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# Comparison of the Calystegine Composition and Content of Potato Sprouts and Tubers from *Solanum tuberosum* Group Phureja and *Solanum tuberosum* Group Tuberosum

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The calystegines detected in tubers from 17 Phureja (*S. tuberosum* Group Phureja) lines and five Tuberosum (*S. tuberosum* Group Tuberosum) cultivars were identified as the $A_3$ and $B_2$ structural types. Their concentration in whole tubers was of a similar order of magnitude in both species, as was the variability in the ratio of $B_2$ to $A_3$. On average, calystegine concentrations in the peel were about 13 times that found in the flesh for the five Tuberosum cultivars, and 4 times higher for four Phureja lines. Removal of the peel reduced the calystegine content by an average of over 50% in Tuberosum but by only 30% in Phureja, despite the latter having the greater proportion of peel. The calystegine content of sprouts was also determined for five Tuberosum cultivars and four Phureja lines and was found to include small amounts of four additional types, $B_3$, $B_4$, $N_1$, and $X_2$, in addition to the more abundant $A_3$ and $B_2$. Concentrations in the sprouts of Tuberosum were on average 100 times higher than that in the tuber flesh and 8 times higher than in the peel, whereas for Phureja, the equivalent values were 30 and 7 times higher, respectively. No correlation was found between sprout concentration and either flesh or peel calystegine concentration.

**KEYWORDS: Potato; *Solanum tuberosum* Group Phureja (*Solanum phureja*); *Solanum tuberosum* Group Tuberosum (*Solanum tuberosum* ssp. *tuberosum*); calystegines; tubers; sprouts**

## INTRODUCTION

Calystegines or calystegins, as they were originally named, were first identified in the roots of *Calystegia sepium*, *Convolvulus arvensis*, and *Atropa belladonna* in 1988 (*1*). The structures of three calystegines ($A_3$, $B_1$, and $B_2$) (**Figure 1**) were elucidated in 1990 and were identified as a family of polyhydroxy-nortropanes (*2*). Numerous structural variants have since been described (*3*), and the calystegines have been found to be widely distributed in the Convolvulaceae, Moraceae, Solanaceae, and Brassicaceae (*3–7*). More recently, an extensive study (*8*) highlighted the prevalence of calystegines in the Erythoxylaceae where in some species they accounted for up to 0.32% of the dry matter.

Calystegines $A_3$ and $B_2$ were identified in tubers of the domesticated potato *Solanum tuberosum* cv. Estima in 1993 and the latter was also found in the leaves of four other cultivars, King Edward, Dunluce, Desiree, and Cara (*9*). Within the tuber, calystegines have been reported (*10, 11*) to be present in the peel, flesh and sprouts, and in a study of eight commercially available American cultivars (*12*) considerable variation was reported in the total calystegine content of flesh (5–316 mg/kg dry matter) and peel (218–2581 mg/kg d.m.). In the majority of the cultivars the concentration of calystegines in the peel was between one and 2 orders of magnitude greater than in the flesh. However, in one cultivar, Russet Burbank, although the level of calystegines was higher in the peel the difference was minimal (flesh 316 mg/kg d.m., peel 462 mg/kg d.m.) and indeed for calystegine $A_3$ the concentration in the flesh of this cultivar was higher than in its peel. These results would suggest that considerable variation exists within the species both in the distribution of calystegines within the tuber and in total amounts.

The role of these compounds within the plant does not appear to have been fully elucidated they but are frequently found (*8*) together with structurally related alkaloids such as cocaine, atropine, and nicotine, all of which have *N*-methylputrescine and the methylpyrrolinium cation as common biosynthetic precursors (*13*). Studies on their interaction with the rhizosphere suggest that they are not involved in nodulation but may be an exclusive source of carbon and nitrogen for soil bacteria having the genes for calystegine catabolism (*5*). Additionally, it has been postulated that these alkaloids may also have allelopathic properties. Indeed the naturally occurring enantiomer of calystegine $B_2$ has been shown to inhibit alfalfa seed germination (*5*).

The most thoroughly investigated property of calystegines has been their ability to act as glycosidase inhibitors. It has been suggested (*14*) that their ability to inhibit rat $\beta$-glucosidases and

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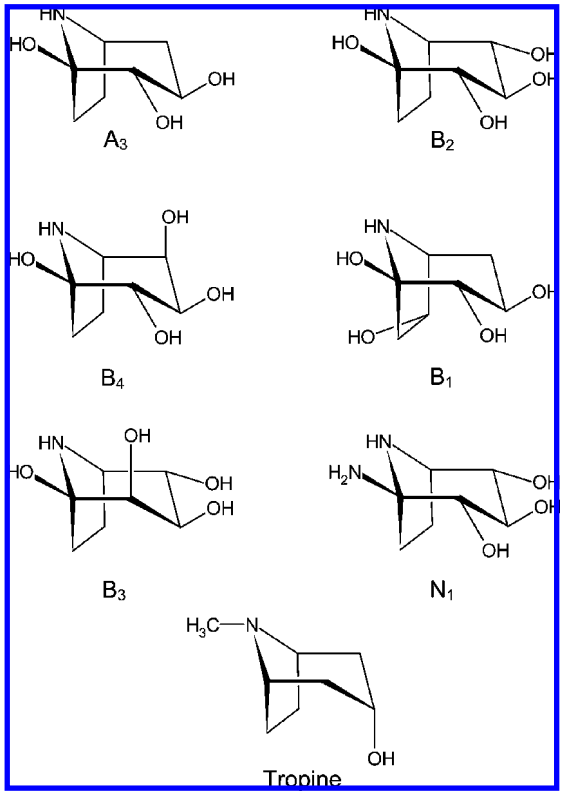


**Figure 1.** Structures of the common calystegines ($A_3$, $B_1$, $B_2$, $B_3$, $B_4$), the amino substituted calystegine $N_1$ and tropine.

α-galactosidases in vitro raises the possibility of toxicity in humans consuming large amounts of these compounds. Inhibition of digestive enzymes would account for episodes of digestive upset associated with eating potato peels.

Although calystegines have been found in plants such as *Solanum dimidiatum* which causes "crazy cow syndrome" and *S. kwebense* that induces "maldronksiekte" in South Africa (*9*), it has not been established that calystegines are the causative agents. A recent review (*3*) concluded that although a lot of suspicion has been pointed at calystegines in inducing intoxication in browsing animals, the proof of action remains elusive and still requires evidence of their absorption from the intestine post ingestion.

