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Effect of Mechanical Injury and Low Temperature Storage on the Accumulation of Glycoalkaloids in the Tubers of 7 Varieties of Potato (*Solanum Tuberosum* L.) grown in Oman

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تأثير جروح وإصابات درنات البطاطس وتخزينها في درجات حرارة منخفضة على تراكم الألكالويدات السكرية في درنات 7 أنواع من البطاطس التي تزرع في سلطنة عمان

الصادق عبدالله الطيب ، سناء سالم السناني وإقرار خان

خلاصة : لقد تمت دراسة صناعة وتراكم الألكالويدات في درنات البطاطس كنتيجة لبعض التأثيرات البيئية مثل التخزين تحت درجة حرارة منخفضة أو كنتيجة للجروح أو أي إصابة للدرنات وهو ما يمكن أن يحدث أثناء النقل والتخزين. وجدت الدراسة أن الجروح والإصابات عموما تزيد من تركيز الألكالويدات. كما وجدت الدراسة أن التخزين في درجة حرارة منخفضة يزيد من تركيز الألكالويدات ولكن مستوى التركيز في درجة 10ºم كان أكبر منه في درجة حرارة 4ºم.

ABSTRACT: Tubers from 7 potato varieties were analyzed for their rates of glycoalkaloid accumulation in response to stresses of three types of mechanical injury and low temperature storage. Mechanical injuries were found to greatly stimulate glycoalkaloid accumulation in both peel and flesh of tubers. The extent of glycoalkaloid accumulation appears to depend on variety, type of mechanical injury, and storage period. Most of the injury-stimulated glycoalkaloid accumulation occurred within 7 and 14 days after treatment. Cutting the tubers resulted in the highest content of glycoalkaloids both in flesh and peel up to levels that exceeded the upper safety limit of 200 mg/kg FW. Injury stimulated α-solanine accumulation in stored potato tubers is more than α-chaconine, resulting in a decrease in the α-chaconine: α-solanine ratio. When tubers were stored at low temperature, the rate of glycoalkaloid accumulation was found to be independent of the glycoalkaloid level at harvest. The greatest increase in total glycoalkaloid content of the seven varieties was found after two weeks of storage at both 4 ºC and 10 ºC. Further storage at these temperatures resulted in a decrease in the rate of glycoalkaloid accumulation in most of them. At 10 ºC glycoalkaloid content tended to increase more rapidly than at 4 ºC. The α-solanine content of the tubers showed an increase following low temperature storage.

KEYWORDS: Potato, *Solanum tubersum* , Glycoalkaloids, Chaconine, Solanine, HPLC, Mechanical Injury and Low Temperature Storage.

1. Introduction

Potato, a member of the Solanaceae family, is consumed by millions of people every day. The potato tuber contains natural bitter-tasting steroidal toxicants, known as glycoalkaloids (GA) (Smith *et al.* 1996). The principal glycoalkaloids found in potato tubers are α-solanine and α-chaconine (Dale *et al.* 1998; Sotelo and Serrano, 2000).

Glycoalkaloids are thought to function in the chemical defense of the plant, as nonspecific protectors or repellents against potential pest and predators (Roddick, 1989). They are found throughout the potato plant with the highest levels observed in those parts of the plant with high metabolic rates (Van Gelder, 1990). Tubers have much lower glycoalkaloid content than foliage and the distribution is not uniform, with higher levels found in the periderm and cortex decreasing markedly towards the pith (Dale *et al.* 1998). The majority of current commercial varieties have been reported to have their average GA content below 150 mg/kg fresh weight (FW) (Van Gelder, 1990). Concentrations in the range 10-150 mg/kg FW are regarded as acceptably low, while glycoalkaloid concentration exceeding 200 mg/kg FW gives a bitter taste and can cause gastroenteric symptoms, coma, and even death (Gregory, 1984). Consequently potato tubers containing >200 mg glycoalkaloids kg^{-1} fresh weight exceed the limit recommended for food safety and should be withdrawn from sale (Percival and Dixon, 1994).

The total glycoalkaloid level in potato tubers is controlled by genetic as well as environmental factors (Dale *et al.* 1998). Potato grown in Oman is faced with varying degrees of heat stress. Breeders have been screening potato varieties adapted to local conditions (Khan *et al.* 2001, 2002).

Potato glycoalkaloids represent a threat not only to certain microbial pathogens and herbivores, but also to man if excessive quantities are inadvertently ingested (Morris and Lee, 1984).

Because of the importance of glycoalkaloids in the quality of potatoes and their resistance to disease, the present investigation was undertaken with a view to finding out the accumulation rate of glycoalkaloids in tubers exposed to postharvest stress such as mechanical damage and low temperature storage.

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). 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, or too little sunshine (Friedman and McDonald, 1997). Also glycoalkaloids, which are usually present at low levels in commercial potatoes, may accumulate as a result of continued biosynthesis. Environmental and storage factors may influence glycoalkaloid formation (Friedman and McDonald, 1997).

