilizing lutein as their substrate, candidate enzymes may include carotenoid cleavage dioxygenases, and/or (iii) transformation of the free carotenoids to fatty acid esters, but if this was the main mechanism, then no change in total carotenoid would have been observed.

It is clear that considerable variation exists for these key dietary constituents in pools of lines of *S. phureja*. Variation also exists for the relative amounts of different types of carotenoid. Among clones with higher levels of carotenoid, three [DB375(1), DB378(1), and DB377(37)] have been selected for commercialization indicating that high levels of tuber carotenoids can be consistent with good agronomic performance. However, extended storage of these short tuber dormancy types of potato remains a problem and reducing storage temperatures to increase useful storage life appears to substantially reduce free carotenoids in the tubers, which is one of their quality attributes.

Light Exposure. The effect of light exposure on potato cultivars is an important characteristic as major retailers often display their products under conditions that expose the tubers to artificial light (*24*). Consequently, the effects of light exposure on total carotenoid content were investigated. The results of exposing 38 *S. phureja* lines continually for 7 days to either mercury or sodium lights indicated (**Table 7**) that there was little difference in the quantity of total carotenoids induced by light exposure. In 21 of the lines, a slightly greater increase in total carotenoids was induced in the samples exposed to sodium light, and indeed, averaged over all lines, the value for the tubers exposed to sodium lights (3.6 μmol 100 g^{-1} FDM) was just ($P = 0.040$) statistically significantly higher than the mean of 3.4 μmol 100 g^{-1} FDM found for the samples exposed to mercury lights.

The effect of light exposure on total carotenoid content appeared to be cultivar-dependent with the magnitude of the increases ranging from just above 1.0 μmol 100 g^{-1} FDM in DB 199(10) to over 5.0 μmol 100 g^{-1} FDM in the line 851 T8. It was of interest to note that these two lines had among the lowest and highest total carotenoid contents, respectively, prior to light exposure (**Table 1**), and indeed, the increase in total carotenoid contents in tubers exposed to both mercury and sodium lights was significantly correlated ($P > 0.001$) with the initial carotenoid content of the tubers (Hg light, $r = 0.565$; Na light, $r = 0.628$). Although regression lines could be fitted between initial carotenoid content and increase in carotenoid content after exposure to either mercury or sodium lights, deviation from the calculated regression lines was, in both instances, large. This indicates that the resulting regression equations had little predictive value reflecting the fact that the percentages of the total variance accounted for by the variation in initial total carotenoid content, for the two light treatments, were less than 40% in both instances.

As expected (*25*, *26*), the value for the chlorophyll content was consistently higher in tubers exposed to mercury lights with the difference between the treatment means, averaged over all lines, being highly significant ($P < 0.001$). The differences found were also significant between lines ($P < 0.001$) with over a 4-fold range in variation being observed under either light source. The increase in total carotenoid content under both light sources correlated ($P < 0.001$) with total chlorophyll content (Hg light, $r = 0.621$; Na light, $r = 0.620$) but again, as can be seen by the r values, the predictive values of the regression equations were low.

Table 7. Induced Increases in the Total Carotenoid (μmol 100 g^{-1} FDM) and in the Total Chlorophyll (mg 100 g^{-1} FDM) Contents in *S. phureja* Tubers Exposed for 7 Days to Either Sodium or Mercury Lights