The majority of the commercial potato varieties grown in the Northern hemisphere belong to the tetraploid taxon *S. tuberosum* spp. *tuberosum*, otherwise known as *S. tuberosum* Group Tuberosum. However, potatoes from a far wider range of species are consumed in South America including diploid Phureja potatoes (*S. tuberosum* Group Phureja previously known as *S. phureja*) which are regarded as being particularly flavorsome and of good eating quality. These observations have been confirmed using trained taste panels who concluded that, as compared with steamed Tuberosum tubers, similarly cooked Phureja tubers had a higher intensity of sensory attributes in terms of appearance, flavor and delicacy (*15, 16*). Consequently, considerable interest is now being shown in this diploid species as both a source of new varieties of table potatoes for the European market and as a resource for improving the flavor characteristics of traditional Tuberosum cultivars by introgression using traditional breeding methods of the appropriate flavor linked genes, once identified.

Comparatively little is known regarding the variation in chemical composition within the species, Phureja. Consequently, the objective of this work was to determine how the levels of calystegines varied within a population of locally adapted Phureja lines, developed at the Scottish Crop Research Institute, and to compare the results with those for tubers of Tuberosum cultivars grown and stored under similar conditions.

## MATERIALS AND METHODS

**Plant Material.** Potato tubers were harvested from replicated field plots grown under normal agronomic practice at Gourdie, Dundee, UK, in 2003 or 2004. The tubers were harvested 2 weeks after foliage burndown and after "curing" by storage at ambient conditions (ca. 8 °C) for 3 weeks, transferred to a 4 °C controlled environment store with relative humidity levels of between 85 and 95%. For the intervarietal comparison of Phureja lines, five average-sized whole tubers were selected at random from each of two field replicates grown in 2003 and manually cut into eighths. Two opposite eighths were taken from each tuber, bulked by replicate, diced, and immersed in liquid nitrogen (*17*). The frozen samples were then lyophilized in a freeze drier (Millitor Engineering Ltd., Manchester, UK), ground in a Model ZM 200 laboratory mill (Retsch (UK) Ltd., Leeds, UK) fitted with a 0.5 mm sieve and stored at below −20 °C until required for analysis.

Peel and flesh samples were prepared from five Tuberosum cultivars and four Phureja lines which had been grown in field plots at Gourdie, Dundee, UK in 2004. Two replicates were prepared from the bulk harvested tubers of each line and cultivar by randomly allocating six average-sized tubers to each subset. The tubers were stored as described above and after 7 weeks (December 2004) the tubers were removed and were manually peeled using a domestic "swivel" hand peeler, which removed the peel to a depth of between 1 and 2 mm. The percentage peel, together with average tuber size for the nine cultivars and lines used are given in **Table 1**. The peel was then weighed, diced, frozen, freeze-dried and milled as for the whole Phureja samples. The peeled tubers were then weighed and chopped into opposite eighths and again treated as described for the Phureja line comparison above.

A further 20 average-sized tubers from the same nine cultivars and lines were removed from the 4 °C store in early February 2005. These were placed on aluminum trays, covered with paper towelling, and left in the dark at room temperature for 46 days. The sprouts produced during this period were harvested, bulked by cultivar or line, weighed, frozen in liquid nitrogen, freeze-dried, and milled as for the potato samples. These were then stored at −20 °C until required for analysis.

**Chemical Analysis.** Dry matter (d.m.) content was determined by measuring the weight loss during freeze-drying and is expressed as a percentage of the initial wet weight (% d.m.).

Calystegines were identified and quantified using a gas chromatography–mass spectrometry (GC-MS) method based on that described by Friedman et al. (*12*) and originally based on that of Nash et al. (*9*). The calystegines were extracted into aqueous methanol and, after concentration by rotary evaporation and adjusting the pH to 4.0, passed through a cation exchange column. After washing with distilled water the calystegines were eluted with ammonia and after concentration by rotary evaporation made up to 10 mL with distilled water. An aliquot (1 mL) was taken to dryness using a centrifugal rotary evaporator and 10 μL of tropine (Extrasynthèse, Genay, France) added as an internal standard. The resulting mixture was then derivatized by the addition of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Pierce) and heating at 50 °C for 30min.

The resulting trimethylsilyl (TMS) ethers were analyzed using a Finnigan Trace DSQ GC-MS (Thermo Electron Corporation, Hemel Hempstead, UK) fitted with a 15m × 0.25 mm i.d., 0.25μm film, DB 5-MS fused silica capillary column (J & W; Crawford Scientific, Strathaven, Scotland, UK). The samples (1 μL) were injected into a programmable temperature vaporizing (PTV) injector with a split of either 80 or 40:1. The PTV conditions were as follows: injection temperature 132 °C held for 1 min, transfer rate 14.5 °C/s, transfer temperature 320 °C for 1 min, clean rate 14.5 °C/s, and a clean temperature of 400 °C for 2 min. Helium at 1.5 mL/min was used as the carrier gas and the GC oven was programmed to increase after an initial 2.1 min hold at 100 °C at 25 °C/min to a final temperature of 320 °C, which was held for a further 3.5 min. The interface temperature was set to 290 °C and the MS was used in EI mode at 70 eV over a

**Table 1.** Mean Tuber Weight, Percentage Peel, Percentage Freeze-Dried Matter, and Sprout Length in Four *S. phureja* Lines and Five *S. tuberosum* Cultivars

