# Chlorophyll, Chlorogenic Acid, Glycoalkaloid, and Protease Inhibitor Content of Fresh and Green Potatoes

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Exposure of commercial White Rose potatoes to fluorescent light for 20 days induced a time-dependent greening of potato surfaces; an increase in chlorophyll, chlorogenic acid, and glycoalkaloid content ($\alpha$-chaconine and $\alpha$-solanine); and no changes in the content of inhibitors of the digestive enzymes trypsin, chymotrypsin, and carboxypeptidase A. The maximum chlorophyll level of the light-stored potatoes was 0.5 mg/100 g of fresh potato weight. Unstored potatoes contained no chlorophyll. Storing potatoes in the dark did not result in greening or chlorophyll formation. Chlorogenic acid and glycoalkaloid levels of dark-stored potatoes did increase but less than in the light-stored potatoes. In the light, chlorogenic acid concentration increased from 7.1 mg/100 g of fresh potato weight to a maximum of 15.8 mg after greening. The corresponding values for $\alpha$-chaconine are 0.66 and 2.03 mg and for $\alpha$-solanine 0.58 and 1.71, respectively, or an approximately 300% increase for each glycoalkaloid. The trypsin, chymotrypsin, and carboxypeptidase A concentrations, respectively, of about 1000, 375, and 100 units/g of dehydrated potato powder were not changed. Experiments on delay of greening by immersion in water suggest that (a) chlorophyll formation and glycoalkaloid synthesis are unrelated physiological processes and (b) the concentration of chlorophyll is 26 times greater, of chlorogenic acid and glycoalkaloids 7–8 times greater, and of protease inhibitors about 2–3 times lower in the peel of the green potatoes than in the whole tuber. The described compositional changes should help define consequences of potato greening for plant physiology, food quality, and food safety.

## INTRODUCTION

Potatoes contain polyphenolic compounds such as chlorogenic acid, the glycoalkaloids $\alpha$-chaconine and $\alpha$-solanine, and proteins, which have the ability to inhibit trypsin, chymotrypsin, and carboxypeptidase (Lisinska and Leszchynski, 1989; Friedman, 1992). Light, mechanical injury, temperature extremes, and sprouting induce an increase in the glycoalkaloid content which, depending on conditions used, can range up to 5 times original levels. Exposure to light after harvest also leads to surface greening and to increases in glycoalkaloid content due to stimulation of chlorophyll biosynthesis. These changes can be minimized by treating potatoes with $\gamma$-radiation, chemicals that inhibit sprouting (Schwimmer and Weston, 1958; Martens and Beardseth, 1987; Swallow, 1991), or oils that cover the surface of potatoes and thus prevent the photoinduction of chlorophyll and glycoalkaloids (Jadhav et al., 1991a,b; Schouten, 1987).

The formation of chlorophyll during greening may be beneficial, since it is used as a natural coloring agent and deodorizer (Martin and Cook, 1962) and can adsorb potentially carcinogenic heterocyclic amines produced during food processing (Weisburger, 1991; Friedman, 1991). However, the increase in glycoalkaloid content is undesirable since it could impart a bitter taste to potatoes (Kaaber, 1993; Sinden et al., 1976) and make them less safe (Morris and Lee, 1984; Keeler et al., 1991). Since chlorogenic acid, glycoalkaloids, and protease inhibitors may act as so-called antifeeding agents in potatoes, protecting potatoes from attack by phytopathogens and insects, the question arises whether suppression of biosynthesis of alkaloids, one of our current objectives (Stapleton et al., 1991, 1992), will result in compensatory changes in the biosynthesis of chlorogenic acid and/or protease inhibitors. To address this issue, potatoes were stored in the dark and under fluorescent light for various time periods and analyzed for chlorophyll, chlorogenic acid, $\alpha$-chaconine, $\alpha$-solanine, and inhibitors of the digestive enzymes trypsin, chymotrypsin, and carboxypeptidase A. Indications are that light induces changes in chlorophyll, chlorogenic acid, and glycoalkaloid levels but not in protease inhibitor levels.

## MATERIALS AND METHODS

**Materials.** Solvents were of HPLC spectroquality grade. Chlorogenic acid was obtained from Aldrich Chemical Co., Milwaukee, WI. $\alpha$-Chaconine, $\alpha$-solanine, trypsin, chymotrypsin, *N*-$\alpha$-tosylarginine methyl ester (TAME), *N*-benzoyl-L-tyrosine ethyl ester (BTEE), tris(hydroxymethyl)aminomethane (Tris), the Kunitz trypsin inhibitor (KTI) and Bowman–Birk inhibitor (BBI) of soybeans, carboxypeptidase A, carboxypeptidase inhibitor from potatoes, hippuryl-L-phenylalanine, and other reagents were purchased from Sigma Chemical Co., St. Louis, MO. White Rose potatoes of uniform size originating from Norm and Nelson Inc., Burlington, WA, and each weighing approximately 80–100 g, were purchased in a local store within a week after shipping.

