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Indices for the Assessment of Glycoalkaloids in Potato Tubers Based on Surface Color and Chlorophyll Content

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Abstract: Glycoalkaloids (GAs) are toxic to humans at higher concentrations. However, studies also suggest the health benefits of GAs depending on the dose and conditions of use. Methods that have been used to determine GA content in potato tubers are destructive and time-consuming and require skilled personnel and high-performance laboratory equipment. We conducted this study to develop indices for the prediction of the level of total GAs in potato tubers at different greening stages based on surface color readings and chlorophyll (Chl) development. Color values (Hunter L*, a*, b*, a*/b*), Chls (Chl a, Chl b, and total Chls) and GA (α-solanine, α-chaconine, and total GAs) content were measured from tubers of 'Atlantic' and 'Trent' potato cultivars at three-week intervals in up to six greening stages during the storage at room conditions (22 °C, 12-h shift of light-dark cycles). The results have revealed that greening, Chls, and GA content significantly increased for the two cultivars as the stage proceeded. The toxic level of GAs (>200 mg kg^{-1} FW) was accumulated at the late greening stages, accompanied by the highest Chl content. Finally, indices were developed based on surface color and Chl content for estimation of the safe GA levels for the consumption of the two commercially and commonly used potato cultivars. Moreover, the developed indices could be used as basic information to adapt to other potato cultivars.

Keywords: α-solanine; α-chaconine; color variables; chlorophyll contents; color index

1. Introduction

Potato (*Solanum tuberosum* L.) is the leading non-grain food commodity in the global food system, with production reaching 368.2 million Mt from 17.6 million ha in 2018 [1]. It is the fourth most important food crop in the world, following maize, wheat, and rice. The Republic of Korea produced 553,596 Mt of potato from 25,772 ha of land in 2018 [1]. Potatoes play a fundamental part in the effort made to ensure food and nutritional security for the increasing world population [2,3]. They are one of the most efficient crops for converting natural resources, labor, and capital into high-quality food. The production of potato is relatively easy, and their genetic complexity allows a diversity of genotypes for any climate, culture, and conditions [3]. They produce more nutritious food in a short growing cycle on small land and in harsher climates than any other major crop [2].

Potato represents half of the root and tuber crops consumed for a carbohydrate food source in the world [4]. Potatoes are rich in carbohydrates, which makes them a good source of energy [2]. They have

the highest protein content, around 2.1 percent on a fresh weight basis, in the family of root and tuber crops and protein of high quality, with an amino-acid pattern that matches human requirements [2,3]. Additionally, potatoes are sources of a variety of essential vitamins and minerals, such as vitamins C and B6 and the minerals potassium, magnesium, and iron [2,3]. A single medium-sized potato contains about half the recommended daily intake of vitamin C and a fifth of the recommended daily value of potassium [2,3]. Potatoes have been traditionally consumed after boiling, frying, or baking as a main dish or with other food types. Different popular snack products, such as crisps or French fries, that appeal to consumers, due to appearance, texture, or flavor, are also processed from potato tubers [3,5].

The potato belongs to the *Solanaceae* family, which is known for producing secondary natural poisonous metabolites called glycoalkaloids (GAs) [5]. GAs play an important defensive role due to their toxic nature against pests [5]. α-solanine and α-chaconine are the two major glycoalkaloids in cultivated potatoes that together account for 95% of the total glycoalkaloid content [6,7]. In the tubers, there is a higher concentration of GAs obtained in the skin, around the eyes, wounded areas, and in the sprouts [8,9]. Exposing potato tubers to light in storage or at home causes greening, and greened tubers accumulate GAs [10]. The processes of greening and GA accumulation are concurrent but independent; the greening shows the formation of chlorophylls (Chls) and it is also considered as an indication for an increase in the level of GAs [11]. GA synthesis can also be elevated by damage to the tubers during post-harvest operations [12].

GAs are toxic to humans at higher concentrations. The safe upper limit for a human is 200 mg kg^{-1} total GAs of fresh tuber weight [13]. Potato tubers that contain over 200 mg kg^{-1} total GAs of fresh tuber weight possess a bitter off-flavor and may cause gastro-enteric symptoms, comas, and even death [14–18]. Symptoms of toxicity include headache, nausea, fatigue, vomiting, abdominal pain, diarrhea, apathy, restlessness, drowsiness, mental confusion, rambling, incoherence, stupor, hallucinations, trembling, and visual disturbances [5,12,14,19]. However, studies suggest that GAs have also health benefits, such as anticancer, antimalarial, anti-inflammatory, hypoglycemic, and hypo-cholesterol emic activities, depending on the dose and conditions of use [14,20]. Therefore, it is crucial to develop an indicative index that can be used easily to avoid the potential toxicity of GAs to human beings.