	carotenoids		chlorophyll			
	total		total		ratio a/b	
S. phureja line	Hg	Na	Hg	Na	Hg	Na
851 T8	5.1	6.0	16.4	14.8	2.6	2.8
DB 377 (4)	4.9	4.8	8.7	6.1	2.9	3.2
DB 175 (5)	4.9	4.6	8.2	5.0	2.6	3.0
DB 170 (35)	4.6	5.3	6.7	5.9	3.2	3.8
DB 358 (24)	4.5	3.5	11.4	7.3	2.7	2.9
DB 257 (28)	4.5	4.3	9.1	6.5	2.8	3.3
80 CP 23	4.3	3.9	5.9	3.2	3.2	4.1
71 T 10	4.3	4.2	6.3	3.9	3.1	3.4
DB 358 (23)	4.3	5.2	8.3	7.5	2.9	3.2
HBX 77 (24)	4.2	4.6	7.5	6.1	1.6	2.0
DB 375 (2)	4.2	4.2	4.7	2.3	3.4	5.9
DB 337 (37)	4.2	4.8	6.6	5.4	3.0	3.7
DB 244 (37)	3.9	4.1	10.4	7.0	2.7	3.1
PHU 950(412)	3.8	4.6	4.8	4.4	2.9	3.4
DB 161 (10)	3.7	4.1	8.3	6.2	2.5	2.5
DB 378 (1)	3.7	3.2	7.1	3.1	2.9	4.2
DB 441 (2)	3.7	4.2	6.6	5.6	3.1	3.5
DB 354(901)	3.6	3.7	4.5	2.2	3.4	4.6
HB 165 (1)	3.6	3.4	6.3	5.0	2.8	3.1
DB 333 (16)	3.4	4.0	5.9	2.8	3.4	4.2
81 S 66	3.3	3.2	8.9	6.4	2.8	3.3
71 T 6	3.3	3.6	5.0	3.4	2.9	3.5
HB 117(55)	3.2	2.7	5.0	3.7	2.9	3.2
DB 358 (30)	3.2	4.7	5.6	5.8	3.2	3.8
DB 207 (35)	3.2	3.9	6.0	4.2	3.4	6.1
DB 375 (1)	3.1	3.2	3.8	2.4	3.5	4.4
PHU 951(901)	2.8	5.1	5.5	5.5	3.2	4.0
DB 226 (70)	2.8	3.1	3.5	2.3	3.2	4.5
DB 323 (3)	2.7	3.5	6.8	5.6	2.7	3.1
842 P 75	2.7	3.2	6.4	6.2	2.8	3.1
71 P 46	2.4	1.9	5.5	3.5	2.8	3.0
DB 168 (11)	2.4	3.1	6.7	4.1	2.5	3.3
DB 270 (43)	2.3	2.3	4.8	3.7	3.4	4.8
DB 271 (39)	2.2	2.0	4.5	2.9	3.4	4.0
TC 43 (45)	2.0	2.3	5.7	4.1	2.8	3.3
DB 299 (39)	1.9	2.0	4.1	2.9	3.1	3.9
HB 171 (13)	1.7	1.3	5.5	3.7	2.3	2.5
DB 199 (10)	1.4	1.1	3.8	1.9	2.8	3.9
mean	3.4	3.6	6.6	4.8	2.9	3.6
LSD ($P = 0.05$)	0.21		0.33		0.15	

The type of light used to illuminate the tubers also affected the ratio of chlorophyll a to chlorophyll b (**Table 7**). Averaged over all *S. phureja* lines, the chlorophyll ratio in the tubers exposed to sodium lights was 3.6 as compared with 2.9 for those exposed to mercury lights. These results are of a similar order of magnitude to the value of 4.5 found in *S. tuberosum* cultivars exposed to sunlight for 10 days (*19*). The observed change in ratio from sodium to mercury lights found in this experiment is consistent with the suggestion (*19*) that if total chlorophyll production is reduced then there is a tendency to maximize light harvesting potential by increasing the a:b ratio. The sodium-illuminated tubers yielded the least total chlorophyll value. Such suggestions would also predict a higher a:b ratio in the *S. phureja* lines, which have the lowest increase in total chlorophyll content, and indeed, statistically significant ($P < 0.01$) negative correlation coefficients (Hg light, $r = -0.443$; Na light, $r =$

