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STUDY TITLE

Compositional Assessment of Z6 Compared to Snowden

AUTHORS

[Personal information]

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PERFORMING LABORATORIES

Eurofins Food Integrity & Innovation, Madison, Wisconsin
Eurofins Food Integrity & Innovation, Greenfield, Indiana

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QUALITY CONTROL STATEMENT

This report was reviewed to assure that it accurately reflects the raw data of this study. The raw data were audited for compliance with the protocol, study notebook, and Standard Operating Procedures where applicable.

Signed	1/8/2020
[Personal information] Quality Control Reviewer	Date

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CERTIFICATION PAGE

I, the undersigned, declare that, to the best of my knowledge, this report provides an accurate evaluation of data in this study.

Signed	Jan 8, 2020
[Personal information] Regulatory Affairs Manager	Date

Signed	Jan 9, 2020
[Personal information] Agronomy Scientist and Statistician	Date

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SUMMARY

Objectives: To evaluate the nutritional composition and trait efficacy of Z6 compared with its control, Snowden, and other conventional potato varieties.

Methods: Z6 and Snowden potato varieties were used in the study.

Field trials were conducted at a total of four sites in potato growing regions of the United States during the 2018 growing season. The field trials were established in a randomized complete block (RCB) design, with four replicates at each site. Harvested tubers of Z6 and Snowden were assessed for analytes (proximates, vitamins, minerals, amino acids, and glycoalkaloids) important to potato nutrition in accordance with OECD guidelines (2002) as well as those related specifically to trait efficacy (free asparagine, reducing sugars) and benefit (acrylamide).

Analytical testing was completed by Eurofins Food Integrity & Innovation, using Eurofins standard analytical methods. Fresh tubers were analyzed after harvest and storage. Samples were processed by grinding six whole tubers, including peel, in liquid nitrogen. Chip samples were prepared by Simplot for acrylamide testing and shipped to Eurofins Laboratories.

Statistical analysis was conducted on data using SAS 9.3 using a linear mixed model. A significant difference was established with a p-value <0.05. Analyte ranges found in the literature were used for compositional data to represent the natural variability among potatoes.

Results: Statistically significant differences between Z6 and Snowden were seen for carbohydrates, calories, moisture, Vitamin B3, Vitamin C, and 16 total amino acids. However, mean values for these analytes were within the combined literature range.

The glycoalkaloid mean concentrations in Z6 and Snowden were not significantly different, fell within the combined literature range, and were lower than the safety limit (20 mg/100 g fresh weight).

The efficacy assessment evaluating free amino acids and reducing sugars in fresh tubers, and acrylamide concentrations in chips, demonstrated that Z6 has significantly lower levels of free asparagine, reducing sugars, and acrylamide than Snowden and Snowden chip products.

Conclusion: Z6 is compositionally equivalent to conventional potatoes, has lower levels of free asparagine and reducing sugars, and lower acrylamide potential than Snowden.

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INTRODUCTION

Z6 was generated by transforming the Snowden variety with plasmids pSIM1278 and pSIM1678 using *Agrobacterium*. Traits conferred by the genetic elements of the inserts are protection against late blight, reduced black spot, lower free asparagine, and lower reducing sugars. Lower acrylamide potential in cooked potatoes is attributed to the decreased levels of free asparagine and reducing sugars. In this assessment of nutritional composition and trait efficacy, Z6 was compared with the Snowden control and conventional potato varieties.

STUDY OBJECTIVES

The objectives of this study were to evaluate the nutritional composition and determine trait efficacy of Z6 with respect to free asparagine and reducing sugars compared with its control, Snowden, and other conventional potato varieties.

STUDY DATES

Fresh tubers were analyzed immediately after harvest in the fall of 2018. Stored tubers were analyzed after six months storage.

PERFORMING LABORATORIES

Eurofins Food Integrity & Innovation, Madison, Wisconsin
Eurofins Food Integrity & Innovation, Greenfield, Indiana

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METHODS AND MATERIALS

Selection of Control

For Z6, the most relevant comparator is Snowden. The only difference between Z6 and Snowden is that Z6 is the product of two successive transformations and contains inserts from pSIM1278 and pSIM1678.