The different methods that have been used to determine the GAs are time-consuming and destructive and require skilled personnel and high-performance laboratory equipment. It is difficult for producers and consumers to determine the safe level of alkaloids with the existing methods of determination. Therefore, the current study was designed to develop indices for the prediction of the level of GAs in potato tubers at different greening stages based on surface color readings and Chl development.

2. Materials and Methods

2.1. Plant Material

Commercially and commonly used ‘Atlantic’ and ‘Trent’ potato cultivars were selected for this study. Tubers of the two cultivars were obtained from the Haitai-Calbee snack factory, Korea. Relatively uniform-sized defect-free tubers were selected for the experiment and stored at room conditions (22 °C, 12-h shift of light-dark cycles) to simulate the consumers’ practice and to allow greening. During sampling, 20 tubers (‘Atlantic’ and ‘Trent’; 10 tubers each) were used for each greening stage. Subsampling was done at six different greening stages at three-week intervals for up to 15 weeks of storage, and representative photos of the tubers from both cultivars were acquired at each greening stage. Samples for the analysis of GAs and Chl content were prepared immediately after taking representative photos and the color reading from the surface of intact tubers. The samples were then frozen by liquid nitrogen and stored in a deep freezer (−80 °C) until analysis [21].

2.2. Color Measurement and Analysis

Intact and undamaged tubers were used to take surface readings of Hunter L*, a*, and b* color variables using CR-400 chroma meter (Minolta, Tokyo, Japan). The Hunter a* represents a chromatic redness parameter that ranges from red to green with positive to negative values, respectively. The Hunter b* represents a chromatic yellowness parameter that ranges from yellow with positive values to blue with negative values, while the Hunter L* represents the degree of brightness, which ranges from black with 0 value to white with 100 value [22]. The color variables were measured and recorded from the surface of each tuber five times and the values were averaged. The color was measured at three-week intervals until the 15th week of storage. During sampling, 20 tubers ('Atlantic' and 'Trent'; 10 tubers each) were used for each greening stage. Hence, a total of 120 tubers (60 tubers from each) were used for the study.

2.3. Extraction and Quantification of Chls and GAs

Chls and GAs were extracted from 20 potato tubers at each stage (10 tubers from each cultivar) for a 15-weeks storage period at three-week intervals. The peripheral 10 mm tissue was sampled, since Chl and GAs are accumulated in the outer cell layer of the tuber [8,9,23]. Dimethyl sulfoxide (DMSO) chlorophyll extraction procedure was used, as described by [24]. The readings were measured at 645 nm and 663 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) against a DMSO blank. Subsequently, Chl a, Chl b, and total Chls were calculated by Arnon [25] using equations as follows:

a. Chl a (mg g^{-1}) peel fresh weight) = [(12.7*A663) − (2.69*A645)]*(V/1000*W)
b. Chl b (mg g^{-1}) peel fresh weight) = [(22.9*A645) − (4.68*A663)]*(V/1000*W)
c. Total Chls = Chl a + Chl b

V = volume of solvent; W = fresh weight of the extracted tissue.

Extraction of GAs was made as stated by Tilahun et al. [9] and quantification of α-solanine and α-chaconine was done by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [26]. The spectrometer was adjusted, as described by Tilahun et al. [9], Zywicki et al. [26], and Nie et al. [27], for detection of α-solanine and α-chaconine.

2.4. Procedure to Develop Indices for the Estimation of Total GAs

The photos of representative potato tubers from both 'Atlantic' and 'Trent' potato cultivars were acquired by a Canon digital camera (EOS 200D, Tokyo, Japan) at the six different greening stages with three-week intervals, after peeling a portion of peripheral 10 mm tissue to show the extent of greening. The average data obtained from the color measurements, total Chls, and total GAs were then matched to the corresponding photo at each stage based on correlation coefficients of color values and Chls content vs. total GAs.

