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Effect of Illumination by Fluorescent Light on the Accumulation of Glycoalkaloids in the Tubers of 7 Varieties of Potato (*Solanum tuberosum* L.) Grown in Oman

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Abstract: The glycoalkaloid content in the tubers of 7 potato varieties grown in Oman was determined by HPLC in both the peel and the flesh. Tubers from these potato varieties were analyzed for their rates of glycoalkaloid accumulation in response to stresses caused by storage under fluorescent light. Exposure to fluorescent light (15.72 $\mu\text{mol m}^{-2} \text{s}^{-2}$ photosynthetically active radiation) was found to result in an increase in the glycoalkaloid concentration within tuber tissue this increase in the total glycoalkaloid content was associated with a decrease in the α -chaconine: α -solanine ratio indicating a more active synthesis of α -solanine and a less active synthesis of α -chaconine.

Key words: Potato, *Solanum tuberosum*, Glycoalkaloids, chaconine, solanine, HPLC

Introduction

Because of the importance of glycoalkaloids in quality and resistance to disease, the present investigation was undertaken with a view to find out the rate of glycoalkaloid accumulation in tubers as a result of postharvest exposure to fluorescent light. The current study aimed at determining the influence of 12 days continuous fluorescent light illumination on glycoalkaloid accumulation in tubers of 7 potato varieties grown in Oman.

Although the nature and relative concentrations of glycoalkaloids are genetically determined, the total concentrations are certainly influenced by environmental factors during the growing period (Friedman and McDonald, 1997). Weather seems to have a considerable effect on glycoalkaloid production. Elevated glycoalkaloid levels may be caused by adverse growing conditions such as prolonged cold, extreme heat, too much water, too little water, too much sunshine and too little sunshine (Friedman and McDonald, 1997). Glycoalkaloids, which are usually present at low levels in commercial potatoes, may accumulate as a result of continued biosynthesis. Factors that influence glycoalkaloid formation include light because illumination stimulates glycoalkaloid synthesis after harvest; this glycoalkaloid stimulation presents a major food safety problem for producers and consumers alike (Percival and Dixon, 1997).

Exposure of post-harvest potato tubers to light, dramatically increase glycoalkaloid synthesis (Friedman and McDonald, 1997). In general, higher intensities result in higher rates of accumulation (Percival, 1999b).

De Maine *et al.* (1988) found that the total glycoalkaloid

content of the tubers exposed to light (9600 lux) for 42 h at 21°C increased significantly.

Maximal rates of glycoalkaloid accumulation were recorded following exposure of tubers to fluorescent or sodium light and minimal rates of accumulation were recorded following exposure of tubers to mercury illumination contains few spectral lines (ultraviolet and infrared).

The usual indication that a potato may have increased glycoalkaloid levels due to light exposure is greening caused by chlorophyll production (Friedman and McDonald, 1997).

Potatoes are commonly displayed under fluorescent light (contains ultraviolet light) during marketing and sale and this may result in higher glycoalkaloid and Chlorophyll accumulation rates.

Percival *et al.* (1993) reported that the removal of tubers from 5 °C and immediate illumination at 24 °C altered the ratios of α -chaconine to α -solanine from 63:37 to 53:47 in some cultivars increasing the synthesis of α -chaconine

Materials and Methods

Potato Samples: Seed tubers of 7 exotic potato varieties were harvested in mid March 2000 and cold-stored up to mid October 2000. The plants were grown in two periods: the first from mid-November 2000 until mid-February 2001 and the second from mid-December 2000 until mid-March 2001 at the Agricultural Experiment Station, Sultan Qaboos University, Muscat, Oman.

The plants were grown in sandy loam soil of 49% sand, 34% silt and 12% clay. Fertilizer was added as N:P:K in the ratio of 2:1:1 with N given as 224 kg ha⁻¹.

Irrigation was scheduled according to evapotranspiration data, which was averaged for 90 days at about $51 \text{ m}^3 \text{d}^{-1} \text{ha}^{-1}$. During that period the total precipitation was 30 mm and the average day temperature was 31°C (overall average temperature was 21°C). The average relative humidity was 62% and the average day-length was 12.5 hours, with average total radiation of 16 cal./hr.cm^2 (186 W/m^2). The average net solar radiation was 7 cal./hr.cm^2 (81.75 W/m^2).

Leaves of 18 potato varieties were collected randomly from the field, sorted into expanding and expanded leaves, which were stored at 4°C before they were analyzed. The harvested tubers of 18 potato varieties were stored in boxes in the dark at $24 \pm 1^\circ \text{C}$ before they were analyzed for their glycoalkaloid content.