| group | cultivar/line | peel (% total) | mean tuber wt (g) | % d.m. peel | % d.m. flesh | % d.m. sprouts | sprout length (cm) |
|---|---|---|---|---|---|---|---|
| Phureja | DB 257–26 | 10.25 | 153.9 | 15.70 | 19.80 | 28.36 | 1–3 |
| | DB 333–16 | 8.75 | 205.6 | 13.05 | 21.35 | 19.65 | 2–6 |
| | DB 337–37 | 11.10 | 159.4 | 16.45 | 23.95 | 21.10 | 2–5 |
| | DB 358–23 | 10.45 | 170.5 | 15.50 | 23.45 | 23.34 | 1–4 |
| group mean | | 10.14 | 172.3 | 15.18 | 22.14 | 23.15 | |
| Tuberosum | Ailsa | 9.90 | 133.6 | 17.65 | 20.70 | 16.79 | 2–7 |
| | Brodick | 7.10 | 214.7 | 14.70 | 22.85 | 18.54 | 2–7 |
| | Cara | 8.80 | 214.5 | 14.90 | 18.30 | 17.06 | 2–5 |
| | M. Piper[a] | 9.15 | 174.9 | 16.60 | 21.85 | 20.92 | 0.5–1.5 |
| | P. Dell[b] | 8.95 | 175.4 | 17.20 | 21.45 | 20.02 | 1–4 |
| group mean | | 8.78 | 182.6 | 16.21 | 21.03 | 18.67 | |
| lsd ($P = 0.05$)[c] | | 1.52 | 66.03[e] | 1.68 | 2.30 | | |
| lsd ($P = 0.05$)[d] | | 0.72 | 31.32[e] | 0.80 | 1.09 | | |
| variance components ($\sigma^2$) | | | | | | | |
| replicate | | −0.021 | 47.21 | −0.059 | 0.020 | | |
| cultivar and line | | 0.435 | 819.81 | 0.532 | 0.994 | | |

[a] Maris Piper. [b] Pentland Dell. [c] Least significant differences between cultivar/line. [d] Least significant differences between groups means. [e] Not statistically significant.

mass range of 50–900 amu with a source temperature of 230 °C. Acquisition rates were set to give approximately 10 data points across each chromatographic peak and the data was acquired and analyzed using the Xcalibur software package (V.1.4)(Thermo Electron Corp., Hemel Hempstead, UK).

The derivatized calystegines were identified on the basis of their individual mass spectra and elution characteristics by comparison with published data for the trimethylsilyl derivatives of calystegines $A_3$ and $B_2$ (*18, 19*), $B_3$ and $B_4$ (*5*), and $N_1$ and $X_2$ (*6*). Representative total ion chromatographic traces showing calystegines in flesh, peel, and sprouts from the Phureja line 333-16 are shown in **Figure 2**, and mass spectra of each calystegine derivative detected are shown in **Figure 3**. Calystegines $B_2$ and $A_3$ both formed two derivatives on silylation, and the derivatization conditions were optimized to maximize formation of the $B_2$ $(TMS)_4$ and $A_3$ $(TMS)_3$ compounds and to minimize formation of the more highly silylated $B_2$ $(TMS)_5$ and $A_3$ $(TMS)_4$. However, data for both were combined during data analysis. For each of the other calystegines, only a single derivative was detected, any others being below the instrumental detection limits. In the absence of purified calystegines, the relative amount of each was expressed in terms of tropine equivalents. Tropine, a commercially available structural analogue of the calystegines (**Figure 1**) which was not detected in any of the lines and cultivars studied, was included as an internal standard of known concentration prior to derivatization. Calystegine contents were then expressed in terms of milligrams of tropine equivalents per kilogram of freeze-dried matter (mg TE/kg d.m.), calculated from the integrated peak areas for individual calystegine and tropine derivatives in the total ion chromatogram. The mass spectrum of derivatized tropine is shown in **Figure 4**.

**Statistical Analysis.** During each experiment, samples from each of two field replicates were injected onto the GC column at two instrumental splits (80:1 and 40:1). In addition, during the comparison of calystegine content within 13 lines of phureja, each injection was made in duplicate.

All data were subject to analysis of variance (ANOVA) using the Genstat (release10.1) software package (Lawes Agricultural Trust, Rothamstead Experimental Station, UK) and lsd values for comparisons between cultivar/line within each group and between group means were determined. To partition the variation within the data between the experimental parameters (split, duplicate injection, field replicate, and cultivar/line), variance components ($\sigma^2$) were calculated for each as appropriate. The relative magnitude of $\sigma^2$ then gives an indication of the significance of the variation attributable to each parameter; negative or small positive values indicate low significance whereas high positive values indicate high significance.

## RESULTS AND DISCUSSION

**Intragroup Variation within Phureja.** Thirteen lines were selected on the basis of their pedigree from the SCRI locally

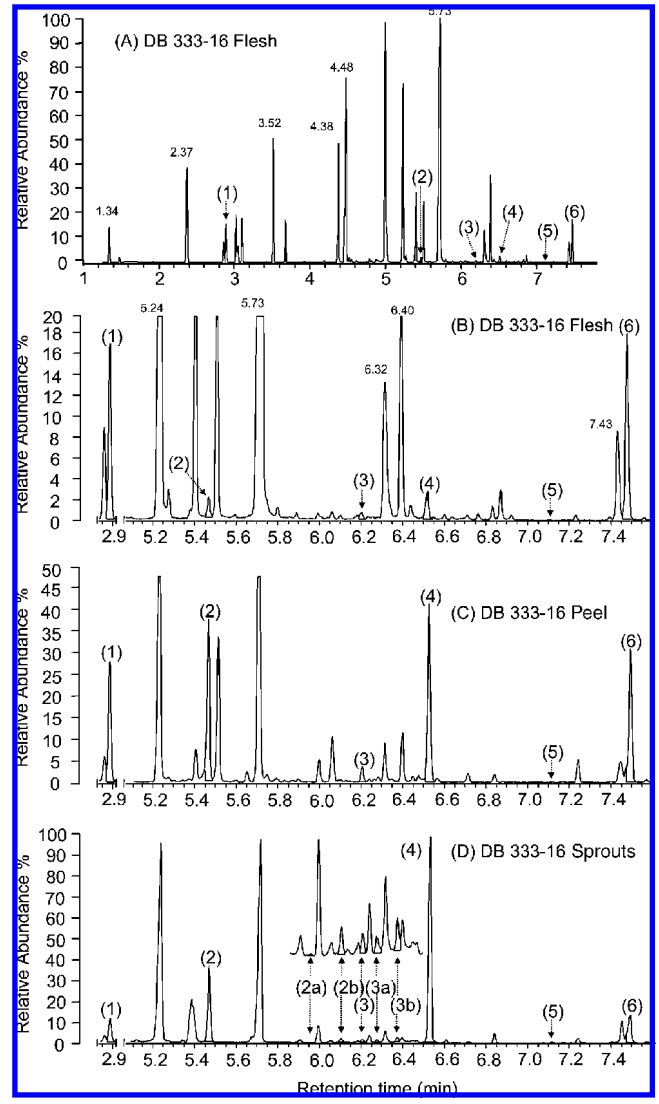


**Figure 2.** Total ion chromatogram (TIC) traces showing the distribution of calystegines in extracts of (**A**, **B**) tuber flesh, (**C**) peel, and (**D**) sprouts of Solanum phureja line DB 333-16. (1) Primary internal standard tropine (TMS); calystegines: (2) $A_3$ $(TMS)_3$, (2a) $B_4$ $(TMS)_4$, (2b) $B_3$ $(TMS)_4$, (3) $A_3$ $(TMS)_4$, (3a) $X_2$ $(TMS)_2$, (3b) $N_1$ $(TMS)_3$, (4) $B_2$ $(TMS)_4$, (5) $B_2$ $(TMS)_5$; (6) secondary internal standard perseitol $(TMS)_6$.