Table 8. Relative Amounts (% Total Carotenoids) and Concentrations (μmol 100 g^{-1} FDM) of Individual Carotenoids Induced in Tubers from Four *S. phureja* Lines in Response to Exposure to Either Mercury or Sodium Lights

	light source	μmol 100 g^{-1} FDM (% total carotenoids)				
		851 T8	80 CP 23	DB 207	DB299	mean
		neoxanthin				
	Hg	0.8 (15%)	0.4 (8%)	0.3 (10%)	0.21 (9%)	0.4 (10%)
	Na	0.6 (9%)	0.1 (4%)	0.1 (3%)	0.04 (2%)	0.2 (4%)
LSD ($P = 0.05$)[a]						0.07 (0.9)
		violaxanthin				
	Hg	0.8 (16%)	1.0 (23%)	0.6 (20%)	0.2 (8%)	0.7 (17%)
	Na	0.5 (2%)	0.0 (0%)	0.0 (1%)	0.0 (1%)	0.1 (2%)
LSD ($P = 0.05$)[a]	0.30 (3.3)					
		antheraxanthin				
	Hg	0.1 (3%)	0.3 (5%)	0.3 (11%)	0.1 (3%)	0.2 (5%)
	Na	0.4 (8%)	0.5 (11%)	0.7 (18%)	0.1 (4%)	0.4 (10%)
LSD ($P = 0.05$)[a]						0.20 (3.4)
		lutein				
	Hg	3.1 (61%)	2.0 (47%)	1.3 (40%)	1.4 (74%)	2.0 (56%)
	Na	4.1 (69%)	1.6 (42%)	1.2 (30%)	1.3 (64%)	2.1 (51%)
LSD ($P = 0.05$)[a]						NS[b] (NS)[b]
		zeaxanthin				
	Hg	0.2 (4%)	0.7 (16%)	0.6 (19%)	0.1 (7%)	0.4 (12%)
	Na	0.3 (5%)	1.8 (45%)	1.9 (49%)	0.5 (27%)	1.1 (32%)
LSD ($P = 0.05$)[a]						0.11 (3.1)

[a] Least significant difference between treatment means averaged over all lines. [b] NS, not statistically significant.

Table 9. Individual Carotenoid Composition (% Total Carotenoids) of Four *S. phureja* Lines Prior to Exposure to Light

	% total carotenoids				
carotenoid	neoxanthin	vioaxanthin	antheraxanthin	lutein	zeaxanthin
80 CP 23	5	33	22	12	28
851 T8	9	55	5	22	9
DB 207	10	46	5	39	tr[a]
DB 299	14	26	7	44	10
mean	10	40	10	29	12

[a] tr, trace.

−0.486) were found between the chlorophyll a:b ratio and total chlorophyll content in both sodium- and mercury-illuminated tubers.

The concentrations of the main individual carotenoids were determined in replicated freeze-dried tuber samples both pre- and postexposure to either mercury or sodium light from five *S. phureja* lines selected to cover the range of values detected for light-induced increases in total carotenoid content (**Table 7**). From these results, the amount of each individual carotenoid produced in response to sodium or mercury illumination was determined by subtraction and the relative amounts (% total carotenoids) of each calculated.

From these results (**Table 8**), it can be seen that for all four *S. phureja* lines, exposure to mercury lights resulted in the induction of all five carotenoids. However, the predominant carotenoid synthesized was lutein, which accounted, depending on the line, for between 40 and 74% of the total mercury light-induced carotenoids. The relative amounts of all of the other carotenoids rarely exceeded 20% on an individual basis.

The overall composition of the light-induced carotenoids did not reflect the carotenoid composition seen in the lines prior to their exposure to light (**Table 9**) but were closer to that reported for leafy vegetables (*27*) where lutein is frequently the predominant carotenoid, accounting for over 50% of the total carotenoids in kale, spinach, endive, and parsley leaf.

The composition of the sodium light-induced carotenoids also did not reflect that seen in the tubers prior to exposure nor did the composition appear to be related to the magnitude of the induced increase. In each of the four cultivars studied, proportionally less neoxanthin and antheraxanthin was induced by sodium lights than was produced in response to mercury illumination and as with exposure to mercury lights lutein accounted for a large proportion (30−70%) of the total carotenoids synthesized. However, averaged over all lines, sodium illumination induced a significantly greater proportion of zeaxanthin (32 cf. 12%) than was seen in the mercury-induced tubers.