Supplies: α -Solanine and α -chaconine standards were obtained from Sigma Chemical Co., St. Louis, MO, USA. Acetonitrile was HPLC grade from BDH Laboratory supplies (Poole, UK). The water used was deionized and further purified using the Milli-Q purification system from Millipore Corp. (Bedford, MA, USA). All other solvents and chemicals used were of standard analytical grades (BDH Laboratory supplies, Poole, UK).

Chemical analysis: α -Solanine and α -chaconine content of leaves and tubers was determined using an improved version of HPLC procedure described by Hellenas *et al.* (1995). 12 tubers of uniform size and without greening or fungal infection were collected from each of the 18 varieties, cleaned, weighed and peeled with a domestic potato peeler. Peels of 2 mm thickness were removed and the ratio of peels to total weight was recorded.

Alkaloid extraction and clean up: Extraction method was a modification of that used by Hellenas *et al.* (1995). Details are as follows: samples (10 g) of leaves, peel and flesh were mixed with 20 ml of water/acetic acid/sodium bisulphate (NaHSO_3), 95:5:0.5 (v/v/w), for five minutes using a blender (Moulinex, France). The mixture was diluted to a final volume of 50 ml with the same solvent and was then vacuum filtered through a Whatman No. 1 filter paper. The sample was cleaned up by centrifuging at $5000g$ (6500 rpm) for 10 minutes in Centrikon T-124 centrifuge (Kontron, Italy). A SepPak Classic C18 extraction cartridge (Waters Corporation, Milford, MA, USA) was conditioned by passing through of 5 ml of acetonitrile, followed by 5 ml of the water/acetic acid/ NaHSO_3 solvent and a 10 ml sample of the tuber extract was then passed through followed by 4 ml water/acetonitrile, 85:15 (v/v), for washing. The glycoalkaloid were eluted from the cartridge with 4 ml of

the acetonitrile/0.022M potassium phosphate buffer, pH 7.6, 55:45 (v/v) and the volume was finally adjusted to 5 ml with the same solvent.

High Performance Liquid Chromatography (HPLC): α -Solanine and α -chaconine were separated and quantified using an HPLC apparatus consisting of a Waters 626 pump, a Waters 717 plus autosampler, a Waters 996 Photodiode Array (PDA) detector and controlled with millennium 32 software. The analytical column was a $250 \times 4.6 \text{ mm}$ id SupelcosilTMLC-NH₂ $5 \mu\text{m}$ (Supleco Park, Bellefonte, PA, USA). The mobile phase was acetonitrile/0.022 M potassium dihydrogenphosphate (KH_2PO_4) buffer, pH 4.7, 75:25 (v/v), pumped at a flow rate of 1.0 ml min^{-1} . The column effluent was monitored for UV absorption at 200 nm wavelength and 0.05 AUFS sensitivity. The volume of the injected samples was $20 \mu\text{L}$. The retention times for α -chaconine and α -solanine were approximately 7 minutes and 10 minutes, respectively. α -Solanine and α -chaconine concentration in sample extracts were quantified by comparing the peak areas with those of injected known amounts of pure standards (Fig. 1). Recovery of glycoalkaloids (Sigma) that have been added to samples averaged (93%).

Illumination: Harvested tubers of 7 potato varieties were placed in a ventilated store at room temperature ($24 \pm 1^\circ \text{C}$) in the dark. Tubers of each variety were selected at random, washed under running tap water and hand dried prior to treatments. Three replicates of tubers from each variety were withdrawn and placed under two 40 W fluorescent tubes (Warm White) with a radiation intensity

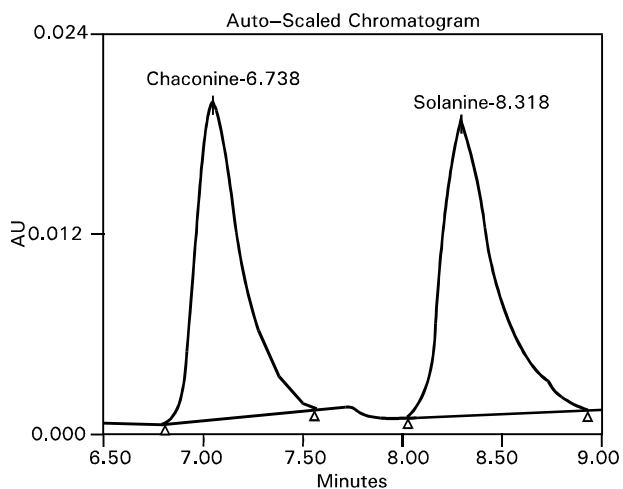


Fig. 1: Separation and quantification of α -Chaconine and α -Solanine by HPLC, for conditions see Experimental