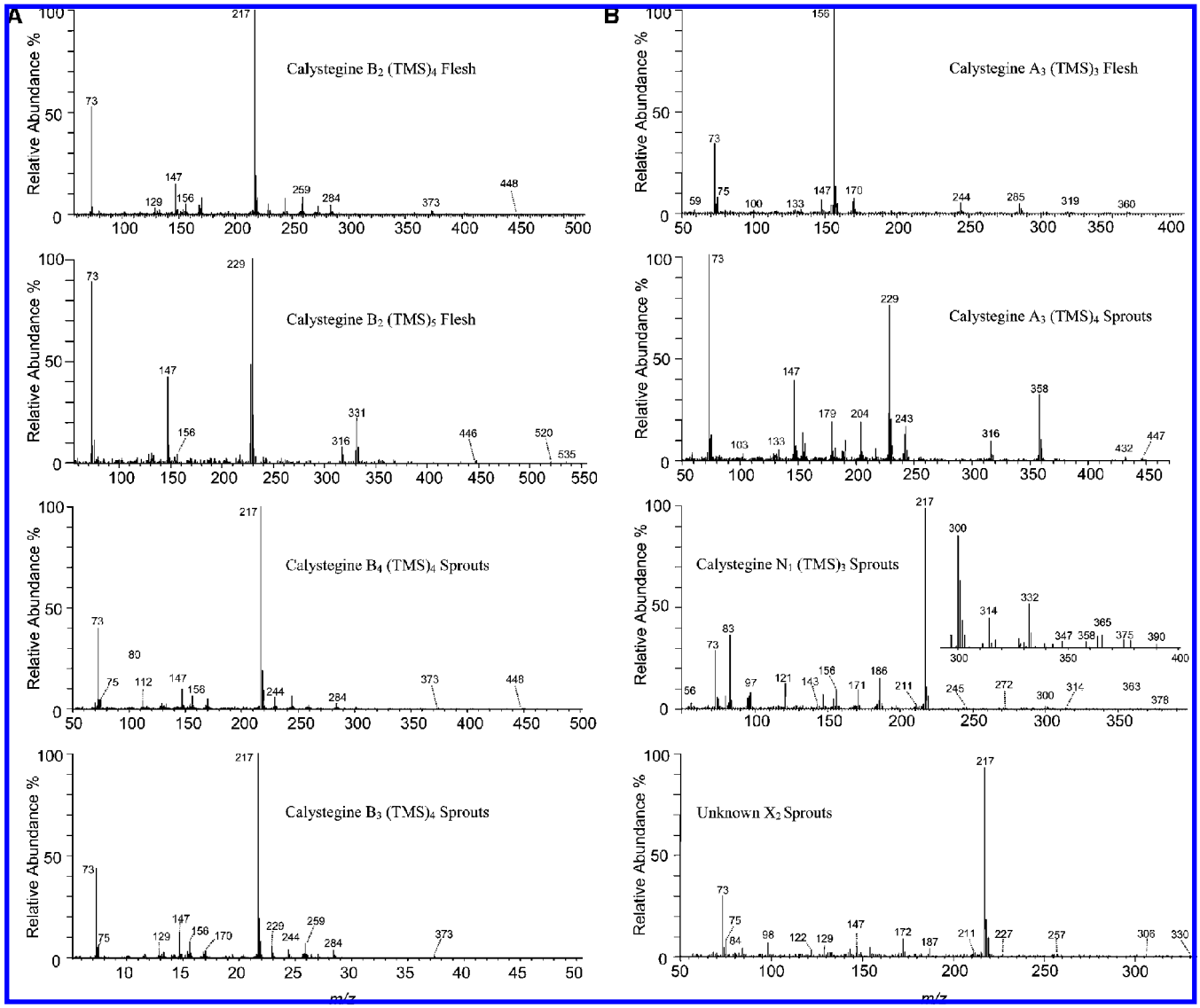


**Figure 3.** Mass spectra of trimethylsilyl derivatives of calystegines $A_3$, $B_1$, $B_2$, $B_3$, $B_4$, the amino-substituted calystegine $N_1$ and unknown compound $X_2$.

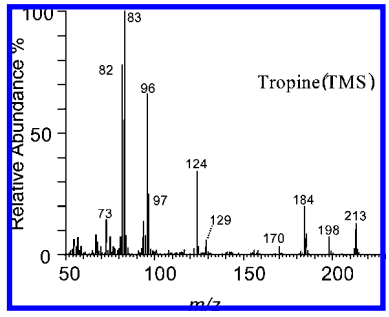


**Figure 4.** Mass spectra of the trimethylsilyl derivative of tropine.

adapted Phureja population such that they represented material with the most diverse origins. GC-MS analysis revealed that the only detectable calystegines present were the $A_3$ and $B_2$ structural types, which have previously been identified as the main constituents of Tuberosum cultivars (*9–12*). The $\sigma^2$ values show that the only significant variation was between individual lines and that the variability due to repeat injections at the same instrumental split and at different splits was similar. Consequently, in subsequent experiments, injections were only made at the two different splits, and these were considered to be equivalent to duplicate injections. Quantification indicated that there was a 6-fold variation in total calystegine content (**Table 2**) with the levels found, varying from 138 mg TE/kg d.m. in the line 80 CP 23 to 881 mg TE/kg d.m. in DB 271–39. A nearly 10-fold range of variation has been previously reported in eight American Tuberosum cultivars (*12*), where the levels ranged from 34 to 326 mg calystegines/kg d.m. However, due to the use of different experimental protocols and different units of measurement, direct comparison of calystegine concentrations between these data for Phureja and tuberosum is not possible.