Field Trials

Field trials were conducted for the purpose of phenotypic and agronomic assessment and to provide tuber samples for compositional analysis. During 2018, Z6 and Snowden were grown at four locations in potato growing regions of the United States (Table 1). Field-grown tuber (G1) seed-pieces were used as planting material at all sites.

Table 1. Field Trial Locations and Study Design for Z6 and Snowden

Year	Site Code	USDA Notification #	State	County	Trial Design[1]	Rows x Seed Pieces per Row[2]
2018	ID-ROSW	18-066-101n	Idaho	Canyon	RCB, 4 Reps	4x20
2018	ID-IDAH	18-066-101n	Idaho	Bonneville	RCB, 4 Reps	4x20
2018	MI-MONT	18-066-101n	Michigan	Montcalm	RCB, 4 Reps	4x20
2018	WI-HANC	18-066-101n	Wisconsin	Waushara	RCB, 4 Reps	4x20

[1]RCB= Randomized complete block.
[2]All material planted was field-grown tubers.

The agronomic practices and pest control measures used were location-specific and typical for all aspects of potato cultivation and included soil preparation, fertilizer application, irrigation, and pesticide application.

The field trials were established in a randomized complete block (RCB) design. The treatments included Z6 and Snowden. Every block (replicate) included a plot of each treatment. The experimental unit was the plot. All plots within each block were independently randomized. Each plot contained four rows. Rows were approximately 20 ft long and the typical seed spacing was one tuber every 10-12 in. The seed tubers were placed by hand or machine to a depth of approximately 6 in.

Each sample is representative of six randomly selected tubers from each replicate at each site (four replicates per site). Because there were four sites used for the field trial, a total of 16 samples were collected for analysis.

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Post-harvest Analysis

Testing Facility. Analytical testing was completed by Eurofins Food Integrity & Innovation. Acrylamide testing was conducted in Greenfield, Indiana. All other analysis occurred in Madison, Wisconsin.

Storage Conditions and Transportation. Fresh tubers from Z6 and Snowden were harvested from the field trial sites and transported to Eurofins Laboratories in Madison, Wisconsin for analytical testing. Tubers from each site were also sent to the Simplot's storage facility in Caldwell, Idaho, where they were held in conditions typical for long-term potato storage at approximately 7 °C for a storage interval of six months.

After the predetermined storage interval, tubers were shipped at ambient temperatures to Eurofins Laboratories in Madison, Wisconsin for analytical testing. Upon receipt, tubers were held under appropriate conditions until processed. All tuber samples from a single site and timing were stored and analyzed in the same way.

Sample Preparation. At Eurofins Laboratories, tuber samples were processed by grinding all tubers together with liquid nitrogen and homogenizing for a composite sample prior to being analyzed. For compositional analysis, a sample consisted of six whole tubers, including the peel.

Chip samples from two sites (Canyon County, ID; and Montacalm County, MI) were prepared for acrylamide testing, frozen, and shipped on dry ice to Eurofins Laboratories in Greenfield, Indiana. These samples were stored in a freezer set at -20±10 °C until analyzed. All chip samples from a single site and timing were stored and analyzed in the same way. Chips were made from fresh tuber samples (not stored) for acrylamide testing.

Analytical Methods

Acrylamide (ACMS). The sample was extracted with water and cleaned by solid phase extraction (SPE). Acrylamide was determined using 13C3-labeled acrylamide as an internal standard. Ions monitored for acrylamide were m/z 55, 44, and 27 and for the internal standard m/z 58. The ratio of peak areas for m/z 55 (acrylamide) and m/z 58 (internal standard) were compared to those for standards over the standard curve range. The results were reported on a fresh weight basis. The limit of quantitation for this study was 10.0 ppb (Musser, 2003; Scheuerell et al., 2002).

Ash (ASHM). The sample was placed in an electric furnace at 550 °C and ignited. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.100% (Method 923.03. AOAC, 2005).

Calories (CALC). Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation.

Calories (Kcal/100 g) = (4 × % protein) + (9 × % fat) + (4 × % carbohydrates)

The limit of quantitation was calculated as 2.00 Kcal/100 g on a fresh weight basis (CFR, 2015).

Carbohydrate (CHO). The total carbohydrate level was calculated by difference using the fresh weight derived data and the following equation.