**Potato Treatments.** The potato tubers were cleaned with a damp paper towel and divided in half. Half of the potatoes were placed in the dark at room temperature and the other in a laboratory hood (70 × 80 × 130 cm; width × height × length) partially opened to access air and equipped with two 90 cm long fluorescent lights. The intensity of light, measured with a greenhouse light meter, was 60 ft-c. The hood fan was shut off. The tubers were rotated around every 24 h. Six randomly exposed tubers were selected after each exposure period of 3, 6, 9, 13, 16, or 20 days at room temperature (22 °C). Three of these were cut up into small cubes, which were then placed into freeze-drying jars and frozen in liquid nitrogen. The frozen samples were lyophilized, and the dried cubes were ground in a Wiley mill to pass a 40-mesh screen. The powders were then stored in a refrigerator until used. The other three tubers were used for chlorophyll determination.

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**Chlorophyll Determination.** The procedure was adapted from that of Moran (1982). Specifically, three tubers were randomly selected at each date to evaluate color. The following sequence of colors on the surface of the potatoes was noted as the light experiment progressed: yellowish green, light green, green, intense green. The skins of each tuber were peeled off and weighed. The skins, about 2 mm thick, were then immersed in 100 mL of *N,N*-dimethylformamide (DMF) and placed in a refrigerator at 4 °C for 48 h. The absorption spectrum of the DMF solution was then obtained with an Perkin-Elmer Lamda UV–vis spectrophotometer equipped with an Epson Equity III computer. The following formula was used to calculate the chlorophyll concentration from the absorption maxima at 664 and 647 nm: $C_t = 7.04A_{664} + 20.27\ A_{647}$, where $C_t$ is the total chlorophyll concentration in mg/L and $A_{664}$ and $A_{647}$ are the maximum absorbances at 664 and 647 nm.

**Delayed Chlorophyll Formation.** Another experiment evaluated chlorophyll synthesis when tubers were partly immersed in water. Four sets of potato samples were studied: A, whole tubers unexposed to light (control); B, whole tubers; C, tubers cut lengthwise and the cut surfaces placed on aluminum foil; D, tubers cut lengthwise and the cut surfaces placed on a glass pan containing a 1 cm deep layer of water which was changed daily. Samples B–D were exposed to light as described earlier. After 5 days, the samples were removed from their environment and peeled. Fresh peels (5 g) were submerged in DMF (10 mL) and stored in a refrigerator at 4 °C for 48 h. The chlorophyll content of the DMF solution was then determined spectrophotometrically. The remaining peels were lyophilized, and the dehydrated powders were analyzed for chlorogenic acid, glycoalkaloids, and protease inhibitors.

**Chlorogenic Acid Analysis.** Chlorogenic acid in potatoes was determined by ultraviolet spectrophotometry as previously described (Dao and Friedman, 1992).

**Determination of Glycoalkaloids.** A Beckman Model 334 liquid chromatograph with a 427 integrator and a 165 UV–vis variable-wavelength detector was used to estimate the α-chaconine and α-solanine content of the potato samples, as described previously (Friedman and Dao, 1992).

**Trypsin Inhibitor Assay.** The assays for inhibition of trypsin and chymotrypsin were adapted from those described for the inhibition of soybean inhibitors (Friedman and Gumbmann, 1986).

Potato powder (100 mg) was suspended in 10 mL of Tris-HCl (pH 8.1) buffer with stirring for 1 h at room temperature. The suspension was centrifuged for 10 min and the supernatant diluted 1:2 with Tris buffer. Twenty microliters of this solution was used for the inhibition assay. The following conditions were used: temperature, 25 °C; buffer, 0.046 M Tris-HCl containing 0.0115 M $CaCl_2$ (pH 8.1); substrate, 10 mM TAME (37.9 mg/10 mL of $H_2O$); enzyme, 1 mg/mL, 1 mM HCl. The enzyme solution was diluted to 10–20 μg/mL. In the absence of inhibitor, 2.6 mL of buffer and 0.3 mL of TAME were added to a 3-mL cuvette followed by 0.1 mL of diluted enzyme solution. The absorbance was recorded at 247 nm ($A_{247}$) for 3 min on a Perkin-Elmer Lambda 6 spectrophotometer. The increase in absorbance was then determined from the initial linear portion of the curve. In the presence of inhibitors, 2.6 mL of buffer, 0.1 mL of enzyme solution, and 20 μL of inhibitor solution (prepared to give 50% inhibition) were preincubated for 6 min. The reaction was started by adding 0.3 mL of TAME; $A_{247}$ was then recorded for 3 min. Values were based on sample dilutions yielding 40–60% inhibition. Enzyme activity is defined by the following equation: units/mg = $(\Delta A_{247}/\text{min} \times 1000 \times 3)/(540 \times \text{mg of enzyme used})$.