2.5. Statistical Analysis

The experiment was conducted in a completely randomized design. The data were subjected to analysis of variance (ANOVA) to determine the significance of differences between cultivars and greening stages at $p < 0.05$ using SAS statistical software (SAS/STAT® 9.1; SAS Institute Inc., Cary, NC, USA), and the Pearson correlation test was used to correlate the collected parameters.

3. Results and Discussion

3.1. Color Variables and GAs

The current study results have revealed a significant difference for Hunter L*, a*, b*, and a*/b* values among the six stages of greening for both 'Atlantic' and 'Trent' potato cultivars. The difference

between the two cultivars was also significant, though not at all greening stages (Table 1). The Hunter L* values decreased across greening stages 1–6, indicating the decrease in tubers' brightness for both cultivars. Grunenfelder et al. [28] also reported a decrease in L* values of tubers as the storage days proceeded from 0–7 days. Hunter a* values, which indicate the chromatic value ranging from red (+a*) to green (−a*), have also shown a decreasing trend from stage 1–6, indicating an increase in the greening of the tubers for both cultivars [22]. The hunter a* values were positive for tubers in stages 1 and 2, and negative hunter a* values were recorded for tubers in the remaining stages. A study conducted by Haase [29] has also shown a significant change of tuber color towards green because of exposure of potato tubers to light. Similar to Hunter a* values, a decreasing trend and significant differences were observed in the Hunter b* values among the six stages for both cultivars. The Hunter a*/b* ratios were significantly different and follow the same trend as Hunter a* color values among the greening stages of both cultivars. Tilahun et al. [30] also reported the same trends of Hunter a* values and a*/b* ratios during the evaluation of color changes in tomatoes.

Table 1. Hunters L*, a*, b*, and a*/b* color values of 'Atlantic' and 'Trent' potato cultivars under six greening stages during storage at room conditions (22 °C) with a 12-h shift of light-dark cycles.

Parameters	Cultivars	Greening Stages					
		1	2	3	4	5	6
L*	Atlantic	58.30_{a}^{A}	56.74_{bc}^{B}	55.66_{bcd}^{A}	55.37_{bcd}^{A}	55.29_{cd}^{A}	54.30_{d}^{A}
	Trent	56.88_{b}^{B}	58.93_{a}^{A}	55.95_{bc}^{A}	55.84_{bc}^{A}	56.28_{bc}^{A}	52.64_{d}^{B}
a*	Atlantic	1.83_{a}^{A}	0.99_{bc}^{A}	-1.23_{cd}^{A}	-1.90_{de}^{A}	-2.20_{de}^{A}	-2.89_{f}^{A}
	Trent	2.10_{a}^{A}	1.01_{bc}^{A}	0.08_{cd}^{B}	-0.55_{de}^{B}	-0.28_{de}^{B}	-3.78_{f}^{A}
b*	Atlantic	26.10_{ab}^{B}	25.21_{bc}^{B}	24.86_{c}^{B}	25.82_{abc}^{B}	23.72_{d}^{B}	22.46_{e}^{A}
	Trent	29.22_{a}^{A}	28.32_{ab}^{A}	27.16_{c}^{A}	27.81_{bc}^{A}	26.41_{cd}^{A}	22.76_{e}^{A}
a*/b*	Atlantic	0.070_{a}^{A}	0.039_{bc}^{A}	-0.049_{cd}^{B}	-0.076_{de}^{B}	-0.093_{de}^{B}	-0.129_{f}^{A}
	Trent	0.072_{a}^{A}	0.036_{bc}^{A}	0.003_{cd}^{A}	-0.020_{de}^{A}	-0.011_{de}^{A}	-0.166_{f}^{B}

The mean with different uppercase letters within the same column is significantly different ($p < 0.05$), while the mean with different lowercase letters within the same row is significantly different ($p < 0.05$).