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% carbohydrates = 100% - (% protein + % fat + % moisture + % ash)

The limit of quantitation for this study was 0.100 % on a fresh weight basis (Merrill and Watt, 1973).

Crude Fiber (CFIB). Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.100% (Method 962.09. AOAC, 2010).

Glycoalkaloids (COID). Glycoalkaloids were extracted from fresh tuber tissue with dilute acetic acid. The extract was concentrated and purified on a disposable solid phase extraction cartridge. Final separation and measurement of α-solanine and α-chaconine were performed by reverse-phase liquid chromatography with ultraviolet detection at 202 nm. The results were reported on a fresh weight basis. The limit of quantitation was 5.00 mg/100 g (Method 997.13. AOAC, 2005).

Free Amino Acid Profile (FAALC). Amino acids were extracted into 0.1N HCl. Samples were deproteinated with molecular weight exclusion filtration. The samples were analyzed by high performance liquid chromatography (HPLC) after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde and the secondary amino acids were derivatized with fluorenylmethyl chloroformate. The results were reported on a fresh weight basis. The limit of quantitation for this study was 10.0 mg/100 g (Henderson et al., 2000; Schuster, 1988).

Fat by Acid Hydrolysis (FAT_AH). The sample was hydrolyzed with hydrochloric acid at an elevated temperature range of 75 to 85 °C. The fat was extracted with ether and hexane. The extract was evaporated on a steam bath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again on a steam bath under nitrogen, dried, and weighed. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.100% (Methods 922.06 and 954.02. AOAC, 2005).

Mineral Analysis by ICP Emission Spectrometry (ICP). The samples were dried, precharred, and ashed overnight in a muffle furnace set to maintain 500 °C. The ashed samples were re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown samples, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions. The results were reported on a fresh weight basis (Methods 984.27 and 985.01. AOAC, 2005). The limits of quantitation were calculated on a fresh weight basis.

Moisture (M100T100). The samples were dried in a vacuum oven at approximately 100 °C. The moisture weight loss was determined and converted to percent moisture. The results were reported on a fresh weight basis. The limit of quantitation was calculated as 0.100% (Methods 926.08 and 925.09. AOAC, 2008).

Protein (PGEN). The protein and other organic nitrogen in the samples were converted to ammonia by digesting the samples with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. Instrumentation was used to automate the digestion, distillation, and titration processes. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.100% (Method Ac 4-91 AOCS, 2011).

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Sugars (LSUG). Sugars in the sample were extracted with a mixture of equal parts water and methanol. Aliquots were taken, dried under inert gas, and then reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucopyranoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoroacetic acid (TFA) as a catalyst, and analyzed by gas chromatography (GC) using a flame ionization detector. The results were reported on a fresh weight basis. The limit of quantitation for this study was 1.20 mg/100 g (Brobst, 1972; Mason and Slover, 1971).

Total Amino Acids (TAALC/TRPLC). All tuber samples were assayed for the total amino acids listed in Table 4. The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 h at approximately 106 to 118 °C. The acid hydrolysis step converts asparagine and glutamine to aspartic acid and glutamic acid, respectively. Phenol was added to the 6N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. Tryptophan was hydrolyzed by heating at approximately 110 °C in 4.2 N sodium hydroxide for approximately 20 h. The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acid (proline) was derivatized with fluorenylmethyl chloroformate (FMOC) before injection. The results were reported on a fresh weight basis. The limit of quantitation for this study was 10.0 mg/100 g (Method 988.15. AOAC, 2006; Barkholt and Jensen, 1989; Henderson and Brooks, 2010; Henderson et al., 2000; Schuster, 1988)

Vitamin B3 (Niacin) (NIAP). The sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interference. The amount of vitamin B3 was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum*, with the growth response of a niacin standard. This response was measured turbidimetrically. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.0300 mg/100 g (Methods 944.13 and 960.46. AOAC, 2005).

Vitamin B6 (Pyridoxine Hydrochloride) (B6A). The sample was hydrolyzed with dilute sulfuric acid in the autoclave and the pH was adjusted to remove interferences. The amount of vitamin B6 was determined by comparing the growth response of the sample, using the yeast *Saccharomyces cerevisiae*, with the growth response of a pyridoxine standard. The response was measured turbidimetrically. Results were reported as pyridoxine hydrochloride. The results were reported on a fresh weight basis. The limit of quantitation for this study was 0.00700 mg/100 g (Method 961.15 AOAC, 2005; Atkin et al., 1943).