A trypsin unit (TU) is defined as the amount of trypsin that catalyzes the hydrolysis of 1 μmol of substrate/min. A trypsin inhibitor unit (TIU) is the reduction in activity of trypsin by 1 TU.

**Chymotrypsin Inhibition Assay.** Potato powder (200 mg) was suspended in 10 mL of Tris-HCl (pH 8.1) buffer with stirring for 1 h at room temperature. The suspension was centrifuged for 10 min. Twenty microliters of the supernatant was used for the inhibition assay. The following conditions were used: buffer, 0.08 M Tris-HCl containing 0.1 M $CaCl_2$ (pH 7.8); substrate, 1.07 mM BTEE (8.4 mg/25 mL of 50% methanol); enzyme, 1 mg/mL, 1 mM HCl. The enzyme solution was diluted to a concentration of 10–20 μg/mL. In the absence of inhibitor, 1.5 mL of buffer, 1.4 mL BTEE, and 0.1 mL of enzyme solution were added and the increase in absorbance at 256 nm ($A_{256}$) was recorded for 3 min. The $\Delta A_{256}$/min was then calculated from the initial linear portion of the curve. In the presence of inhibitor, 1.5 mL of buffer, 0.1 mL of enzyme solution, and 20 μL of inhibitor were incubated for 6 min before the addition of 1.4 mL of BTEE and recording as above. Buffer plus substrate served as controls for all measurements. Values were based on sample dilutions yielding 40–60% inhibition. Enzyme activity is defined by the following equation: units/mg = $(\Delta A_{256}/\text{min} \times 1000 \times 3)/(964 \times \text{mg of enzyme used})$.

One chymotrypsin unit (CU) is defined as the amount of chymotrypsin that catalyzes the hydrolysis of 1 μmol of substrate/min. A chymotrypsin inhibitor unit (CIU) is the reduction in activity of chymotrypsin by 1 CU.

**Carboxypeptidase Inhibition Assay.** The method was adopted from that of Worthington Biochemical Products (1982). Potato powder (300 mg) was suspended in 10 mL of Tris-HCl (pH 7.5) buffer with stirring for 1 h at room temperature. The suspension was centrifuged for 10 min. One hundred microliters of the supernatant was used for the inhibition assay. Reagents were 0.025 M Tris-HCl buffer containing 0.5 M NaCl (pH 7.5), 1 mM hippuryl-L-phenylalanine in 0.025 M Tris-HCl buffer, and 10% LiCl.

The enzyme was dissolved in 10% LiCl to a concentration of 1–3 units/mL. The rate of hydrolysis of hippuryl-L-phenylalanine by carboxypeptidase in the absence and presence of inhibitors was determined by measuring the increase in absorbance at 254 nm. Specifically, into each cuvette was pipetted 2.9 mL of substrate solution, which was then incubated at 25 °C for 3–4 min to reach temperature equilibrium. The diluted enzyme solution (0.1 mL) was then added, and the absorbance at 254 nm was recorded for 3–5 min. The change in absorbance ($\Delta A_{254}$) was calculated from the initial portion of the curve.

One carboxypeptidase unit is defined as the amount of carboxypeptidase that catalyzes the hydrolysis of 1 μmol of hippuryl-L-phenylalanine/min at pH 7.5 and 25 °C.

Pure soybean Kunitz trypsin inhibitor (KTI) and pure soy Bowman–Birk chymotrypsin inhibitor (BBI) were used as standards with each trypsin and chymotrypsin inhibition assay. The calculated values are based on the individual inhibitor control values. For carboxypeptidase, enzyme inhibition was standardized with the carboxypeptidase inhibitor from potatoes.

**Statistics.** The data were analyzed using SAS PROC GLM (SAS/STAT, 1987) fitting time as a continuous effect where appropriate and storage conditions as a discrete effect. The following is a brief summary of the results from the statistical tests.