of $15.72 \mu\text{mol m}^{-2} \text{s}^{-2}$ photosynthetically active radiation measured by SKP 200 measuring device (Skye instruments LTD) at the tuber surface with temperature of $24 \pm 1 \text{ }^\circ\text{C}$. Tubers were rotated at 24 h intervals ensuring complete exposure to light. Batches of 3 tubers were withdrawn at 0, 3, 6, 9, 12-day intervals for glycoalkaloid analysis. Control tubers were stored in the dark at $24 \pm 1 \text{ }^\circ\text{C}$. Samples were analysed statistically using the computer software SPSS.

Results and Discussion

Results presented in Fig. 2 show that the tubers of different varieties respond differently to light. There were significant differences between the tuber total glycoalkaloid of cultivars and between total glycoalkaloid content of tubers after light treatment and at harvest. Exposure of potato tubers to fluorescent light resulted in a substantial increase in total glycoalkaloid concentrations than those recorded at harvest for all varieties except L. Clair, Aida and Atlas, where there were some decreases. The highest glycoalkaloid accumulation was recorded after 3 days exposure to fluorescent light, however glycoalkaloid accumulation decreased upon continued illumination after this period for most of the varieties.

The results showed that there were large differences between varieties in the initial total glycoalkaloid contents

of dark stored tubers. The initial total glycoalkaloid concentrations of most varieties were higher than the maximum recommended level of 200 mg/kg FW, except that of L. Rosseta. The highest concentration was in tubers of Atlas with a level about 3 times higher than that of L. Rosseta, the variety with the lowest amount. Differences in the percentage increase in total glycoalkaloid on exposure to light were also significant, with L. Rosseta showing the greatest increase of 195% after 3 days exposure to light, followed by 92% for Turbo, 78% for Diamant and 18% for Spunta. This indicates the absence of correlation between the initial total glycoalkaloid contents of the tubers and glycoalkaloid accumulation in response to light. For example, L. Rosseta, which was proven to have the lowest total glycoalkaloid content, was shown to be the most sensitive to light treatment. Indicating that light may have an effect on glycoalkaloid synthesis by the tubers.

After 12 days of exposure to fluorescent light, the rate of glycoalkaloid accumulation in most of the varieties decreased but the total glycoalkaloid concentrations remained above the maximum recommended level of 200 mg kg⁻¹ FW.

After 3 days of exposure to fluorescent light, the flesh total glycoalkaloid content was about 12 times higher for L. Rosseta, 5 times for Diamant and 3 times for Spunta. In varieties whose total glycoalkaloid content was not

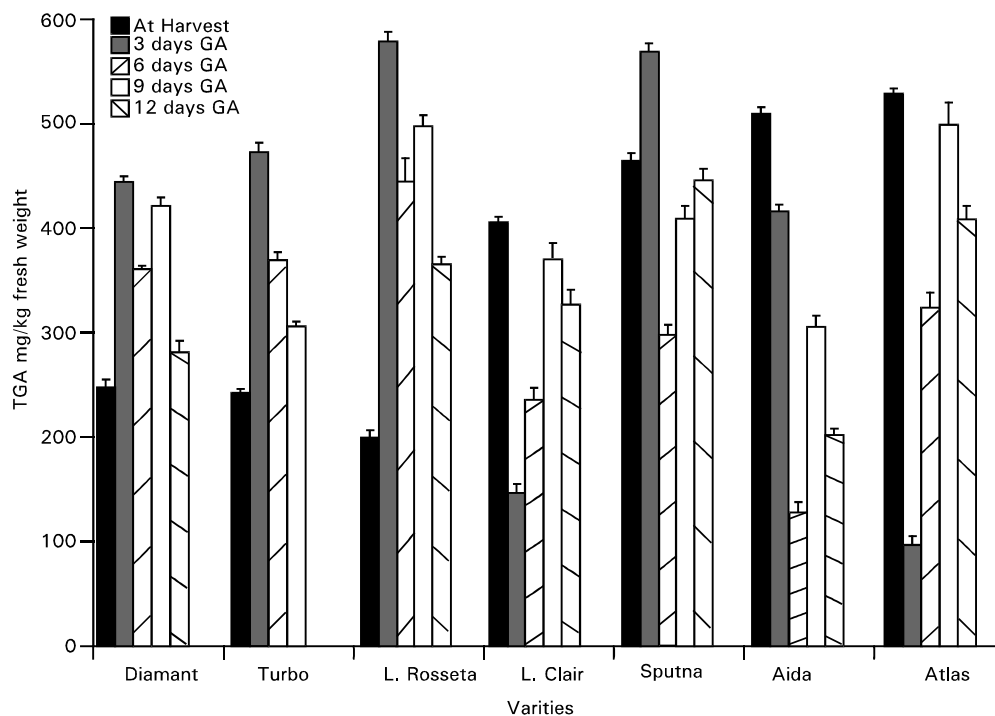


Fig. 2: Effect of exposure to fluorescent light on the accumulation of glycoalkaloids in the tubers from 7 potato varieties grown in Oman