More glycoalkaloid concentrations were reported as a result of storage at lower temperatures than at higher temperatures by Griffiths *et al.* (1997). Any injury or damage to the potato plant or tuber is thought to result in glycoalkaloid accumulation. Thus disease or insect attack to the plant as well as rough tuber handling during harvest or distribution will initiate glycoalkaloid synthesis (Maga, 1994).

2. Experimental

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 to mid-February 2001 and the second from mid-December 2000 to 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 $m^3d^{-1}ha^{-1}$. 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).

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, U.K.). 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: Tuber α-Solanine and α-chaconine content 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: The extraction method was a modification of that used by Hellenas *et al.* (1995). Details are as follows: samples (10 g) of 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 5000*g* (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 the 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 glycoalkaloids 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 x 4.6 mm id Supelcosil^{TM} LC-NH_2 5 µm (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 µL. 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. Recovery of glycoalkaloids (Sigma) that were added to samples averaged (93%).

Mechanical Damage: Potato tubers of each variety without any mechanical and pathological injuries were randomly selected and hand washed prior to treatments. Four treatments including controls and three types of mechanical injuries on potato tubers were arranged. There were three replicates. The mechanical injury treatments included dropping, puncturing with a nail and cutting. Dropping was done by dropping each tuber 6 times from a height of 2 m, cutting was done by cutting lines 1 cm deep and 1 cm apart into tubers (6 lines/tuber) and puncturing was done by making a hole of 3 mm diameter to the depth of 1 cm with two holes cm^{-2} (max. 10 holes tuber^{-1}). Zero time samples were taken from each variety. Tubers were sampled immediately and after 7, 14 and 21 days of storage. Peel and flesh were separated and analyzed for their glycoalkaloid content.

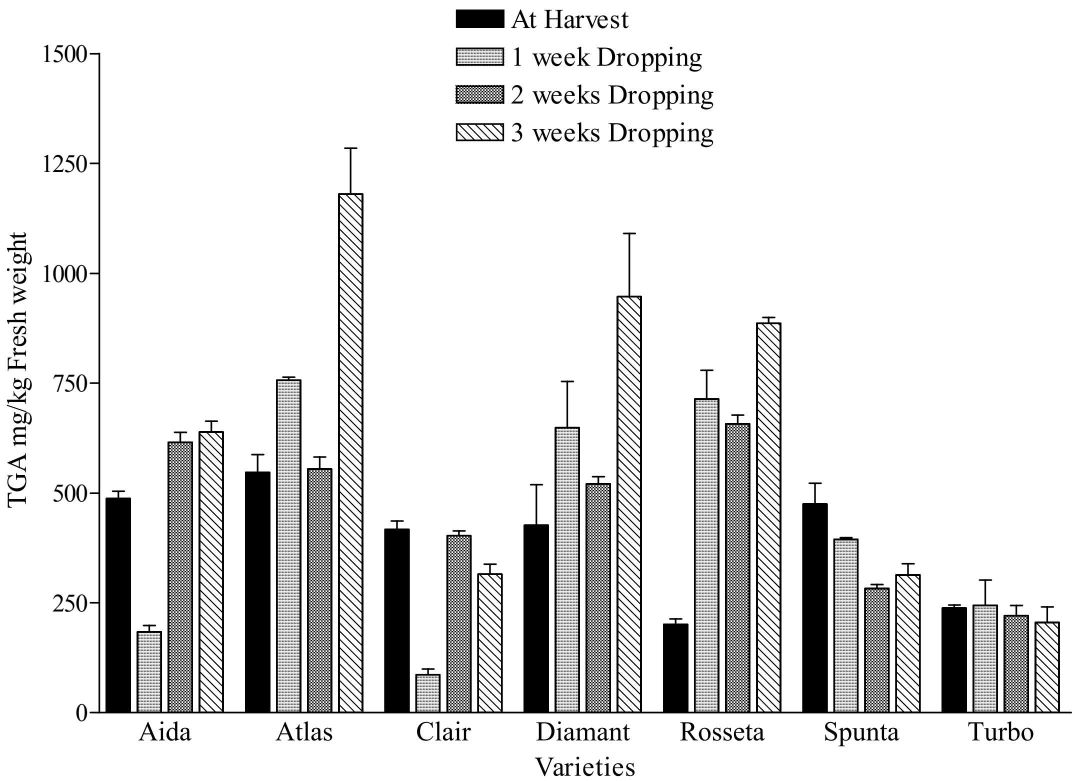
Temperature: Tubers from each variety were randomly selected and hand washed prior to treatments. Three replicates from each variety were stored at the following temperatures:
Constant 4 ºC for 0, 2, 4, 6 weeks, constant 10 ºC for 0, 2, 4, 6 weeks, variable: a) 3 weeks at 4 ºC followed by 3 weeks at 10 ºC and b) 3 weeks at 10 ºC followed by 3 weeks at 4ºC.
Peel and flesh tissues were separated and analyzed for their glycoalkaloid content.

Figures and statistical Analysis: Figures were prepared using the computer software GraphPad Prism version 2.01, which has a statistical package for the analysis of data in the graph.