In the majority of the Phureja lines studied, the levels of the two calystegines ($A_3$ and $B_2$) were very similar, and consequently for these lines the ratio of the two calystegines to each other did not differ greatly from unity (**Table 2**). The sole exception to this was the line PHU 950-412 where the concentration of $B_2$ was 2.5 times less than that of $A_3$. When the results from the 13 lines were utilized, calystegine $A_3$ and calystegine $B_2$ contents were both statistically significantly correlated ($r = 0.96$, $P < 0.001$ and $r = 0.95$, $P < 0.001$, respectively) with the total concentration of calystegine. Removal of the values for line PHU 950-412 improved both correlation coefficients which increased to $r = 0.99$ for both compounds.

**Table 2.** Individual Calystegine Contents (mg TE/kg d.m.) of Whole Tubers Harvested from 13 Phureja Lines

| line | calystegine $A_3$ | calystegine $B_2$ | ratio ($B_2/A_3$) |
|---|---|---|---|
| DB 271-39 | 478.2 | 403.1 | 0.87 |
| DB 207-35 | 389.3 | 410.2 | 1.02 |
| 81 S 60 | 352.4 | 446.7 | 1.26 |
| DB 378-01 | 382.7 | 339.8 | 0.88 |
| DB 244-37 | 343.4 | 339.7 | 1.06 |
| 71 T 46 | 328.4 | 333.2 | 1.02 |
| DB 266-70 | 334.2 | 315.4 | 0.92 |
| PHU 950-412 | 435.1 | 171.6 | 0.39 |
| 84 2P 75 | 233.6 | 258.4 | 1.21 |
| DB 161-10 | 148.8 | 145.6 | 0.98 |
| 71 P 10 | 93.0 | 99.5 | 1.12 |
| DB 168-11 | 84.1 | 99.8 | 1.18 |
| 80 CP 23 | 78.4 | 59.6 | 0.78 |
| group mean | 283.2 | 263.2 | 0.98 |
| lsd ($P = 0.05$)[a] | 92.7 | 94.3 | 0.18 |
| variance components ($\sigma^2$) | | | |
| split | −67.35 | −7.74 | 0.001 |
| injection | 94.27 | −57.26 | −0.001 |
| field replicate | −202.80 | −295.63 | −0.001 |
| line | 8687.63 | 8999.81 | 0.032 |

[a] Least significant differences between lines.

**Table 3.** Individual and Total Calystegine Contents (mg TE/kg d.m.) of Whole Tubers from Phureja and Tuberosum Cultivars and Lines Grown in 2004

| group | cultivar/line | calystegine $A_3$ | calystegine $B_2$ | ratio ($B_2/A_3$) |
|---|---|---|---|---|
| Phureja | DB 257-26 | 170.2 | 334.0 | 1.91 |
| | DB 333-16 | 172.9 | 204.7 | 1.19 |
| | DB 337-37 | 100.1 | 118.8 | 1.23 |
| | DB 358-23 | 136.0 | 312.3 | 2.33 |
| group mean | | 144.8 | 242.5 | 1.67 |
| Tuberosum | Ailsa | 97.8 | 114.0 | 1.17 |
| | Brodick | 99.1 | 123.3 | 1.23 |
| | Cara | 146.3 | 130.3 | 0.90 |
| | M. Piper | 63.6 | 72.8 | 1.14 |
| | P. Dell | 93.7 | 239.3 | 2.54 |
| group mean | | 100.1 | 135.9 | 1.40 |
| lsd ($P = 0.05$)[a] | | 46.55 | 86.58 | 0.32 |
| lsd ($P = 0.05$)[b] | | 22.08 | 41.07 | 0.15 |
| variance components ($\sigma^2$) | | | | |
| split | | 130.39 | −201.16 | −0.000 |
| field replicate | | −3.18 | 750.34 | −0.003 |
| line/cultivar | | 1017.40 | 3519.30 | 0.047 |

[a] Least significant differences between cultivar/line. [b] Least significant differences between groups means.

**Intergroup Comparison.** In 2004, a further four Phureja lines were grown in field plots together with five Tuberosum cultivars (**Table 3**), and as found previously, only calystegines $A_3$ and $B_2$ were detected. Values for $\sigma^2$ show that the variation between line and cultivar was considerably more significant than that due to field replicate or injection split. The mean value for the total combined calystegine ($A_3$ and $B_2$) content of the four Phureja lines (387 mg TE/kg d.m.) was slightly lower than that for the 13 lines grown in the previous year (546 mg TE/kg d.m.), but all values fell within the range found in 2003. The ratio of the two major calystegines $B_2$ and $A_3$ was close to 1 for two of the lines grown in 2004, as for the 2003 samples, but in the other two lines, DB 257-26 and DB 358-23, the concentration of $B_2$ was almost double that of $A_3$.

The mean total calystegine content of the five Tuberosum (**Table 3**) cultivars, grown, harvested, and stored under identical conditions to the four Phureja lines, was 236 mg TE/kg d.m., which was statistically significantly lower ($P < 0.05$) than the mean of the Phureja lines. However, the ranges for the two species overlapped when considered on an individual cultivar and line level with, for example, the total calystegine content of the Phureja line, DB 337-37, being less than that of three of the Tuberosum cultivars Brodick, Cara, and P. Dell.

The presence of calystegines $A_3$ and $B_2$ in the Tuberosum cultivars studied here was in agreement with compositional data reported for other cultivars within the species (*9–12*). In four out of the five cultivars, the ratio of $B_2$ to $A_3$ was close to unity, with only Pentland Dell showing a concentration of $B_2$ more than twice that of $A_3$. When averaged over lines and cultivars respectively, a small but significant increase was found in the mean ratio for the Phureja group relative to the Tuberosum group. The mean value for the calystegine ratio $B_2/A_3$ for the Tuberosum cultivars (1.40) was less than that reported in the study of eight American cultivars where the ratios ranged from 1.7 to 6.1, while Nash et al. (*9*) reported a value of 2 for the ratio of these calystegines in the peel of the European cultivar, Estima.