Table 1: Glycoalkaloids in the tubers of 7 potato varieties grown in Oman following storage under fluorescent light

| Variety | 3 Days | | | | 6 Days | | | |
|--------------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| | Flesh TGA | Peel TGA | C:S Ratio | Flesh GA% | Flesh TGA | Peel TGA | C:S Ratio | Flesh GA% |
| Diamant | 68.64 | 364.5 | 59:41 | 16 | 53.8 | 300.7 | 55:45 | 15.2 |
| Turbo | 34.53 | 434 | 71:29 | 7.4 | 51.8 | 302.7 | 58:42 | 14.6 |
| Lady Rosseta | 96.7 | 468.7 | 57:43 | 17.1 | 67.4 | 355.2 | 57:43 | 16 |
| Lady Clair | 28.3 | 101.7 | 73:27 | 21.8 | 34 | 175.9 | 61:39 | 16.2 |
| Spunta | 109.7 | 438.8 | 69:31 | 20 | 34.8 | 252.3 | 68:32 | 12 |
| Aida | 60.84 | 351 | 72:28 | 14.8 | 4 | 113.9 | 38:62 | 3.4 |
| Atlas | 8.71 | 77.5 | 61:39 | 10.1 | 2.7 | 321.3 | 44:56 | 1 |
| | 9 Days | | | | 12 Days | | | |
| Diamant | 61.5 | 415.1 | 67:33 | 13 | 122.5 | 161.5 | 45:55 | 43 |
| Turbo | 60 | 242 | 77:23 | 20 | nd | nd | nd | nd |
| Lady Rosseta | 95.4 | 395 | 68:32 | 19 | 66.7 | 306.3 | 39:61 | 18 |
| Lady Clair | 33.6 | 380.2 | 69:31 | 8 | 54.2 | 401 | 68:32 | 12 |
| Spunta | 39 | 317.7 | 65:35 | 11 | 71.2 | 270.1 | 49:51 | 21 |
| Aida | 25.5 | 275.6 | 61:39 | 8 | 71.8 | 133 | 66:34 | 35 |
| Atlas | 102.7 | 419.7 | 57:43 | 20 | 149 | 266.7 | 36:64 | 36 |

C:S Ratio = Chaconine : Solanine ratiound = Not Detected

affected by light, their flesh total glycoalkaloid was also not affected after 3 days in Turbo, whereas increased from 0 to 28.3 mg kg⁻¹ FW in L. Clair and decreased from 79 to 9 mg kg⁻¹ FW in Atlas and from 97.8 to 60.8 mg kg⁻¹ FW in Aida (Table 1).

After 12 days of exposure to fluorescent light, the rate of glycoalkaloid accumulation increased in some of the varieties studied such as Diamant, Clair and Atlas. However in other varieties such as Rosseta, Spunta and Aida the rate of glycoalkaloid accumulation decreased.

After 3 days of exposure to fluorescent light, the peel total glycoalkaloid content was about 3 times higher than that of control for L. Rosseta and 2 times for Diamant and Turbo. In varieties whose tuber total glycoalkaloid content was not affected by light, their peel total glycoalkaloid was also not affected. These varieties are L. Clair, Aida, Atlas and Spunta. However, after 12 days of exposure to light, the total glycoalkaloid contents decreased in the peel of most of the 7 varieties (Table 1). The results also revealed that the ratio of α -chaconine: α -solanine in the tubers decreased over 12 days of illumination indicating enhancement of α -solanine synthesis more than α -chaconine. After 12 days of light exposure the two major glycoalkaloids varied between varieties, but in general α -solanine content of the tubers increased to be between 32% for Spunta and 64% for Atlas compared to that of 19-29% α -solanine before treatment (Table 1).