3. Results and Discussion

3.1 Dropping

Figure 1 and tables 1-3 show the results of dropping on the glycoalkaloid contents of whole tubers, peel and flesh of potato tubers after 3 weeks of storage. The total glycoalkaloid (TGA) content of four of the treated varieties increased significantly as a result of dropping while the remaining three Turbo, L. Clair and Spunta varieties were less sensitive to this treatment and their total glycoalkaloid content decreased by 43, 86 and 176 mg/kg FW, respectively. Most of the glycoalkaloid accumulation occurred within 2 weeks after treatment with some further increase after 2 weeks (Table 2). With L. Rosseta, Aida, and Atlas varieties, however, the rate of glycoalkaloid accumulation decreased after this period for the rest of the varieties treated.



L. Rosseta and Atlas were the most sensitive to the dropping treatment, as after three weeks their tubers contained 4.6 times and 2.3 times glycoalkaloid as before treatment. Diamant and Aida showed a slight increase of 1.7 times and 1.3 times respectively.

After one week of storage, the total glycoalkaloid content in the flesh of the dropping treated potatoes was 11 times higher than before treatment for L. Rosseta, 8 times higher for Diamant, 3 times for Spunta and Atlas. The flesh total glycoalkaloid content of L. Clair increased from 0 to 34 mg/kg after one week of the dropping treatment. The flesh glycoalkaloid content of all varieties decreased during the last two weeks of the treatments. This decrease coupled with high tuber TGA indicates that the glycoalkaloid synthesis was stimulated and continues in the peel during this period. With the exception of Atlas, the flesh glycoalkaloid content of most of the dropping treated potatoes remained below the upper safety limit of 200 mg/kg FW.

Higher glycoalkaloid content was detected in the peel tissue than in the flesh (Table 2). The extent of glycoalkaloid accumulation depends on the cultivar and duration of storage.

The relative α-solanine content of the dropping treated potato tubers increased after 3 weeks to between 35 - 41% (α-chaconine content 59 - 65%) compared to that of 19 - 29% α-solanine before treatment (71 - 81% α-chaconine). This synergy is still considered high (Table 3). Because it has been demonstrated that the maximum synergy was exhibited between 60:40 and 40:60 (α-solanine: α-chaconine) ratios (Fewell and Roddick, 1993), these authors also reported that as little as 10-20% of either glycoalkaloid in the mixture is sufficient to initiate a marked synergism, especially chaconine, which suggests a significant saving in metabolic cost by the plant through the operation of the synergism.

Table 1: TGA levels (mg/kg Fresh Weight) in the tubers of 7 potato varieties grown in Oman after 3 types of injury.

	Cutting						Puncturing						Dropping					
	1 wk		2 wks		3 wks		1 wk		2 wks		3 wks		1 wk		2 wks		3 wks	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Diamant	117	323	254	680	206	603	113	310	101	340	156	406	113	266	123	367	50	348
Turbo	92	377	92	316	110	295	90	57	92	166	36	254	30	194	41	171	36	165
L. Rosseta	208	727	237	322	165	616	91	539	50	532	36	464	76	208	155	504	134	750
L. Clair	82	255	204	484	213	522	31	346	64	266	60	206	34	44	76	311	26	293
Spunta	161	434	233	474	124	67	45	31	85	253	55	270	132	258	62	222	75	213
Aida	279	369	219	555	228	562	122	555	161	393	93	422	26	118	124	494	191	442
Atlas	250	435	326	541	381	572	157	203	285	469	301	1126	208	556	149	397	412	774

F = Flesh, P = Peel, TGA = The sum of a-solanine and a-chaconine.

Table 2: Percentage Peel and Flesh TGA of 7 potato varieties grown in Oman after 3 types of injury.

	Cutting						Puncturing						Dropping					
	% Flesh GA			% Peel GA			% Flesh GA			% Peel GA			% Flesh GA			% Peel GA		
	1 wk	2 wk	3 wk	1 wk	2 wk	3 wk	1 wk	2 wk	3 wk	1 wk	2 wk	3 wk	1 wk	2 wk	3 wk	1 wk	2 wk	3 wk
Variety	27	27	26	73	73	74	27	23	28	73	77	72	30	25	13	70	75	87
Diamant	20	22	27	80	78	73	61	36	12	39	64	88	13	19	18	87	81	82
Turbo	22	42	21	78	58	79	14	8	7	86	92	93	13	24	15	87	76	85
L. Rosseta	24	30	29	76	70	71	8	22	22	92	78	78	43	20	8	57	80	92
L. Clair	27	33	65	73	67	35	57	25	17	43	75	17	34	22	26	66	78	74
Spunta	43	28	29	57	72	71	18	29	18	82	71	82	18	20	30	82	80	70
Aida	36	38	40	64	62	60	44	38	21	56	62	79	27	27	35	73	73	65
Atlas	27	27	26	73	73	74	27	23	28	73	77	72	30	25	13	70	75	87

3.2 Cutting

After one week of treatment all of the cut potatoes contained higher glycoalkaloid content than the control ones; however, after three weeks of storage the rate of glycoalkaloid accumulation decreased in the Diamant, Turbo, L. Rosseta, and Spunta varieties. Some varieties showed an increase in glycoalkaloid content after one week of storage reaching 744 mg/kg in L. Rosseta, 225 mg/kg in Turbo, 196 mg/kg in Diamant, 159 mg/kg in Atlas, 154 mg/kg in Aida, and 132 mg/kg in Spunta, whereas a decrease in total glycoalkaloid content of L. Clair was observed after one week of treatment. (Figure 2 and Tables 1-3).