For chlorophyll, there was significant linear regression across time for the light storage treatment ($p \leq 0.001$). The intercept was not significantly different from zero ($p = 0.40$). Following are the parameter estimates and standard errors, respectively: intercept, −0.033, 0.036; slope, 0.0259, 0.0029. For the delayed greening experiment, Duncan's multiple-range test provides evidence that all differences among sample means are significant at the 5% level for chlorophyll.

For chlorogenic acid, a linear model adequately fits the data, with an almost significant storage × time linear interaction ($p = 0.064$). The intercepts are also not significantly different ($p = 0.099$). The slope for the dark storage treatment is not significantly different from zero ($p = 0.077$), while that for the light storage treatment is highly significant from zero ($p \leq 0.001$). Following are the parameter estimates and standard errors: intercept, dark, 7.12, light, 8.97, SE, 0.72; slope, dark, 0.122, light, 0.305, SE, 0.62.

For α-chaconine and α-solanine, a quadratic model across time was required to adequately fit the data, with the storage × time interactions not significant ($p > 0.57$). In both cases the overall difference between the light and dark storage is significant at the 5% level. Following are the least-squares means and standard errors: α-chaconine, dark, 1.306, light, 1.544, SE, 0.066; α-solanine, dark, 1.034, light, 1.239, SE, 0.054.

For protease inhibitors, there was no evidence of any significant continuous time effect. The trypsin data were transformed by ranks because of an extreme value. The data were subjected to

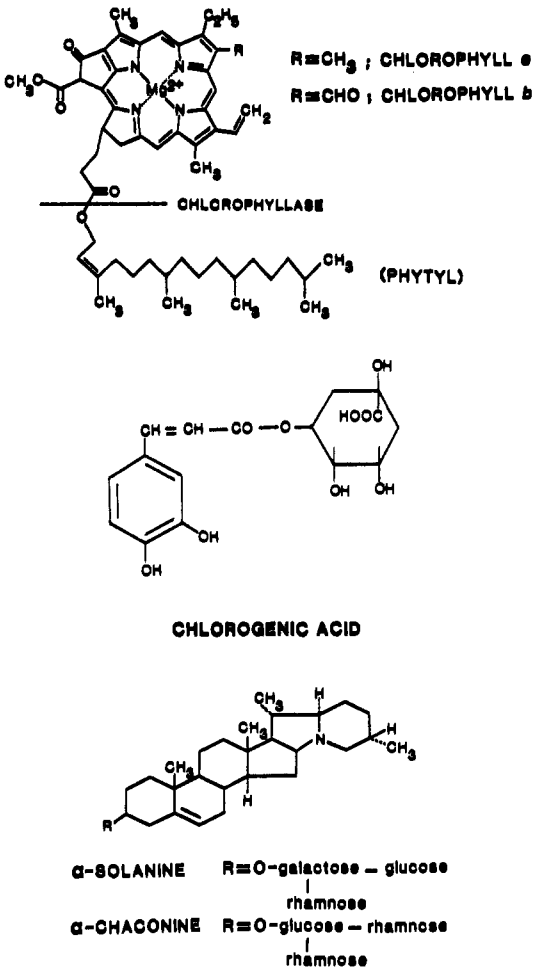


**Figure 1.** Structures of chlorophylls, chlorogenic acid, and glycoalkaloids.

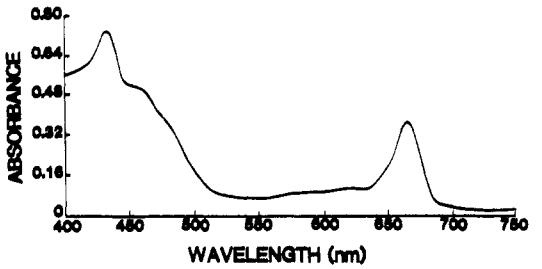


**Figure 2.** Visible absorption spectrum of a potato chlorophyll extract in DMF. The spectrum represents a composite of four possible chlorophyll species in White Rose potatoes: chlorophyll *a*, chlorophyll *b*, chlorophyllin *a*, and chlorophyllin *b*. The latter two are derived from removal of the phytol group as shown in Figure 1.

two-way analyses of variance, which resulted in nonsignificant treatment effects ($p > 0.18$), i.e., both main effects and interactions.

## RESULTS AND DISCUSSION

To place our findings in perspective, we briefly discuss how each of the potato constituents evaluated in this study may affect food quality, food safety, and nutrition. We then discuss the observed changes following potato greening.