From the results presented in this study, it would appear that the consumption of whole Phureja tubers from the locally adapted population developed at SCRI, by either animals or man, would result in a total calystegine intake of approximately the same order of magnitude to that currently received from eating whole potato tubers from current commercially available Tuberosum cultivars. However, it would be premature to conclude that, whatever the toxicity profiles of calystegines, the development of new varieties based on this Phureja population is unlikely to result in any serious toxicity problems attributable to these water-soluble alkaloids. The genetic influences on calystegine biosynthesis have not yet been studied at the molecular level, and it is possible that some crosses could yield high levels of calystegines.

**Intratuber Distribution.** Whole tubers from the five Tuberosum cultivars and four Phureja lines utilized in the interspecies comparison were manually peeled to produce separate samples of peel and flesh. The proportion of peel (**Table 1**) removed from tubers of the two species differed significantly ($P < 0.05$), with the tubers from the Phureja lines containing on average 10.1% peel as compared with a mean value of 8.8% for the Tuberosum cultivars. This appeared to reflect the lower mean tuber weight recorded for the Phureja lines but analysis revealed that this difference was not statistically significant. However, when mean tuber weights and percent peel for each cultivar and line were correlated, a statistically significant negative correlation was found ($r = -0.76$, $P < 0.02$).

The individual and combined concentration of calystegines $A_3$ and $B_2$ in the flesh samples (**Table 4**) were significantly lower for the Tuberosum group, when averaged over all cultivars (114 mg TE/kg d.m.), than for the Phureja group, averaged over all lines (296 mg TE/kg d.m.). The ratios of the calystegines $A_3$ and $B_2$ was, as might be expected, similar to that found for the whole tubers (**Table 3**). Individual calystegine concentrations in flesh from all cultivars and lines were lower than those in the corresponding peel samples (**Table 4**). In contrast to the flesh samples, the mean total calystegine concentration of the peel from the Phureja lines were significantly lower than those of the Tuberosum cultivars, primarily due to variation in the levels of calystegine $A_3$. The concentrations of calystegines $A_3$ and $B_2$ in peel (**Table 4**) differed significantly between both individual lines and cultivars. In the case of the Phureja lines, their combined concentrations almost span a 6-fold range (362−2075 mg TE/kg d.m.). This is comparable to a greater than 10-fold range in variability reported in a study of the calystegine content of peel and flesh from eight American

**Table 4.** Individual and Total Calystegine Contents of Tuber Flesh and Peel from Phureja and Tuberosum Cultivars and Lines

| | | calystegine $A_3$ | | | calystegine $B_2$ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (mg TE/kg d.m.) | | peel % | (mg TE/kg d.m.) | | peel % | ratio ($B_2/A_3$) | |
| group | cultivar/line | flesh | peel | whole tuber | flesh | peel | whole tuber | flesh | peel |
| Phureja | DB 257-26 | 114.3 | 667.2 | 42.0 | 224.1 | 1408 | 45.2 | 1.93 | 2.06 |
| | DB 333-16 | 97.8 | 846.7 | 43.1 | 140.2 | 761 | 32.7 | 1.46 | 0.90 |
| | DB 337-37 | 86.2 | 204.5 | 22.0 | 113.3 | 158 | 14.2 | 1.38 | 0.77 |
| | DB 358-23 | 122.9 | 257.0 | 20.0 | 285.5 | 572 | 19.5 | 2.35 | 2.25 |
| group mean | | 105.3 | 494 | 31.8 | 190.5 | 725 | 27.9 | 1.78 | 1.50 |
| Tuberosum | Ailsa | 37.0 | 701.0 | 68.2 | 49.3 | 695 | 59.2 | 1.37 | 1.01 |
| | Brodick | 45.4 | 772.2 | 56.0 | 70.8 | 812 | 47.7 | 1.59 | 1.05 |
| | Cara | 100.8 | 611.4 | 34.9 | 91.5 | 527 | 34.6 | 0.91 | 0.88 |
| | M. Piper | 32.1 | 364.2 | 51.9 | 29.7 | 467 | 58.4 | 0.92 | 1.28 |
| | P. Dell | 34.7 | 639.8 | 61.2 | 80.0 | 1777 | 64.5 | 2.34 | 2.73 |
| group mean | | 50.0 | 617.7 | 54.4 | 64.2 | 856 | 52.9 | 1.42 | 1.39 |
| lsd ($P = 0.05$)[a] | | 32.9 | 162.0 | 9.23 | 60.4 | 301.3 | 8.9 | 0.29 | 0.25 |
| lsd ($P = 0.05$)[b] | | 15.6 | 76.8 | 4.38 | 28.6 | 142.9 | 4.2 | 0.14 | 0.12 |
| variance components ($\sigma^2$) | | | | | | | | | |
| split | | 91.7 | −998.5 | 13.04 | 227.9 | −4783.4 | 15.79 | 0.002 | 0.017 |
| field replicate | | 98.1 | −91.2 | 0.15 | 252.7 | 19581.4 | −1.62 | −0.004 | 0.002 |
| line/cultivar | | 509.4 | 26190.6 | 40.04 | 1710.3 | 90626.1 | 36.80 | 0.040 | 0.062 |

[a] Least significant differences between cultivar/line. [b] Least significant differences between groups.

**Table 5.** Individual and Total Calystegine Contents (mg TE $kg^{-1}$ FDM) of Sprouts Harvested from Phureja and Tuberosum Cultivars and Lines