Table 3: Chaconine: Solanine ratio in the tubers of 7 potato varieties grown in Oman after 3 types of injury.

Variety	Cutting			Puncturing			Dropping		
	Chaconine: Solanine ratio			Chaconine: Solanine ratio			Chaconine: Solanine ratio		
	1 wk	2 wks	3 wks	1 wk	2 wks	3 wks	1 wk	2 wks	3 wks
Diamant	59:41	63:37	65:35	53:47	59:41	57:43	52:48	68:32	62:38
Turbo	64:36	73:27	60:40	69:31	65:35	66:34	81:19	64:36	60:40
L. Rosseta	60:40	46:54	61:39	51:49	60:40	64:36	53:47	67:33	59:41
L. Clair	65:35	69:31	65:35	66:34	85:15	67:33	71:29	74:26	71:29
Spunta	68:32	65:35	79:21	88:12	75:25	87:13	61:39	61:39	64:36
Aida	67:33	70:30	64:36	73:27	61:39	69:31	94:06	63:37	65:35
Atlas	62:38	60:40	60:40	72:28	62:38	62:38	66:34	67:33	61:39

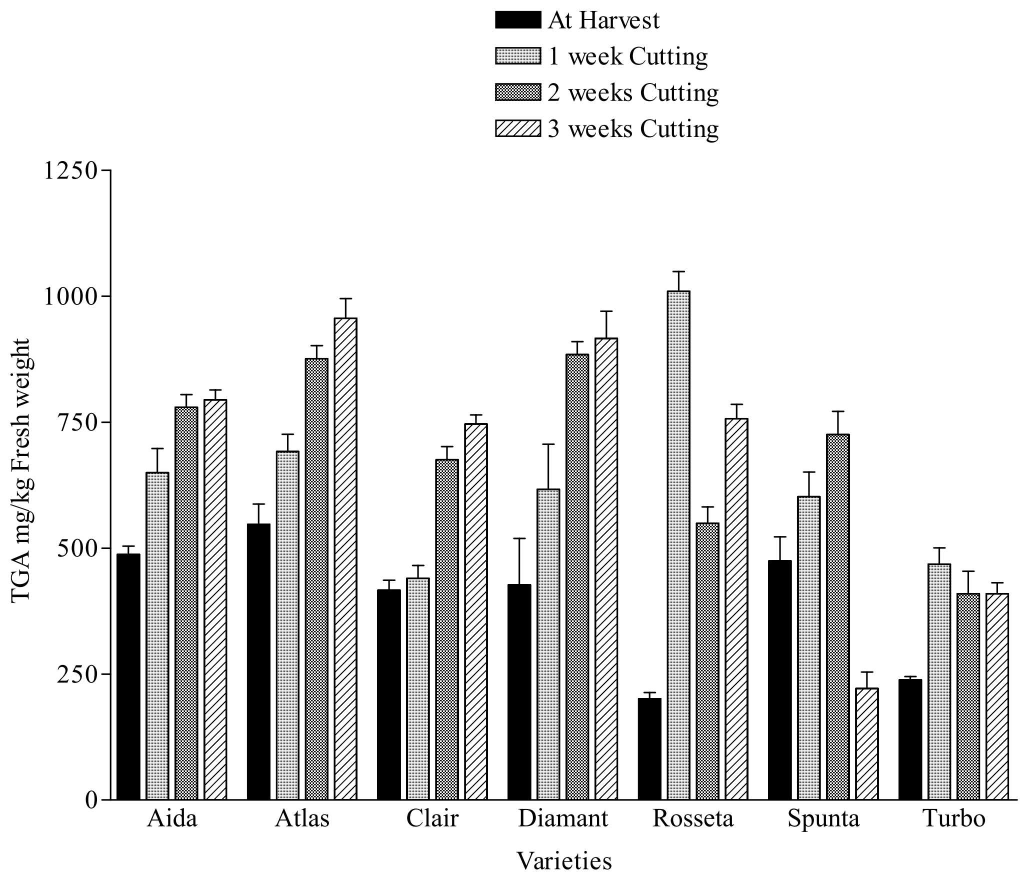


Figure 2. Effect of cutting on the accumulation of glycoalkaloids in the tubers from 7 potato varieties grown in Oman.

After one week of treatment, the content of glycoalkaloid in L. Rosseta was 5 times higher than before treatment while it was 4 times higher for Diamant and only two times higher for Turbo. Varieties continued to accumulate glycoalkaloids after three weeks L. Clair and Atlas 1.8 times, Aida 1.6 times. L. Rosseta and Diamant were the most sensitive varieties to the cutting treatment and showed the highest increase in total glycoalkaloid contents of 5 times and 4 times respectively, whereas Spunta and Aida were the least sensitive which showed the lowest increase in total glycoalkaloid of 1.5 times as before the treatment.