These results might suggest, assuming that carotenoid ester breakdown is not a major effect, that both sodium and mercury lights result in an activation of all enzymes up to and including the hydroxylases required for the conversion of α- and β-carotene to their dihydroxy analogues lutein and zeaxanthin, respectively. The oxidation of zeaxanthin to the diepoxide, violaxanthin, via the monoepoxide, antheraxanthin, is catalyzed by the enzyme zeaxanthin epoxidase (ZEP 1) while neoxanthin is produced from violaxanthin by a process of proton extraction and rearrangement (*1*). These processes both appear to be stimulated by mercury light, but sodium light appears to result only in an increase in the monoepoxide, antheraxanthin, and a build up of the precursor, zeaxanthin.

Differential expression of the genes for carotenoid biosynthesis enzymes under different light qualities may be mediated by elements within the gene promoter. In the promoter of the phytoene synthase gene in *Arabidopsis*, the key enzyme initiating the carotenoid biosynthesis pathway, a number of elements that respond differentially to blue, red, and far red light, have been identified (*28*). Motifs from the phytoene synthase promoter are shared with promoters of genes involved in other carotenoid, tocopherol, and some photosynthesis-related genes, and these observations may explain the differential response to different light qualities.

The difference in carotenoid profiles following exposure to mercury or sodium light suggests that our current understanding of the biosynthetic pathway (*2*) may be incomplete. The increase in the monoepoxide antheraxanthin and the build-up of its predecessor zeaxanthin together, without a strong influence on the levels of the diepoxide violaxanthin, are incompatible with the view that the same enzyme drives both epoxidation steps and that a second enzyme drives both reverse de-epoxidation reactions. Clearly, more detailed enzymological and molecular studies are required to elucidate more precisely the mechanisms involved.

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LITERATURE CITED

(1) van den Berg, H.; Faulks, R.; Fernando Granado, H.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food Agric.* **2000**, *80*, 880−912.

(2) Taylor, M. A.; Ramsay, G. Carotenoid biosynthesis in plant storage organs: Recent advances and prospects for improving plant food quality. *Physiol. Plant.* **2005**, *124*, 143−151.

(3) Bouvier, F.; Isner, J.-C.; Dogbo, O.; Camara, B. Oxidative tailoring of carotenoids: A prospect towards novel functions in plants. *Trends Plant Sci.* **2005**, *10*, 187−194.