| | | calystegine (mg TE/kg d.m.) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| group | cultivar/line | $A_3$ | $B_2$ | $B_3$ | $B_4$ | $N_1$ | $X_2$ | ratio ($B_2/A_3$) |
| Phureja | DB 257–26 | 2063 | 9163 | 80.6 | 95.6 | 22.8 | 13.9 | 4.45 |
| | DB 333–16 | 1988 | 5158 | 121.7 | 9.9 | 169.9 | 83.7 | 2.61 |
| | DB 337–37 | 2846 | 5120 | 44.9 | 12.0 | 27.2 | 10.6 | 1.80 |
| | DB 358–23 | 1621 | 7167 | 74.4 | 177.9 | 12.5 | 10.6 | 4.51 |
| group mean | | 2129 | 6652 | 80.4 | 73.4 | 58.1 | 29.7 | 3.34 |
| Tuberosum | Ailsa | 3885 | 7606 | 103.5 | 259.1 | 17.4 | 12.8 | 1.97 |
| | Brodick | 2171 | 6832 | 39.0 | 382.4 | 15.1 | 14.7 | 3.15 |
| | Cara | 7752 | 10145 | 125.3 | 526.5 | 22.0 | 22.4 | 1.31 |
| | M. Piper | 5352 | 5993 | 70.4 | 383.5 | 7.4 | 9.8 | 1.12 |
| | P. Dell | 834 | 6551 | 93.8 | 45.3 | 12.0 | 9.5 | 7.90 |
| group mean | | 3999 | 7425 | 86.4 | 319.4 | 14.8 | 13.8 | 3.09 |
| lsd ($P = 0.05$)[a] | | 526.0 | 1696 | 31.5 | 53.91 | 29.55 | 27.33 | 0.81 |
| lsd ($P = 0.05$)[b] | | 249.5 | 804 | 14.9 | 25.60 | 14.02 | 12.96 | 0.38 |
| variance components ($\sigma^2$) | | | | | | | | |
| split | | −2772 | 373951 | 3.87 | −5.06 | 21.19 | −18.05 | 0.29980 |
| field replicate | | −5847 | − 146184 | −34.49 | −139.25 | −1.98 | 1.89 | −0.0196 |
| line/cultivar | | 129895 | 1350366 | 464.73 | 1365.09 | 410.06 | 350.57 | 0.3053 |

[a] Least significant differences between cultivar/line. [b] Least significant differences between groups.

Tuberosum cultivars (*12*), where values ranged from 218 mg/kg d.m. for the cultivar Red Lasoda to 2581 mg/kg d.m. for Ranger Russet. However, as mentioned previously, the actual concentrations measured in the two studies are not directly comparable due to the use of different methodology and units of measurement.

For all lines and cultivars in both species, the ratio of calystegines $B_2$ to $A_3$ in the peel was similar to that in the corresponding flesh sample. When the data from all lines and cultivars was used, the ratios of calystegines in the peel was statistically significantly correlated ($r = 0.85$, $P < 0.01$) with that in the flesh samples. As might therefore be expected, both peel and flesh ratios were highly correlated with that for the whole tuber sample ($r = 0.96$, $P < 0.001$ and $r = 0.95$, $P < 0.001$ respectively). This might suggest that the ratio of calystegines in both peel and flesh were under the same genetic control. In the model for calystegine biosynthesis and transport proposed recently by Richter et al. (*11*), in the developing potato plant tuber calystegines are formed in the stolon from tropinone, which is tranlocated there from the site of tropinone synthesis in the roots.

A comparison of the proportion of the total tuber calystegines present in the peel (**Table 4**) indicated statistically significant differences between the two groups when averaged over all lines or cultivars. Despite having a higher proportion of peel in their tubers, the Phureja lines (**Table 1**) had, on average, a lower proportion of the total calystegines in the peel (30%) than the Tuberosum cultivars, which had 54% of the tuber calystegines in the peel. This clearly indicates that peeling, prior to consumption, significantly reduces calystegine intake in both species. However, on average, the observed reduction would be expected to be greater for the more commonly consumed Tuberosum cultivars.

**Calystegine Content of Sprouts.** Sprouts were harvested from tubers from the five Tuberosum and four Phureja lines used in the previous studies. The main calystegines in both species were $B_2$ and $A_3$. In addition, four other calystegines were detected at lower concentrations. Of these the most abundant were $B_3$ and $B_4$. There were smaller amounts of $N_1$, the amino analog of $B_2$ (*6*) and an unidentified compound which was tentatively identified as being the same component designated $X_2$ by Bekkouche et al. in their study of calystegines

present in *Hyoscyamus albus* (*6*) (**Figures 13**). Calystegine $B_4$ had been previously detected as a constituent in some, but not all, sprout samples derived from the European Tuberosum cultivar Liu (*10*).

Quantification (**Table 5**) revealed that for the Phureja lines the combined concentrations of calystegines $B_2$ and $A_3$ were 20–40 (mean 30) times greater in the sprouts than in the whole tuber flesh, and 4–20 (mean 7) times higher than in the peel samples (**Table 4**). For the Tuberosum cultivars their levels in sprouts were respectively 80–600 (mean 100) times greater than in flesh, and 3–100 (mean 8) times greater than in peel. Analysis of variance indicated that for the Phureja lines the mean concentration of calystegine $A_3$ was significantly lower than that for the Tuberosum cultivars. However, some differential behavior was observed, with for example, the concentration of calystegine $A_3$ in sprouts from the Tuberosum cultivar, P. Dell, being considerably lower than those found for any of the Phureja lines. Comparison of sprout calystegine concentration with the levels of total calystegines in the flesh, peel, and whole tuber samples did not produce any statistically significant correlations, indicating that sprout calystegine concentration could not be predicted from the tuber composition.

The ratio of the two major calystegines in the sprouts ($B_2$ and $A_3$) of both species varied considerably (**Table 5**), but for all cultivars and lines the concentration of calystegine $B_2$ was greater than that of calystegine $A_3$. However, the group average ratio for the Tuberosum cultivars did not differ significantly from that of the Phureja lines. For each species the ratio of calystegine $B_2/A_3$, was on average 2 times greater in sprouts (**Table 5**) than flesh or peel (**Table 4**). Translocation of calystegines from stems to sprouting eyes and de novo synthesis of calystegines from tropinone in developing sprouts are both features of the model for calystegine biosynthesis and transport described by Richter et al. (*11*). An increase in the ratio $B_2/A_3$ may therefore be indicative of enhanced translocation of calystegine $B_2$ from peel to sprout and/or biosynthesis within sprouts relative to that of $A_3$. A similar increase in the ratio $B_2/A_3$ in elongating sprouts was previously observed for the European cultivar Liu (*10*).

There were also inter- and intraspecies variations in the distribution of minor calystegines. Whereas the concentration ranges found for $B_3$ in sprouts across the Tuberosum cultivars and Phureja lines were broadly similar, calystegine $B_4$ was more concentrated in sprouts from the Tuberosum cultivars, with the exception of P. Dell. In the latter cultivar, the $B_4$ concentration was less than the Phureja group mean. There was also considerable variability between the Phureja lines, two of which had very low levels of $B_4$. Concentrations of calystegine $N_1$ and unknown $X_2$ were generally very low in sprouts from both species, except for Phureja line DB 333-16, in which the measured levels were several times greater than in any of the other lines or cultivars.