Our results are in agreement with other workers showing the effect of light as a positive elicitor of glycoalkaloid synthesis in potato tubers (De Maine *et al.*, 1988; Dale *et al.*, 1993; Griffiths *et al.*, 1994; Dao and Friedman, 1994; Percival and Dixon, 1994; Percival, 1999a; b). Light had a significant effect on total glycoalkaloid content increasing it in most of the varieties with some differences between the varieties in their rates of increase in total glycoalkaloid

contents. Light effect appears to be limited to a very short period of time during which it is exerted as no increase in the total glycoalkaloid content was observed following 3 days illumination of Clair, Aida and Atlas, while Spunta showed the smallest increase in total glycoalkaloid of 18.3%. Rosseta on the other hand, showed the highest increase of 195%, with Turbo and Diamant in between with total glycoalkaloid increase of 92 and 78%, respectively. This again confirms that the extent of light enhanced total glycoalkaloid accumulation is cultivar specific (De Maine *et al.*, 1988; Dale *et al.*, 1993; Dao and Friedman, 1994; Percival and Dixon, 1994; Percival, 1999a; b) and it is likely that cultivars Clair, Aida and Atlas potatoes do not accumulate total glycoalkaloid strongly.

The glycoalkaloid accumulation in most of the varieties decreased after 3 days exposure to fluorescent light. A similar phenomenon was identified by Percival *et al.* (1993) in cultivar Disiree, where glycoalkaloid concentration did not increase significantly after 96 h exposure to fluorescent light. On the other hand our results disagree with findings by Percival (1999a;b) who reported that in virtually all cases studied, glycoalkaloid concentrations within tuber tissue increased steadily with time during light exposure with no indication of cessation. An observation that we did not make may be because some of these varieties were insensitive to light.

Clearly flesh glycoalkaloid production is light sensitive for most of the varieties even those whose tuber total glycoalkaloid did not increase in response to light exposure. In all cases, exposure of potato tubers to fluorescent light even of low intensity resulted in substantial increases in glycoalkaloid of the varieties tested. This may be due to the fact that fluorescent light contain ultraviolet light of <300 nm wavelength, which is considered to be active elicitors of glycoalkaloid

synthesis compared with other wavelengths (Percival and Dixon, 1994; Percival, 1999a; b). Since during marketing and sale, potatoes are displayed particularly under fluorescent light, this will result in higher glycoalkaloid accumulation rates. It is therefore suggested the replacement of fluorescent by mercury illumination would reduce glycoalkaloid synthesis in tubers.

Initial total glycoalkaloid levels did not have any effect on their response to fluorescent light, which is in agreement with Dale *et al.* (1993); Griffiths *et al.* (1994) and Percival, (1999a). The present investigation revealed that, in all varieties, exposure to continuous illumination decreased the ratio of α -chaconine: α -solanine, indicating synthesis of α -solanine was enhanced more than α -chaconine to be between 32% for Spunta and 64% for Atlas compared to that of 19-29% α -solanine before treatment. Similar results have been reported by Percival and Dixon (1994) and Percival (1999b) possibly indicating a conversion of starch to sugar following tuber stress. As a result, a higher proportion of reducing sugars such as galactose are more freely available compared with rhamnose resulting in the sequential addition of monosaccharide units to the aglycone in favor of α -solanine production. Alterations in the relative proportions of glycoalkaloids as a result of exposure to light may influence toxicity more than absolute total glycoalkaloid concentrations, with consequential implications for the overall recommendation of 200 mg/kg FW for food safety (Percival, 1999a; b). For example, with erythrocytes and fungal protoplasts, the synergism was maximal with mixtures containing approximately 70% α -chaconine, whereas with beet cells it peaked at approximately 40% α -chaconine (Roddick *et al.*, 1988). Our results are in disagreement with the results of Edwards and Cobb (1997) who reported that α -solanine to α -chaconine ratios appear to be stable and were not affected by light treatment. They have also suggested that the tuber glycoalkaloid are stable and not rapidly metabolized, even with increasing metabolic activity.

A highly significant variety, light and variety-light interaction was found. Varieties which accumulated glycoalkaloid more rapidly in response to light exposure also tended to accumulate them more rapidly in response to other types of stress factors such as damage and temperature. L. Rosseta was recorded to be the most sensitive of the seven varieties to the three types of stress tested in this investigation. So not only varieties with low initial glycoalkaloid content, but also those with low rates of increase in glycoalkaloid in response to light, temperature, or damage should be used in breeding programmes for human consumption.

Clearly in the long term we would agree with (Griffiths *et al.*, 1998) that the consumer would be best served by

the production of varieties with inherently low levels of glycoalkaloids and with low rates of glycoalkaloid accumulation in response to damage, low temperatures and light exposure.

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