Vitamin C (VCF). The vitamin C in the samples was extracted, oxidized, and mixed with o-phenylenediamine to produce a fluorophor whose fluorescence was proportional to the concentration. In addition, a blank was prepared with an aliquot of each sample extract where development of the fluorescence compound with the vitamin was prevented by forming a boric acid-dehydroascorbic acid complex prior to addition of the o-phenylenediamine solution. Results were calculated using a standard curve. The results were reported on a fresh weight basis. The limit of quantitation was 1.00 mg/100 g (Method 967.22. AOAC, 2005).

Statistical Analysis. All attributes were analyzed using SAS 9.3 (SAS Institute, Cary, NC) by combining data from multiple locations using the following linear mixed model.

$$Y_{ijkl} = \alpha_i + \beta_j + \gamma_{k(j)} + (\alpha\ \beta)_{ijl} + \varepsilon_{ijkl}$$

α = mean of treatment (fixed)

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β = effect of site (random)
γ = rep[site] (random)
ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), β_j denotes the effect of the j^{th} site (random effect), $\gamma_{k(j)}\gamma_{k(i)}$ denotes the random rep effect (within site), $(\alpha\ \beta)_{ijl}$ denotes the interaction between the i^{th} treatment and random j^{th} site effect, and ε_{ijkl} denotes the residual random error.

For the by-site analyses, data from each location were analyzed using the following linear mixed model.

$Y_{ij} = \alpha_i + \gamma_j + \varepsilon_{ijk}$

α = mean of treatment (fixed)
γ = effect of rep (random)
ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), γ_j is the random rep effect, and ε_{ijk} denotes the residual random error.

Means shown in the data tables in the results section were taken from the statistical analysis output and are least square means. Least square means are the same as means when no data are missing. When data are missing, least square means are a statistical estimate of the mean based on the available data.

A significant difference was established with a p-value < 0.05. Every effort was made to generate p-values to aid in the interpretation of the data. Some departures from the assumptions of normality and equal variances were allowed since the results were always interpreted in the context of variation observed in the conventional varieties.

A step-wise approach was used to interpret any differences between Z6 and Snowden. First, statistical significance ($p < 0.05$) was determined for each attribute. If the p-value indicated no statistical significance, then Z6 was considered equivalent to Snowden. Next, if the p-value indicated statistical significance, mean values were compared with the combined literature range. If the means were within the combined literature range, they were considered within the normal range for potatoes.

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RESULTS

A summary of analytes tested can be found in Table 2.

These analytes were selected by considering the important nutritional components of potatoes (OECD, 2002), the analytes expected to be altered based on the inserted DNA, and those analytes considered important in the potato industry. By-site data tables can be found in APPENDIX A.

Table 2. Tuber Composition Analytes Measured

Proximates and Fiber (7)		
Protein	Fat	Ash
Crude Fiber	Carbohydrates	Calories
Moisture		
Vitamins (3)		
Vitamin B3	Vitamin B6	Vitamin C
Minerals (3)		
Copper	Magnesium	Potassium
Total Amino Acids (18)		
Alanine	Histidine	Proline
Arginine	Isoleucine	Serine
Aspartic Acid + Asparagine	Leucine	Threonine
Cystine (including cysteine)	Lysine	Tryptophan
Glutamic Acid + Glutamine	Methionine	Tyrosine
Glycine	Phenylalanine	Valine
Free Amino Acids (4)		
Asparagine	Aspartic Acid	Glutamic Acid
Glutamine		
Sugars (2)		
Fructose + Glucose[1]	Sucrose[1]	
Anti-Nutrients (1)		
Glycoalkaloids		
Fried Product Assessment (1)		
Acrylamide[2]		

[1]Analyzed in fresh tissue.
[2]Analyzed in processed materials from fresh tissue.

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NUTRITIONAL ANALYSIS RESULTS FOR SNOWDEN AND Z6

These analyses were conducted to confirm that composition of Z6 remained within the normal levels for potato when compared to Snowden, and conventional potatoes. The compositional assessments determined the following concentrations.