**Chlorophyll.** Figure 1 shows the structures of three of the four classes of potato constituents evaluated in this study. Figure 2 shows the visible spectrum of chlorophyll obtained from green potato peels. The green color is due to the absorbance peak near 670 nm. Table 1 shows the chlorophyll content of potatoes subjected to fluorescent light and stored in the dark. While potatoes stored in the dark contained no measurable chlorophyll, there was a continuous increase in chlorophyll content of potatoes subjected to light from 0 to 0.5 mg/100 g of fresh weight of the potatoes. The potato skins exposed to light were intense green. This observation shows that light is important for the biosynthesis of chlorophyll during postharvest storage of potatoes (Kozukue and Mizuno, 1990; Jadhav et al., 1991a,b).

Chlorophyll, structurally a dihydroporphyrin magnesium chelate derivative located in parts of plant cells called chloroplasts, functions as a photosynthetic pigment at the "front end" of life processes that depend on the utilization of light energy. Here, the property of intense absorption of porphyrin derivatives is used to transform the energy of photons to chemical energy which catalyzes photosynthesis in plants (Stryer, 1988). Chlorophylls exist in plants as a mixture of chlorophyll *a* and chlorophyll *b* (Figure 2). Chlorophyll *a* differs from *b* by having a methyl group ($CH_3$) of the porphyrin ring replaced by a formyl (CHO) group. The plant enzyme chlorophyllase can hydrolyze the ester linkage by which the phytyl side chain is attached to the porphyrin ring, forming chlorophyllide and phytol (Phan, 1987). Thus, both chlorophylls *a* and *b* and their hydrolysis products may all be present in green potatoes. The chlorophyll content shown in Table1 reflects the sum of all the chlorophyll species, since the calculated values are based on the absorption spectra of the porphyrin ring that they all share.

**Chlorogenic Acid.** Chlorogenic acid (5-*O*-caffeoylquinic acid) (Figure 1) constitutes about 90% of the total polyphenolic content of potatoes (Malmberg and Theander, 1985; Mondy and Gosselin, 1988; Ramamurthy et al., 1992; Dao and Friedman, 1992). The compound may be responsible for the bluish gray coloration of boiled or steamed potatoes following exposure to air (Swain, 1962) and for enzymatic browning (Schwimmer, 1981; Hurrell and Finot, 1984). It also may be involved in the defenses of potatoes against insects and phytopathogens (Deshpande et al., 1984; Sinden et al., 1988). It affects the taste and flavor of potatoes (Martens and Baardseth, 1987; Sinden et al., 1976), is a strong antioxidant (Pratt, 1993), and inhibits phorbol-ester-induced skin tumor promotion in mice (Huang et al., 1992).

Table 1 and Figure 3 show trends in chlorogenic acid content of potatoes following dark or light storage. The chlorogenic acid content of potatoes stored in the dark increased from 7.1 to 9.4 mg/100 g of fresh weight after 20 days. The increase for the light-stored potatoes was greater, more than double, from 7.1 to 15.8 mg/100 g of fresh weight. These results suggest that light stimulates the biosynthesis of chlorogenic acid in potatoes after harvest. The small increase in chlorogenic acid content in dark-stored potatoes is difficult to explain. One possibility is that presprouting activities in these tubers cause this increase, since we have previously shown that the chlorogenic acid content of sprouts is much greater than that of tubers (Dao and Friedman, 1992). However, we have no evidence of the potatoes breaking dormancy.

**Glycoalkaloids.** The two major glycoalkaloids of potatoes, α-chaconine and α-solanine (Figure 1), are reported to exert strong pharmacological and toxicological effects in animals (Friedman, 1992; Jadhav et al., 1991; Keeler et al., 1991). Previously, we reported that α-chaconine is more toxic than α-solanine to frog embryos (Friedman et al., 1992), induces greater amounts of the enzyme hepatic ornithine decarboxylase in rats (Caldwell

Table 1. Effect of Greening on Chlorophyll, Chlorogenic Acid, and Glycoalkaloid Contents of White Rose Potatoes[a]