A significant difference was also observed in GA content among the six stages for the two cultivars (Figure 1). The α-solanine, α-chaconine, and total GA contents increased as it went from stage 1−6. The α-solanine and α-chaconine content were cultivar-dependent in the present study. α-solanine was lower than α-chaconine in the 'Atlantic' cultivar throughout the greening stages. However, the ratio of α-solanine to α-chaconine was nearly 1:1 in the 'Trent' cultivar. Tajner-Czopek [31] reported cultivar-dependent ratios of α-solanine to α-chaconine, ranging from 1:1.9 to 1:2.5 for early potato cultivars. In this study, both cultivars accumulated more than the recommended safe level (>200 mg/kg) of glycoalkaloids at the late stages [7,9,13]. 'Atlantic' accumulated a toxic level of total GAs (296 mg kg^{-1}) at the sixth stage with a Hunter a* value of −2.89, indicating the tuber color change into green. 'Trent' accumulated a toxic level (205.38 mg kg^{-1}) of GAs at stage 5, earlier than 'Atlantic', and reached (225.96 mg kg^{-1}) stage 6 with Hunter a* values of −0.28 and −3.78, respectively (Table 1 and Figure 2). Hunter L* values showed a decrease in the brightness of the tuber at the late stages (Table 1).

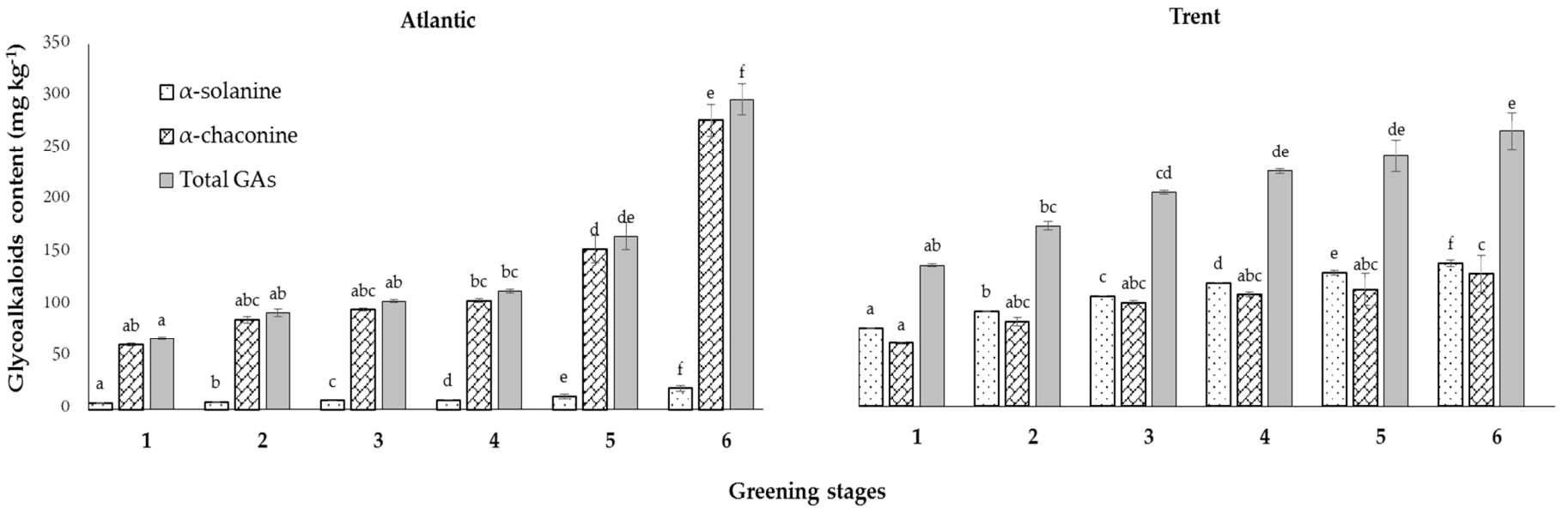


Figure 1. Glycoalkaloids content of 'Atlantic' and 'Trent' potato cultivars under six greening stages during storage at room conditions (22 °C) with a 12-h shift of light-dark cycles. The bars with different letters indicate a significant difference ($p < 0.05$) between the greening stages. The vertical bars represent the standard error of the mean (n = 10).