Most of the glycoalkaloid accumulation in the flesh occurred within 2 weeks of treatment, although there was further increase after 3 weeks, but that was rather small in Turbo, L. Clair, Aida and Atlas. However, the glycoalkaloid level in the flesh of Diamant and L. Rosseta started to decrease after 3 weeks of storage (Table 1). The highest increase in glycoalkaloid accumulation was recorded for the flesh of L. Clair, as its total glycoalkaloid increased from 0 to 213 mg/kg FW after 3 weeks of the cutting treatment. This was followed by L. Rosseta, which contained 29 times as much glycoalkaloid, 18 times for Diamant and 6 times for Spunta after 2 weeks. After 3 weeks, the flesh TGA content of Atlas was 5 times higher than before treatment, 3 times for Turbo and 2.3 times for Aida. In general, after the cutting treatment, the flesh of most of the seven varieties (except Turbo) contained total glycoalkaloid content, which was well above the upper safety limit of 200 mg/kg FW.

Cutting also stimulated glycoalkaloid synthesis and accumulation in peels of potato tubers. After one week peels from the cut potatoes of L. Rosseta contained 4 times as much glycoalkaloid as before treatment and twice as much for Turbo, but after 2 weeks they contained 4 times TGA for Diamant and 1.1 times for Spunta. After 3 weeks Aida peels contained 1.4 times TGA content as before the treatment, 1.3 times for L. Clair and 1.2 times for Atlas.

The effect of the cutting treatment on the relative content of α-solanine was similar to that of the dropping treatment.

3.3 Puncturing

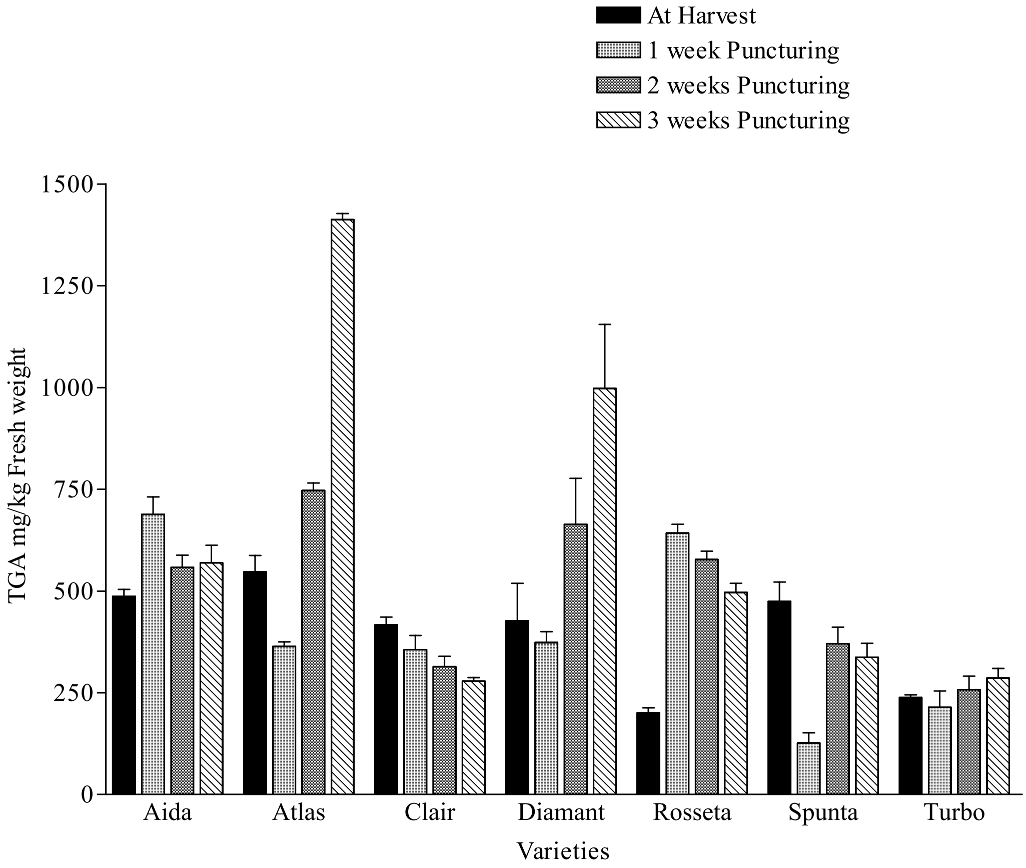
After one week of treatment, puncturing stimulated synthesis and accumulation of glycoalkaloid in the tubers of most of the potato varieties with the exception of L. Clair and Spunta, where the TGA content decreased by 28 mg/kg FW and 386 mg/kg FW, respectively. After a further 3 weeks of storage, an increase in the total glycoalkaloid content was recorded for Diamant, Turbo, and Atlas, but the total glycoalkaloid content for L. Rosseta and Aida decreased. The total glycoalkaloid content of the punctured potatoes was found to be 3.3 times as much TGA for L. Rosseta and 1.4 times for Aida after one week, whereas it was 2.7 times for Atlas, 2.3 times for Diamant, and 1.2 times for Turbo after three weeks. This indicates that L. Rosseta and Atlas were the most sensitive to the puncturing treatment, while Turbo was the least sensitive.