| | chlorophyll | | chlorogenic acid | | α-chaconine | | α-solanine | |
|---|---|---|---|---|---|---|---|---|
| time (days) | dark | light | dark | light | dark | light | dark | light |
| 0 | ND[b] | 0 | 7.1 ± 0.1 | 7.1 ± 0.1 | 0.66 ± 0.05 | 0.66 ± 0.05 | 0.58 ± 0.05 | 0.58 ± 0.05 |
| 3 | ND | 0.077 ± 0.002 | 7.4 ± 0.3 | 11.9 ± 0.9 | 1.02 ± 0.13 | 1.15 ± 0.02 | 0.68 ± 0.07 | 0.88 ± 0.01 |
| 5 | ND | 0.096 ± 0.011 | 7.6 ± 0.0 | 10.7 ± 0.7 | 1.02 ± 0.23 | 1.75 ± 0.05 | 0.81 ± 0.25 | 1.38 ± 0.04 |
| 9 | ND | 0.134 ± 0.007 | 8.7 ± 0.1 | 12.8 ± 1.0 | 1.30 ± 0.03 | 1.81 ± 0.07 | 1.06 ± 0.04 | 1.39 ± 0.07 |
| 13 | ND | 0.341 ± 0.024 | 8.3 ± 0.7 | 11.8 ± 1.3 | 1.71 ± 0.47 | 1.82 ± 0.05 | 1.38 ± 0.28 | 1.39 ± 0.01 |
| 16 | ND | 0.368 ± 0.068 | 9.4 ± 0.2 | 12.8 ± 1.7 | 1.84 ± 0.07 | 2.03 ± 0.09 | 1.43 ± 0.06 | 1.71 ± 0.04 |
| 20 | ND | 0.498 ± 0.008 | 9.4 ± 0.2 | 15.8 ± 0.1 | 1.59 ± 0.07 | 1.59 ± 0.13 | 1.30 ± 0.08 | 1.34 ± 0.09 |

[a] Listed values are in mg/100 g of fresh potato weight ± standard deviations for three separate determinations. [b] ND, not detected.

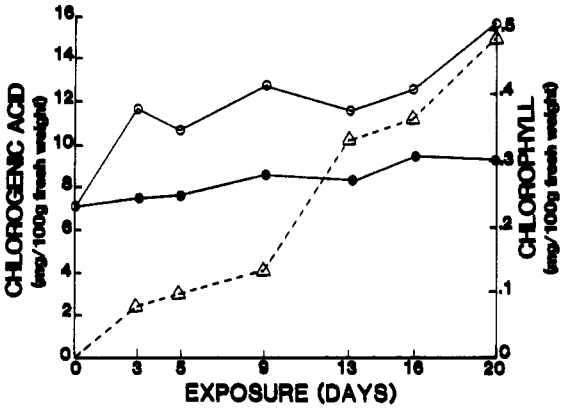


Figure 3. Chlorophyll (△) and chlorogenic acid contents of White Rose potatoes stored under fluorescent light (○) and in the dark (●).

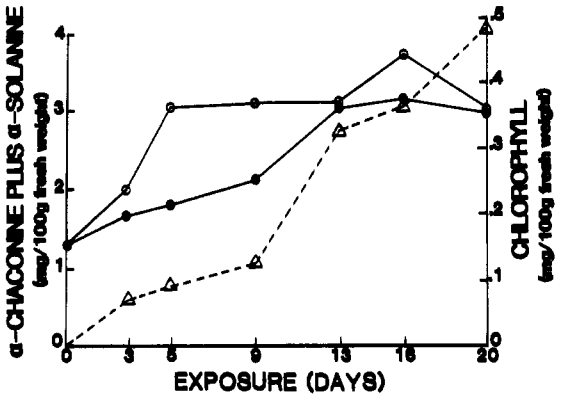


Figure 4. Chlorophyll (△) and total glycoalkaloid contents (sum of α-chaconine and α-solanine) of White Rose potatoes stored under fluorescent light (○) and in the dark (●).

et al., 1991), and has a greater effect on the membrane potential of frog embryo cells (Blankemeyer et al., 1992). We also reported that the aglycons (lacking carbohydrate residues) are less toxic than the glycosides (glycoalkaloids) from which they are derived (Friedman et al., 1991, 1992) and that the alkaloids are neither genotoxic nor chromosome-damaging (Friedman and Henika, 1992). Since biological potencies are strongly influenced by the chemical structures of the alkaloids, it is important to ascertain changes in composition of specific glycoalkaloids—not just total glycoalkaloid content—both during growth and during postharvest storage and processing of potatoes (Friedman and Dao, 1992).

Glycoalkaloids are also reported to affect the taste and flavor of potatoes (Kaaber, 1993; Sinden et al., 1976; Zitnak and Filadelfi-Keszi, 1988).