(4) Paulsen, H. *The Photochemistry of Carotenoids*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999.
(5) Breithaupt, D. E.; Bamedi, A. Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): New insights into an ancient vegetable. *J. Agric. Food Chem.* **2002**, *50*, 7175−7181.
(6) Morris, W. L.; Ducreux, L.; Griffiths, D. W.; Stewart, D.; Davies, H. V.; Taylor, M. A. Carotenogenesis during tuber development and storage in potato. *J. Exp. Bot.* **2004**, *55*, 975−982.
(7) Lu, W.; Haynes, K.; Wiley, E.; Clevidence, B. Carotenoid content and color in diploid potatoes. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 722−726.
(8) Landrum, J. T.; Bones, R. A. Carotenoids in the retina and lens: Possible acute and chronic effects on human visible performance. *Arch. Biochem. Biophys.* **2001**, *385*, 28−40.
(9) Seddon, J. M.; Ajani, U. A.; Sperduto, R. D.; Hiller, R.; Blair, N.; Burton, T. C.; Farber, M. D.; Gargoudas, E. S.; Haller, J.; Miller, D. T.; Yanuzzi, L. A.; Willet, W. Dietary carotenoids, Vitamins A, C and E and age-related macular degeneration. Eye disease case-control study group. *J. Am. Med. Assoc.* **1994**, *272*, 1413−1420.
(10) Brown, C. R.; Edwards, C. G.; Yang, C. P.; Dean, B. B. Orange flesh trait in potato: Inheritance and carotenoid content. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 145−150.
(11) Romer, S.; Lubeck, J.; Kauder, F.; Steiger, S.; Adomat, C.; Sandmann, G. Genetic engineering of zeaxanthin-rich potato by anti-sense inactivation and co-suppression of carotenoid epoxidation. *Metab. Eng.* **2002**, *4*, 263−272.
(12) Ducreux, L. J. M.; Morris, W. L.; Hedley, P. E.; Shepherd, T.; Davies, H. V.; Millam, S.; Taylor, M. A. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. *J. Exp. Bot.* **2004**, *56*, 81−89.
(13) Brown, J.; Mackay, G. R.; Bain, H.; Griffiths, D. W.; Allison, M. J. The processing potential of tubers of the cultivated potato, *Solanum tuberosum L.*,after storage at low temperatures. 2. Sugar concentration. *Potato Res.* **1990**, *33*, 219−227.
(14) De Wilde, T.; De Meulenaer, B.; Mestdagh, F.; Govaert, Y.; Vandebeurie, S.; Ooghe, W.; Fraselle, S.; Deneulemeester, K.; Van Peteghem, C.; Calus, A.; Degroodt, J.-M.; Verhe, R. Influence of storage practices on acrylamide formation during potato frying. *J. Agric. Food Chem.* **2005**, *53*, 6550−6557.
(15) Griffiths, D. W.; Bain, H.; Dale, M. F. B. Effect of storage temperature on potato (*Solanum. tuberosum* L.) tuber glycoalkaloid content and subsequent accumulation of glycoalkaloids and chlorophyll in response to light exposure. *J. Agric. Food Chem.* **1998**, *46*, 5262−5268.
(16) Griffiths, D. W.; Bain, H.; Deighton, N.; Robertson, G. W.; Dale, M. F. B. Photo-induced synthesis of tomatidenol-based glycoalkaloids in *Solanum phureja* tubers. *Phytochemistry* **2000**, *53*, 739−745.
(17) Griffiths, D. W.; Dale, M. F. B. Effect of light exposure on glycoalkaloid content of *Solanum phureja* tubers. *J. Agric. Food Chem.* **2001**, *49*, 5223−5227.
(18) Hendry, G. A. F.; Price, A. H. Stress indicators: Chlorophyll and carotenoids. In *Methods of Comparative Plant Ecology: A Laboratory Manual*; Hendry, G. A. F., Grime, G. P., Eds.; Chapman and Hall: London, United Kingdom, 1993; pp 148−152.
(19) Edwards, E. J.; Saint, R. E.; Cobb, A. H. Is there a link between greening and light enhanced glycoalkaloid accumulation in potato (*Solanum tuberosum* L.) tubers? *J. Sci. Food Agric.* **1998**, *76*, 327−333.
(20) Kimura, M.; Rodriguez-Amaya D. B. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem.* **2002**, *78*, 389−398.
(21) von Euler, L.; Ahlstroem, L.; Hoegberg, B.; Tingstam, Sv. Vitamin content of foodstuffs I. *Ark. Kemi. Mineral. Geol. Ser. B* **1943**, *11* (8).
(22) Cadwell, J. S.; Brunstetter, B. C.; Culpepper, C. W.; Ezell, B. D. Causes and control of discoloration in dehydration of white potatoes. *Canner* **1945**, *100*, 112−122.
(23) Dale, M. F. B.; Griffiths, D. W.; Todd, D. T. Effects of genotype, environment and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum*) tubers. *J Agric. Food Chem.* **2003**, *51*, 244−248.
(24) Akeley, R. V.; Houghland, G. V. C.; Schark, A. E. Genetic differences in potato-tuber greening. *Am. Potato J.* **1962**, *39*, 409−417.
(25) Conner, H. W. Effect of light on solanine synthesis in the potato tuber. *Plant Physiol.* **1937**, *12*, 79−98.
(26) Jadhav, S. J.; Sharma, R. P.; Salhunke, D. K. Naturally occurring toxic alkaloid in food. *CRC Crit. Rev. Toxicol.* **1981**, *9*, 21−67.
(27) Müller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm Unters. Forsch. A* **1997**, *204*, 88−94.
(28) Welsch, R.; Medina, J.; Giuliano, G.; Beyer, P.; von Lintig, J. Structural and functional characterization of the phytoene synthase promoter from *Arabidopsis thaliana*. *Planta* **2003**, *216*, 523−534.

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