After one week the flesh total glycoalkaloid content of the punctured potatoes was 11 times higher than before treatment for L. Rosseta, whereas after 2 weeks it was 3 times higher for Turbo, and 1.6 times for Aida. After 3 weeks, the flesh total glycoalkaloid content of Diamant was 11 times higher than before treatment and 4 times for Atlas. Although puncturing greatly stimulated glycoalkaloid synthesis and accumulation in the flesh of most of the varieties, the flesh total glycoalkaloid content of these varieties was still below the upper safety limit of 200 mg/kg FW with the exception of Atlas, which had TGA content higher than the upper limit of food safety after the treatment (Tables 1-3, Figure 3).

The TGA accumulation that was stimulated in the peels by the puncturing treatment after one week was 3 times higher for L. Rosseta and 1.4 times for Aida, but after 3 weeks it was 2.5 times higher for Atlas, twice for Diamant, and 1.2 times for Turbo.

The relative content of α-solanine of the punctured potato tubers increased after 3 weeks to be between 31-43% (corresponding to a relative α-chaconine content between 57-69%) compared to that of 19-29% α-solanine before treatment (corresponding to 71-81% α-chaconine).

Our results show that the extent of glycoalkaloid accumulation depends on variety, type of mechanical injury and duration of storage. Following treatment, the flesh and peel of most of the mechanically injured potatoes contained higher total glycoalkaloid content than the control. These results agree with the conclusions made by Wu and Salunkhe (1976) from three cultivars; Ahmed and Muller (1978) from two cultivars; Olsson (1986) from a range of cultivars; and of Dale *et al.* (1998). This increase could be attributable to either localized synthesis in response to damage triggered by some form of elicitor or to diffusion or transfer of synthesized glycoalkaloids from the damaged areas.



Injury stimulated glycoalkaloid accumulation in flesh and peels occurred within 7-14 days after treatment, which is consistent with previously reported work (Wu and Salunkhe, 1976).

Of the three treatments, cutting the tubers appeared to be the most effective as it resulted in the highest total glycoalkaloid content in both flesh and peel of potato tubers. Cutting greatly stimulated glycoalkaloid synthesis and/or accumulation in the flesh of most potato varieties to very high and unsafe levels of much more than 200 mg/kg FW. Puncturing and dropping also stimulated glycoalkaloid synthesis and accumulation in the flesh but to a lesser extent than cutting and to TGA levels that do not exceed the upper safe limit.

Glycoalkaloid accumulation in the flesh after the puncturing and dropping treatments was higher than in the peel, which indicates that puncturing and dropping caused more injury and as a result more glycoalkaloid accumulation in the flesh than in the peel.

Glycoalkaloid accumulation as a result of dropping seems to depend on the severity of the damage as well as damage susceptibility of the variety. Dropping damage during harvest and storage operations can result in various types of injury including bruising, shattering, cracking and splitting leading to glycoalkaloid accumulation.

There was a wide variation between varieties in their susceptibility to the types of injury tested in this work. Of the seven varieties, L. Rosseta was the most sensitive to all three types of injury treatments. However, Diamant was highly sensitive to the cutting treatment, whereas Spunta and Aida were the least sensitive to this treatment. L. Clair and Spunta were insensitive to the puncturing treatment, while Atlas was highly sensitive to it. Atlas was more sensitive to the dropping than the cutting treatment, whereas Turbo was insensitive to the dropping while L. Clair and Aida accumulated glycoalkaloid to a lesser extent than the other varieties in response to dropping. The observed low increase is probably attributable to the severe tissue reaction and cell death throughout the tuber in response to severe damage.

No relationship was found between the initial total glycoalkaloid levels and the extent of glycoalkaloid accumulation after injury treatment. For example, L. Rosseta, which had the lowest initial total glycoalkaloid content (< 200 mg/kg FW) was the most sensitive to the three types of injury and accumulated glycoalkaloid content to levels of more than 200 mg/kg FW, and Spunta, which had very high initial total glycoalkaloid content of more than 200 mg/kg FW, was least sensitive to both puncturing and dropping, and was the least sensitive of the seven varieties to the cutting treatment in which it accumulated only 1.5 times as much glycoalkaloid as that before treatment. These results disagree with those previously reported by Sinden and Webb (1972) and Olsson (1986) where the resulting high total glycoalkaloid contents were more common in cultivars with inherently high contents than in those cultivars with low contents.