Table 1 and Figure 4 show that exposure of potatoes to fluorescent light induces a progressive increase in both α-chaconine and α-solanine. The sum of these two glycoalkaloids increased by 62% after 3 days and by 300% after 16 days. This value then decreased somewhat after

Table 2. Protease Inhibitor Content of Stored White Rose Potatoes[a]

| | inhibitors (units/g) | | | | | |
|---|---|---|---|---|---|---|
| | trypsin | | chymotrypsin | | carboxypeptidase A | |
| time (days) | dark | light | dark | light | dark | light |
| 0 | 1020 ± 28 | 2020 ± 28 | 362 ± 15 | 362 ± 15 | 121 ± 5.6 | 121 ± 5.6 |
| 3 | 1056 ± 23 | 1056 ± 23 | 368 ± 7 | 367 ± 23 | 100 ± 9.5 | 130 ± 7.8 |
| 5 | 1076 ± 5 | 1113 ± 0 | 390 ± 5 | 387 ± 9 | 111 ± 7.1 | 112 ± 5.6 |
| 9 | 1040 ± 0 | 1093 ± 28 | 380 ± 5 | 394 ± 13 | 106 ± 2.1 | 114 ± 0 |
| 13 | 988 ± 18 | 943 ± 33 | 363 ± 14 | 368 ± 7 | 125 ± 0 | 134 ± 0 |
| 16 | 960 ± 56 | 945 ± 33 | 393 ± 0 | 363 ± 0 | 112 ± 6.4 | 102 ± 16 |
| 20 | 1008 ± 46 | 1056 ± 23 | 358 ± 7 | 378 ± 7 | 107 ± 9.9 | 116 ± 0.7 |

[a] Values are averages from two separate determinations ± standard deviation for dehydrated potatoes.

20 days of exposure. Figures 3 and 4 also show that during greening of potatoes, chlorophyll, glycoalkaloid, and chlorogenic acid contents all increase with time of exposure to light. However, these increases are not a linear function of exposure time, and they do not parallel each other.

Table 1 also shows that the α-chaconine and α-solanine contents of the potatoes increased with time when stored in the dark, although less than in the light. As was suggested for chlorogenic acid, glycoalkaloid synthesis may be stimulated by pre-prouting around the eyes of potato tubers during storage in the dark. Potato sprouts stored in the dark or light both contain high levels of glycoalkaloids (unpublished results).

In contrast to *Solanum* potato glycoalkaloids, where storage under light induces a greater amount of glycoalkaloid synthesis than storage in the dark, Hobbs (1988) reports that nicotine and other alkaloids produced in cultures of the Solanaceous plant *Nicotiana rustica* are induced in the dark and suppressed by light. Although possible reasons for this effect in the *Nicotiana* plant are not immediately apparent, the author suggests that light may affect the activities of enzymes involved in the synthesis of the alkaloids.

Guidelines for the upper limit of total glycoalkaloid content of 20 mg/100 g fresh or 100 mg/100 g of dry potatoes have been widely accepted (Kaaber, 1993; Morris and Lee, 1984). The total glycoalkaloid levels of major commercial potato varieties such as Idaho Russett (Friedman and Dao, 1992) and White Rose (Table 1) are less than 2 mg/100 g. The 300% increase which occurred during greening of White Rose potatoes under the conditions of this study brings the total to about 6 mg/100 g, still low in terms of the guideline. Therefore, greening of low-glycoalkaloid potatoes may not always have a significant adverse effect on food safety. Moreover, since cultivars differ significantly in their ability to produce greening-related glycoalkaloids (Dale et al., 1993), it may be possible to find potato varieties with low rates of postharvest glycoalkaloid synthesis.

**Protease Inhibitors.** Potatoes contain significant amounts of inhibitors of digestive enzymes such as trypsin, chymotrypsin, and carboxypeptidase A (Pearce et al., 1984;

**Table 3. Effect of Delayed Greening on Chlorophyll, Chlorogenic Acid, Glycoalkaloid, and Protease Inhibitor Contents of White Rose Potatoes**

| sample | chlorophyll[a] | chlorogenic acid[a] | glycoalkaloids[a,b] | inhibitors[c] | | |
|---|---|---|---|---|---|---|
| | | | | trypsin | chymotrypsin | carboxypeptidase A |
| A[d] | 0 | 54.1 ± 0.6 | 16.5 ± 2.1 | 312 ± 4 | 195 ± 0 | 63.2 ± 4.5 |
| B[e] | 6.03 ± 1.3 | 60.4 ± 0.3 | 24.6 ± 0.6 | 316 ± 9 | 238 ± 20 | 80.4 ± 9.6 |
| C[f] | 6.65 ± 4.6 | 60.8 ± 0.7 | 25.7 ± 1.7 | 236 ± 20 | 159 ± 4 | 72.5 ± 7.8 |
| D[g] | 2.51 ± 2.3 | 54.0 ± 0.6 | 24.6 ± 0.6 | 231 ± 13 | 132 ± 4 | 54.8 ± 1.7 |

[a] Values are averages in mg/100 g of fresh peel weight from three separate determinations ± standard deviation. [b] Sum of α-chaconine and α-solanine. [c] Values are in units/g of dehydrated peel weight from two separate determinations ± standard deviation. [d] Control. [e] Whole tubers exposed to light. [f] Tubers were cut lengthwise, placed on aluminum foil, and exposed to light for 5 days. [g] Tubers were cut lengthwise, partially immersed in water, and exposed to light for 5 days.