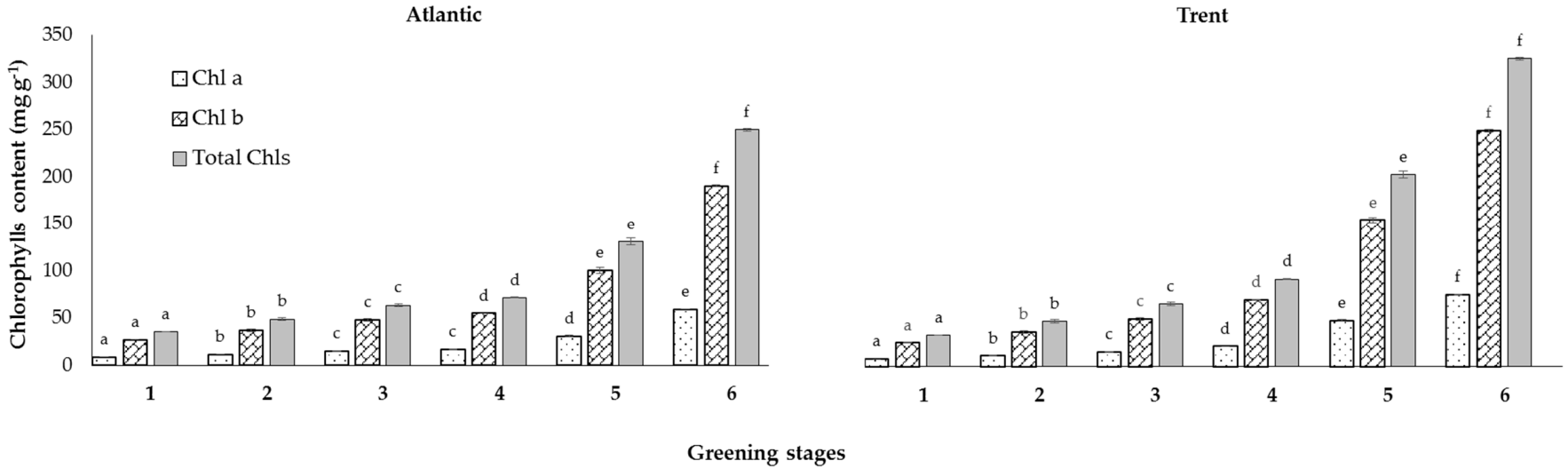
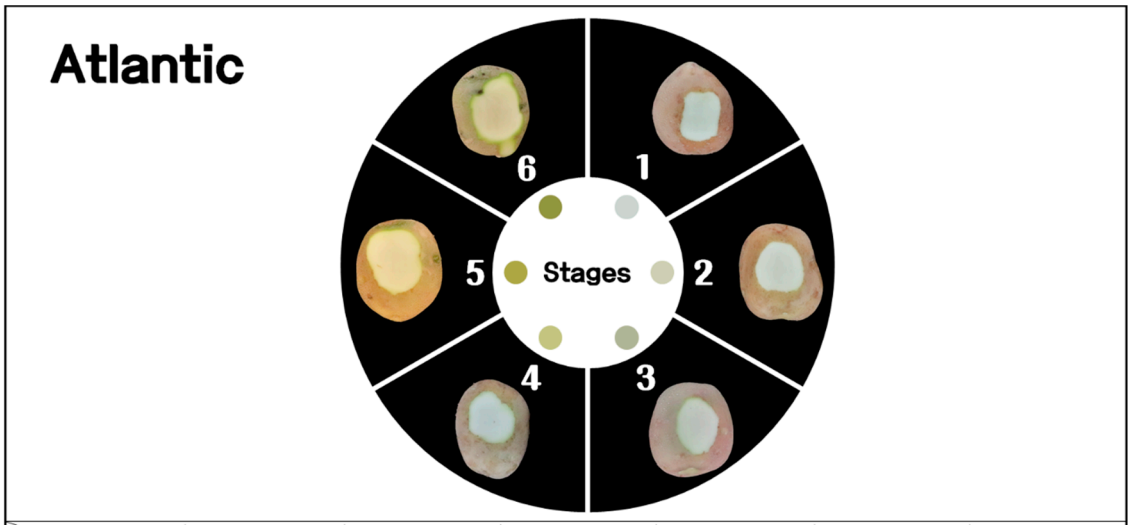


Figure 2. Chlorophyll content of 'Atlantic' and 'Trent' potato cultivars under six greening stages during storage at room conditions (22 °C) with a 12-h shift of light-dark cycles. The bars with different letters indicate a significant difference ($p < 0.05$) between the greening stages. The vertical bars represent the standard error of the mean (n = 10).