3.4 Chaconine: Solanine Ratio

Differences between varieties in respect of their α-chaconine: α-solanine ratios are in Table 3. The cultivar appears to be the principal significant factor in determining the chaconine**:** solanine ratio. Generally, all types of injury increased the relative content of α-solanine in the tubers of all varieties tested to be between 31%-43% (corresponding to α-chaconine content 57-69%) compared to that of 19-29% α-solanine (corresponding to 71-81% α-chaconine) before treatment. This indicates that the injury stimulated α-solanine synthesis and/or accumulation in tubers more than α-chaconine, possibly indicating a conversion of starch to sugar following tuber stress. As a result a higher proportion of reducing sugars such as galactose is more freely available compared with rhamnose resulting in the sequential addition of monosaccharide units to the aglycone in favor of α-solanine production (Percival, 1999a; b). Alterations in the relative proportions of glycoalkaloids as a result of injury 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). This ratio appears enough for obtaining maximum synergy as reported by Roddick (1996).

Potato tissue injuries in commercial harvesting, grading, shipping, and storage practice may not be as severe as those produced in this study; however, our results indicate that the three types of mechanical injury (cutting, puncturing, and dropping) stimulated glycoalkaloid synthesis and accumulation in both peel and flesh.

3.5 Effect of storage at 4 ºC

Results in Table 4 show that cultivar, storage time, and storage temperature affect the TGA content of the potato tubers of the seven varieties tested. At harvest the total glycoalkaloid contents of all seven varieties were higher than the recommended maximum level of 200 mg/kg FW, except for the total glycoalkaloid content of L. Rosseta, which was slightly below that maximum.

Immediate postharvest storage of potato tubers at 4 ºC for a period of 6 weeks resulted in a significant increase in total glycoalkaloid of the tubers. TGA levels increased to the highest levels after 2 weeks of storage at 4 ºC, with TGA concentration reaching 740 mg/kg FW in Spunta, followed by total glycoalkaloid content of 660 and 620 mg/kg FW in Diamant and L. Rosseta respectively. Aida was the only variety to show a decrease in total glycoalkaloid content after two weeks of storage at 4 ºC with total glycoalkaloid content decreasing from 494 mg/kg to 424 mg/kg. Further storage at 4 ºC resulted in a decrease in the rate of glycoalkaloid accumulation in all of the seven varieties and the total glycoalkaloid content was found to be below the upper safety limit for Aida and Atlas, but the concentrations of the other varieties remained above that safety limit.

Table 4: TGA levels in the tubers of potato varieties grown in Oman stored at different temperatures.

Variety	4 ºC Storage period (weeks)				10 ºC Storage period (weeks)				Variable Temperature		
	0	2	4	6	0	2	4	6	At Harvest	4-10 ºC	10-4 ºC
Diamant	244	660	489	614	244	1120	477	616	244	577	455
Turbo	245	424	243	283	245	611	476	329	245	63	178
L. Rosseta	192	620	407	354	192	912	463	547	192	955	454
L. Clair	405	580	332	481	405	1114	444	442	405	508	389
Spunta	464	740	446	668	464	73	527	565	464	636	80
Aida	494	424	264	83	494	393	397	104	494	311	218
Atlas	526	648	91	26	526	60	516	1521	526	530	482

These results agree in principle with previously reported studies (Griffiths *et al.* 1997) of six other cultivars stored at 4 ºC, whose total glycoalkaloid content increased after 6 weeks of storage.

The relative α-solanine content of the potato tubers after two weeks of storage at 4 ºC increased slightly to between 21%-34% compared to that of 19%-29%, however, it slightly decreased again after 6 weeks to between 13 %-31 % of the total glycoalkaloid content (Table 5).

Table 5: Chaconine: Solanine ratio in the tubers of 7 potato varieties grown in Oman stored at low temperatures.

Variety	4 °C Chaconine: Solanine			10 °C Chaconine: Solanine			Variable Temperature Chaconine: Solanine		
	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	4 – 10 °C	10 – 4 °C	2 wks
Diamant	76:24	67:33	74:26	82:18	63:37	71:29	73:27	66:34	76:24
Turbo	78:22	71:29	76:24	87:13	67:33	79:21	64:36	81:19	78:22
L. Rosseta	70:30	71:29	81:19	78:22	67:33	69:31	84:16	57:43	70:30
L. Clair	79:21	77:23	80:20	83:17	71:29	79:21	76:24	79:21	79:21
Spunta	69:31	70:30	73:27	50:50	72:28	66:34	74:26	81:19	69:31
Aida	72:28	68:32	87:13	72:28	71:29	45:55	74:26	53:47	72:28
Atlas	66:34	61:39	69:31	46:54	67:33	67:33	67:33	70:30	66:34

3.6 Effect of storage at 10 ºC:

Storage at 10 ºC for a period of 6 weeks resulted in high increases in the TGA content of Diamant, L. Rosseta, L. Clair, and Turbo. The TGA content of these varieties increased rapidly in the first two weeks of storage, reaching 1120 mg/kg FW in Diamant, 1114 mg/kg FW in L. Clair, 912 mg/kg FW in L. Rosseta and 611 mg/kg FW in Turbo. Thereafter, the rate of glycoalkaloid accumulation in the tubers dropped but TGA levels remained above the recommended safety limit in all varieties. In Spunta and Atlas, great decreases in total glycoalkaloid concentrations were observed during the first two weeks of storage at 10 ºC, but upon continued storage the total glycoalkaloid content increased again to 101 mg/kg FW higher than at harvest in Spunta and 996 mg/kg FW higher in Atlas. However, after 6 weeks of storage the only variety with TGA levels below the upper safety limit was Aida (Table 4).