Rackis et al., 1986). The presence of such inhibitors in foods may impair the nutritional quality and possibly the safety of foods (Gumbmann et al., 1990). However, unlike soybean inhibitors of trypsin and chymotrypsin, the inhibitors of these two enzymes in potatoes are largely inactivated through denaturation by food processing. This is not the case for potato inhibitors of carboxypeptidase (Brown et al., 1986).

Table 2 shows that the dark and light storage conditions used in this study had no effect on the content of inhibitors of trypsin, chymotrypsin, and carboxypeptidase A, which remained virtually unchanged. This finding shows that of the three classes of postulated potato antifeeding compounds, chlorogenic acid and glycoalkaloids increase over time during postharvest storage, whereas inhibitors of digestive enzyme inhibitors do not.

The combined values for trypsin and chymotrypsin inhibitors in dehydrated White Rose potatoes determined in this study (about 1400 units/g, Table 2) fall between those of White Russet potatoes (1100 units/g) and the experimental potato variety No. 3194 (1700 units/g) reported previously (Friedman, 1992). The trend for the carboxypeptidase inhibitor content of the three varieties is similar. Evidently, different potato cultivars have different inhibitor contents.

Comparison of the trypsin and chymotrypsin inhibitor contents of soybeans (Domagalski et al., 1992) and potatoes shows that dehydrated potatoes contain about 25% and fresh potatoes about 6% of the corresponding values of soybeans. It would be worthwhile to determine whether any of the potato protease inhibitors can prevent cancer, as appears to be the case with the Bowman–Birk inhibitors of soybeans (Billings and Habers, 1992).

**Significance for Plant Physiology.** Since chlorogenic acid, glycoalkaloids, and protease inhibitors have been postulated to act individually or jointly in defending potato plants against attack by phytopathogens and insects, the following questions arise:

1. Are these three classes of compounds under the same genetic control in the plant?
2. Do these compounds act independently or synergistically during growth of the plant and after harvest during storage?
3. What is the relationship between the postharvest synthesis of chlorophyll in potato tubers and the synthesis of chlorogenic acid, glycoalkaloids, and protease inhibitors?
4. How will suppression of biosynthesis of glycoalkaloids influence the biosynthesis of the other compounds?

This study showed that light stimulates synthesis of chlorophyll, chlorogenic acid, and glycoalkaloids but not of protease inhibitors.

We carried out an additional experiment to investigate whether these three classes of compounds are under the same genetic control. Table 3 shows that partial immersion of cut potato tubers in water for 5 days (sample D) resulted in formation of only 2.51 mg of chlorophyll/100 g of fresh peel weight compared to 6.65 for the sample placed on aluminum foil (sample C). Since the glycoalkaloid contents of the two samples were identical, this result suggests that chlorophyll and glycoalkaloid biosynthesis appear to be under separate genetic control.

In addition to light and temperature, water could also affect postharvest chlorophyll formation in potatoes by controlling access of air necessary for photosynthesis. This suggestion is supported by our observation that cut surfaces of water-immersed potatoes (sample D in Table 3) did not green, whereas the surfaces of cut potatoes placed on aluminum foil (sample C in Table 3) did during the 5-day storage time. Additional studies showed that water-immersed potatoes also did not brown compared to the cut potatoes placed on aluminum foil which did. Water evidently blocks oxygen needed for enzymatic browning (Molnar-Perl and Friedman, 1990). However, we cannot rule out the possibility of diffusion of water-soluble components from potatoes, although we did not see any change in water color (Mondy and Chandra, 1974).

Tables 1–3 show that after 5 days of light exposure, the 2-mm peel part of the green potatoes contained about 26 times more chlorophyll than the whole potato tubers. The corresponding chlorogenic acid and glycoalkaloid concentrations are 7–8 times greater and the protease inhibitor levels about 2–3 times lower. Possible reasons for these variations and the genetic mechanisms controlling the distribution of these compounds within the body of the potato merit further study.

In conclusion, the described studies on the influence of potato greening on compositional changes in potatoes demonstrate for the first time possible relationships between four classes of potato constituents. Our findings should facilitate further definition of the significance of chlorophyll, chlorogenic acid, glycoalkaids, and protease inhibitors in the physiology of the potato plant, both during growth and after harvest, and help assess the relative contributions of these compounds to the quality and safety of potatoes. This study can also serve as a model for "fingerprinting" the composition of new potato cultivars including transgenic potatoes.

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