3.2. Chls and GAs

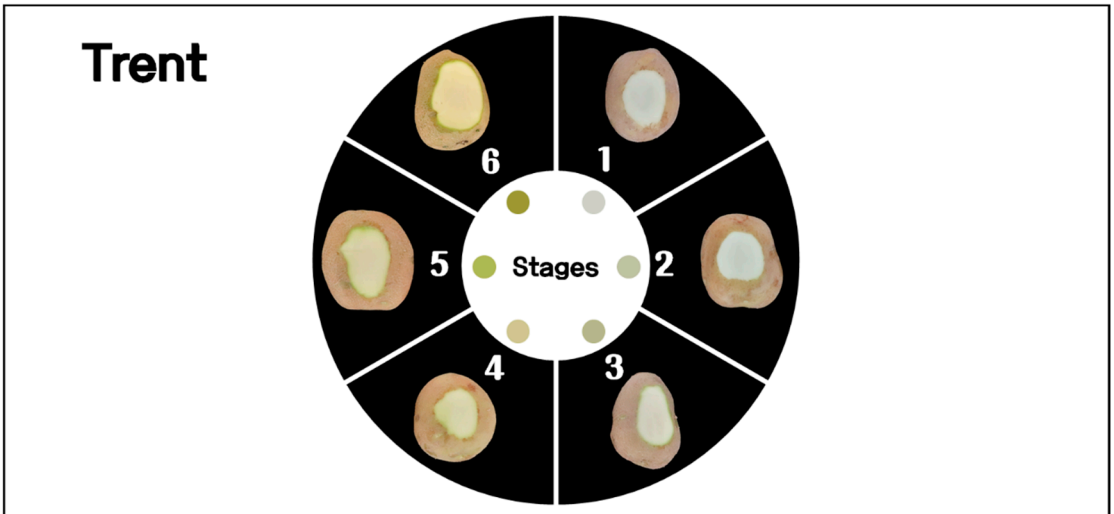
Exposure of potato tubers to light causes greening due to the formation of Chls and accumulation of toxic GAs [10,11]. The results of this study have revealed that Chl and GA content were significantly different among the six greening stages for both 'Atlantic' and 'Trent' potato cultivars. An increasing trend in total Chl and total GA content was observed as the greening stages went from stage 1 to 6 for both cultivars (Figures 1 and 2). Similar results were reported by Percival [32], in which Chl and total GA concentrations steadily increased in three potato cultivars in response to light over time. Okamoto et al. [33] have also shown the dependence of tuber Chl and total GA accumulation on light and confirmed the major role of light in both greening and GAs synthesis. They also observed no Chls under the absence of light, while there was an increase in accumulation of Chls under high light intensity. In the present study, the highest Chl a, Chl b, and total Chl content were recorded on the sixth stage for both 'Atlantic' and 'Trent' cultivars (Figure 1).

The total Chls increased from 35.71 and 33.57 mg g^{-1} in the first stage to 249.86 and 326.86 mg g^{-1} in the sixth stage for 'Atlantic' and 'Trent' cultivars, respectively. Concurrently, the total glycoalkaloids increased from 67.52 and 115.63 mg kg^{-1} in the first stage to 296.9 and 225.96 mg kg^{-1} in the sixth stage for 'Atlantic' and 'Trent' cultivars, respectively. The safe level of total glycoalkaloid content accepted for consumption (200 mg kg^{-1}) was recorded at stage 1–5 for 'Atlantic' and stage 1–4 for 'Trent'. 'Atlantic' accumulated the toxic level of glycoalkaloids at stage 6 and 'Trent' at stages 5 and 6. Therefore, it is safe to consume the potato tubers of both cultivars up to greening stage 4 (Figures 3 and 4).



Parameters \ Stages	1	2	3	4	5	6
a*	1.83	0.99	-1.23	-1.90	-2.20	-2.89
a* / b*	0.070	0.039	-0.049	-0.076	-0.093	-0.129
Total Chls	35.71	48.74	63.50	71.61	130.90	249.86
Total GAs	67.52	92.79	103.72	113.04	165.70	296.90

Figure 3. Index for the estimation of total glycoalkaloids (Gas) of 'Atlantic' potato cultivar at six greening stages based on color values and chlorophyll (Chl) content.



Parameters \ Stages	1	2	3	4	5	6
a*	2.10	1.01	0.08	-0.55	-0.28	-3.78
a* / b*	0.072	0.036	0.003	-0.020	-0.011	-0.166
Total Chls	33.57	48.30	66.26	92.51	204.37	326.86
Total GAs	115.63	147.72	175.52	192.76	205.38	225.96

Figure 4. Index for the estimation of total GAs of 'Trent' potato cultivar at six greening stages based on color values and Chl content.