The effect of storage at 10 ºC for 6 weeks on α-solanine content was similar to that of storage at 4 ºC during the first two weeks (Table 5).

It is also of interest to note that at either constant storage temperature there was no relationship between TGA concentration at harvest and the rate of accumulation in response to temperature, and that varieties which have previously been shown to accumulate lower glycoalkaloid in response to injury treatments also appear to be the least sensitive to storage temperature and length of storage time.

3.7 Storage at 10 ºC prior to storage at 4 ºC:

As can be seen in Table 4, storage for a period of three weeks at 10 ºC prior to three weeks at 4 ºC resulted in a decrease in TGA content of all of the seven varieties as compared to those of the tubers stored continuously at 10 ºC, but the TGA content in these tubers remained above the maximum recommended level except that of Turbo and Spunta. If these results are compared to that of tubers placed immediately at 4 ºC, a fluctuation between varieties is observed.

The effect of this treatment on α-solanine content of the tubers varied widely between varieties to be between 19% for Spunta and 47% for Aida compared to that of 19%-29% α-solanine before treatment (corresponding to 71%-81% α-chaconine).

3.8 Storage at 4 ºC prior to storage at 10 ºC:

The results of storage for a period of three weeks at 4 ºC prior to three weeks at 10 ºC fluctuated between the seven varieties. For example, the TGA content of Diamant, Turbo, and Spunta was lower than those of tubers stored continuously at 4 ºC, but it was higher for L. Rosseta, L. Clair, Aida, and Atlas. If these results are compared with those of tubers placed immediately at 10 ºC, a fluctuation between varieties is also observed. Storage of 3 weeks at 4 ºC prior to 3 weeks at 10 ºC resulted in a decrease in the TGA content of Diamant, Turbo, L. Clair, and Atlas to levels lower than those of tubers stored immediately at 10 ºC, and the reverse is observed for L. Rosseta, Spunta, and Aida.

This treatment increased the relative α-solanine content to between 24%-36% (corresponding to α-chaconine content 64%-76%) compared to that of 19%-29% α-solanine before treatment, indicating better synergy for the protection of the tuber.

Our results indicate that the rate of glycoalkaloid accumulation in potato tubers is dependent both on variety and storage temperature and demonstrate that selection of new varieties based solely on TGA content at harvest is insufficient to ensure consumer safety. These results are consistent with the results reported by Griffiths *et al.* (1997). Tubers placed immediately at 10 ºC storage can, as in the case of Diamant, L. Rosseta, L. Clair, and Turbo rapidly accumulate high glycoalkaloid levels, while Aida tubers did not accumulate glycoalkaloid in response to this treatment. The highest rate of accumulation was observed in Diamant, L. Clair and L. Rosseta varieties stored at 10 ºC for 2 weeks. Prior storage at 4 ºC reduces the magnitude of this effect in the varieties Diamant, Turbo, L. Clair, and Atlas. Storage at 10 ºC prior to storage at 4 ºC decreased the glycoalkaloid accumulation in all varieties to levels lower than those obtained for samples stored continuously at 10 ºC.

Continuous storage of potato tubers at 4 ºC stimulated glycoalkaloid synthesis rapidly, but to a lesser extent than in those stored continuously at 10 ºC. This glycoalkaloid accumulation was observed in most of the varieties except in Aida, whose TGA content decreased in response to this treatment. The greatest increase was found after 2 weeks of storage at 4 ºC, again with the highest being for Diamant and L. Rosseta. Prior storage at 10 ºC was found to reduce the magnitude of the effect of storage at 4 ºC for Diamant, Turbo, L. Clair, and Spunta. Storage at 4 ºC prior to storage at 10 ºC reduced the rate of glycoalkaloid accumulation in Diamant, Turbo, and Spunta to levels lower than those obtained for tubers stored continuously at 4 ºC.

This indicates that the transfer of tubers stored for 3 weeks at 10 ºC to colder storage conditions results in lower glycoalkaloid accumulation rates than those placed in similar conditions soon after harvest, which is in agreement with conclusions reported by Griffiths *et al.* 1997. This may offer a viable strategy for maintaining low levels of total glycoalkaloid in these varieties.

Exposure of potato tubers to stress such as mechanical injury and low temperature storage appears to increase the total glycoalkaloid levels in both peel and flesh of potatoes, the magnitude of the effect being dependent on the variety, treatment and duration of exposure. L. Rosseta was the most sensitive of the seven varieties, whereas Aida was the least sensitive to the three types of stress treatments. The cultivars that did not accumulate glycoalkaloid rapidly in response to low temperature (e.g. Aida) were the most stable and least sensitive to stress caused by mechanical injury, while in almost all cases the observed increases in total glycoalkaloid levels exceeded the maximum levels currently judged to be safe for human consumption (200 mg/kg FW).

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