The results presented would suggest that considerable variation for calystegine content exists within both species, which could be utilized to get a better understanding of the role played by these compounds within the tuber. The steroidal glycoalkaloids, α-solanine, and α-chaconine, commonly associated with potato tubers of both species, are known to increase in response to various environmental stresses such as low temperature (*17*), light exposure (*20, 21*), and bruising (*22*) and it would clearly be of interest to determine whether these water soluble alkaloids are similarly affected. Recently, Richter et al. (*11*) demonstrated that calystegine levels in sucrose synthase antisense tubers increase with increasing sucrose levels. These findings suggests a connection between calystegine biosynthesis and sugar metabolism, which would a good subject for further investigation.

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## LITERATURE CITED

(1) Tepfer, D.; Goldmann, A.; Pambokdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Dénarié, J.; Rosenberg, C. A plasmid of Rhizobium meliloti 41 encodes catabolism of two compounds from root exudates of Calystegium sepium. *J. Bacteriol.* **1988**, *170*, 1153–1161.

(2) Goldmann, A.; Milat, M.-L.; Ducrot, P.-H.; Lallemand, J.-Y.; Maille, M.; Lepingle, A.; Charpin, I.; Tepfer, D. Tropane derivatives from Calystegia sepium. *Phytochemistry* **1990**, *29*, 2125–2127.

(3) Drager, B. Chemistry and biology of calystegines. *Nat. Prod. Rep.* **2004**, *21*, 211–223.

(4) Moyneux R. J.; Nash, R. J.; Asano, N. *The chemistry and biological activity of calystegines and related Nortropane alkaloids. Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier Science Ltd.: Oxford, UK, 1996; *Vol. II* pp 303–343.

(5) Schimming, T.; Tofern, B.; Mann, P.; Richter, A.; Jenett-Siems, K.; Dräger, B.; Asano, N.; Gupta, M. P.; Correa, M. D.; Eich, E. Distribution and taxonomic significance of calystegines in the Convolvulaceae. *Phytochemistry* **1998**, *49*, 1989–1995.

(6) Bekkouche, K.; Daali, Y.; Cherkaoui, S.; Veuthey, J.-L.; Christen, P. Calystegine distribution in some solanaceous species. *Phytochemistry* **2001**, *58*, 455–462.

(7) Brock, A.; Herzfeld, T.; Paschke, R.; Koch, M.; Dräger, B. Brassicacea contain nortropane alkaloids. *Phytochemistry* **2006**, *67*, 2050–2057.

(8) Brock, A.; Bieri, S.; Christen, P.; Dräger, B. Calystegines in wild and cultivated Erythroxylum species. *Phytochemistry* **2005**, *66*, 1231–1240.

(9) Nash, R. J.; Rotschild, M.; Porter, E. A.; Watson, A. A.; Waigh, R. D.; Waterman, P. G. Calystegines in Solanum and Datura species and the death's head hawkmoth. *Phytochemistry* **1993**, *34*, 1281–1285.

(10) Keiner, R.; Dräger, B. Calystegine distribution in potato (Solanum tuberosum) tubers and plants. *Plant Sci.* **2000**, *150*, 171–179.

(11) Richter, U.; Sonnewald, U.; Dräger, B. Calystegines in potatoes with genetically engineered carbohydrate metabolism. *J. Exp. Botany* **2007**, *58*, 1603–1615.

(12) Friedman, M.; Roitman, J. N.; Kozuke, N. Glycoalkaloid and calystegine contents of eight potato cultivars. *J. Agric. Food Chem.* **2003**, *51*, 2964–2973.

(13) Stenzel, O.; Teuber, M.; Dräger, B. Putrescine N-methyltransferase in Solanum tuberosum L., a calystegine-forming plant. *Planta* **2006**, *223*, 200–212.

(14) Asano, N.; Kato, A.; Matsui, K.; Watson, A. A.; Nash, R. J.; Molyneux, R. J.; Hackett, L.; Topping, J.; Winchester, B. The effects of calystegines isolated from edible fruits and vegetables on mammalian liver glycosidases. *Glycobiology* **1997**, *7*, 1085–1088.

(15) De Maine, M. J.; Lees, A. K.; Muir, D. D.; Bradshaw, J. E.; Mackay, G. R. Long-day adapted phureja as a resource for potato breeding and genetic research. In *Potato, Global Research and Development*; Khurana, S. M. P., Shekhawat, G. S., Singh, B. P., Pandey, S. K., Eds.; Indian Potato Association: Shimla, India, 2000; *Vol. 1*, pp 134–137.

(16) Winfield, M.; Lloyd, D.; Griffiths, W.; Bradshaw, J.; Muir, D.; Nevison, I.; Bryan, G. Assessing organoleptic attributes of Solanum tuberosum and S. phureja potatoes. *Aspects Appl Biol.* **2005**, *76*, 127–135.

(17) Griffiths, D. W.; Bain, H.; Dale, M. F. B. The effect of low-temperature storage on the glycoalkaloid content of potato (Solanum tuberosum L.) tubers. *J. Sci. Food Agric.* **1997**, *74*, 301–307.

(18) Molyneux, R. J.; Pan, Y. T.; Goldmann, A.; Tepfer, D. A.; Elbein, A. D. Calystegins, a novel class of alkaloid glycosidase inhibitors. *Arch. Biochem. Biophys.* **1993**, *1*, 81–88.

(19) Dräger, B. Identification and quantification of calystegines, polyhydroxyl nortroppane alkaloids. *Phytochem. Anal.* **1995**, *6*, 31–37.

(20) Griffiths, D. W.; Bain, H.; Dale, M. F. B. The effect of storage temperature on potato (Solanum tuberosum L.) tuber glycoalkaloid content and the subsequent accumulation of glycoalkaloids and chlorophyll in response to light exposure. *J. Agric. Food Chem.* **1998**, *46*, 5262–5268.

(21) Griffiths, D. W.; Dale, M. F. B. Effect of light exposure on the glycoalkaloid content of Solanum phureja tubers. *J. Agric. Food Chem.* **2001**, *49*, 5223–5227.

(22) Dale, M. F. B.; Griffiths, D. W.; Bain, H. Effect of bruising on the total glycoalkaloid and chlorogenic acid content of potato (Solanum tuberosum) tubers of five cultivars. *J. Sci. Food Agric.* **1998**, *77*, 499–505.

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