3.3. Correlation of the Evaluated Parameters and Indices for the Estimation of Total GAs

The correlation result between all the color values vs. the total GAs showed a significant inverse relationship, except for the relationship between Hunter b* and total GAs (Table 2). The Hunter L* color values and total GA correlation (−0.62) indicated a decrease in brightness as the total glycoalkaloid content increased. Correspondingly, the significant correlation (−0.65) between the Hunter a* value and total GA content showed an increase in the greening of the tubers as the glycoalkaloids accumulation increased. The significant correlation (−0.67) between the Hunter a*/b* ratio and total GA content implies that a*/b* ratio could be a good indicator of GA content. In agreement with our results, the previous studies by Arias et al. [34], Helyes et al. [35], and Tilahun et al. [30] reported the Hunter a* and a*/b* ratio as good indicators of lycopene content (redness) in tomatoes. Conversely, the correlation between Chl a, Chl b, and total Chl vs. total GA in the current study showed a highly significant positive relationship, indicating an increase in total GA content as the total Chl content increased (Table 2). Total Chl content showed the highest (r = 0.84) correlation to the total GA contents. This finding agrees with Spoladore et al. [36], who reported a strong positive relationship between Chl and GA content in potato tubers. In summary, the overall results of the evaluated parameters indicated that the Hunter a* values, a*/b* ratio, and Chl content could be used as the key indicators of the total GA contents. Hence, we used the above parameters to develop indices for the estimation of total GA content, as shown in Figures 3 and 4.

Table 2. Correlation between color values, Chls, and GA contents of 'Atlantic' and 'Trent' potato cultivars under six greening stages during storage at room conditions (22 °C) with a 12-h shift of light-dark cycles.

	Hunter L*	Hunter a*	Hunter b*	a*/b*	Chl a	Chl b	Total Chl	α-solanine	α-chaconine	Total GA
Hunter L*	1	0.89 ***	0.73 **	0.90 ***	−0.78 **	−0.79 **	−0.80 **	-0.16^{ns}	-0.55^{ns}	−0.62 *
Hunter a*		1	0.82 ***	0.99 ***	−0.78 **	−0.78 **	−0.82 ***	-0.05^{ns}	−0.67 *	−0.65 **
Hunter b*			1	0.83 ***	−0.67 *	−0.67 *	−0.69 *	0.35^{ns}	−0.71 **	-0.40^{ns}
a*/b*				1	−0.80 **	−0.80 **	−0.84 ***	-0.06^{ns}	−0.69 *	−0.67 **
Chl a					1	0.99 ***	0.99 ***	0.39^{ns}	0.60 *	0.82 **
Chl b						1	0.99 ***	0.39^{ns}	0.59 *	0.81 **
Total Chl							1	0.38^{ns}	0.63 *	0.84 ***
α−solanine								1	-0.25^{ns}	0.47^{ns}
α−chaconine									1	0.74^{**}
Total GA										1

ns, *, **, and *** indicate non-significant and significant differences at $p < 0.05$, 0.01, and 0.001, respectively.

4. Conclusions

The current study has tried to develop simple indices to detect the toxic level of GAs in potato tubers using surface color and Chl development. Potato tubers from 'Atlantic' and 'Trent' cultivars were used to determine the greening stages at which toxic levels of GAs can be accumulated. The greening, Chls, and GA contents significantly increased for the two cultivars as the stage proceeded. The toxic level of GAs was accumulated at the late greening stages of the tubers, accompanied by the highest Chl content. Taken together, indices were developed based on surface color and Chl content for estimation of the safe GA levels for the consumption of the two commercially and commonly used potato cultivars. The developed indices can be used easily to avoid the potential toxicity of GAs to human beings. Moreover, the developed indices could be used as basic information to adapt it to other potato cultivars.

Author Contributions: Conceptualization, S.T., H.S.A., and C.S.J.; methodology, S.T. and H.S.A.; execution of experiment, S.T., M.W.B., and H.C.L.; software, S.T. and T.S.; formal analysis, S.T., T.S., and H.R.C.; resources, C.S.J.; original draft preparation, T.S., H.S.A., and H.R.C.; review and editing S.T. and T.S.; supervision, C.S.J.; project administration, M.W.B.; funding acquisition, C.S.J. All authors have read and agreed to the published version of the manuscript.

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