1. Proximates, vitamins, and minerals (Table 3)
2. Total amino acids (Table 4)
3. Glycoalkaloids (Table 5)

Proximates, Vitamins, and Minerals

Statistically significant differences between Z6 and Snowden were observed for carbohydrates, calories, moisture, Vitamin B3, and Vitamin C (Table 3). However, mean values for each of these analytes were within the combined literature range. These results indicate that Z6 was equivalent to conventional potatoes. The remainder of the analytes presented in Table 3 showed no statistically significant difference between Z6 and Snowden.

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Table 3. Proximates, Vitamins, and Minerals in Tubers of Z6 and Snowden

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Combined Literature Range[2]	
						Min	Max	Min	Max
Protein (%)	Z6	2.33	0.7562	0.174	16	2.11	2.73	0.7	4.6
	Snowden	2.31		0.149	16	2.08	2.61		
Total Fat (%)	Z6	0.158	0.1983	0.102	16	0.100	0.370	0.02	0.74
	Snowden	0.178		0.118	16	0.100	0.420		
Ash (%)	Z6	0.931	0.4871	0.0900	16	0.792	1.10	0.15	2.0
	Snowden	0.905		0.158	16	0.462	1.11		
Crude Fiber (%)	Z6	0.609	0.8192	0.0990	16	0.453	0.870	0.17	3.5
	Snowden	0.603		0.105	16	0.425	0.780		
Carbohydrates (%)	Z6	19.3	**0.0002**	2.41	16	16.2	23.3	3.68	29.4
	Snowden	18.1		2.56	16	14.5	24.5		
Calories (kcal/100 g)	Z6	87.4	**0.0006**	9.49	16	73.9	103	22.4	110
	Snowden	83.2		9.97	16	68.5	107		
Moisture (%)	Z6	77.4	**0.0003**	2.37	16	73.4	80.6	71.8	86.0
	Snowden	78.5		2.50	16	72.4	82.1		
Vitamin B3 (mg/100 g)	Z6	1.58	**0.0071**	0.243	16	1.28	2.05	0.88	3.43
	Snowden	1.46		0.275	16	1.15	2.00		
Vitamin B6 (mg/100 g)	Z6	0.142	0.0605	0.0130	16	0.121	0.160	0.065	0.204
	Snowden	0.133		0.0110	16	0.105	0.150		
Vitamin C (mg/100 g)	Z6	26.7	**0.0395**	3.03	16	21.5	31.2	6.97	51.4
	Snowden	24.8		3.01	16	19.7	30.1		
Copper (mg/100 g)	Z6	0.113	0.2273	0.128	16	0.0320	0.570	0.04	2.05
	Snowden	0.0831		0.0440	16	0.0250	0.160		
Magnesium (mg/100 g)	Z6	23.8	0.0809	2.10	16	20.1	28.2	14.6	40.6
	Snowden	22.6		2.20	16	20.0	29.4		
Potassium (mg/100 g)	Z6	479	0.1082	34.0	16	405	527	291	765
	Snowden	461		22.7	16	409	492		

[1]P-values indicating significant differences are underlined and in bold.
[2]Combined literature ranges are from ILSI, 2019 and OECD, 2002.

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Total Amino Acids

Significantly lower aspartic acid + asparagine and significantly higher glutamic acid + glutamine was noted between Z6 and Snowden. These results were expected because of the down regulation of asparagine synthetase in Z6. The means for these analytes in Z6 were within the CLR.

A significant difference between Z6 and Snowden was also noted for all other total amino acids, with the exception of histidine and tryptophan (Table 4). In all cases, the mean for Z6 was within the CLR, so Z6 was considered equivalent to conventional potatoes.

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Table 4. Total Amino Acids in Tubers of Z6 and Snowden

Variable	Variety	Mean (mg/100 g)	P-Value[1]	Standard Deviation	N	Range (mg/100 g)		Combined Literature Range (mg/100 g)[2]	
						Min	Max	Min	Max
Alanine	Z6	74.8	**0.0007**	8.4	16	61.6	89.6	10.0	145
	Snowden	62.5		5.13	16	54.8	69.7		
Arginine	Z6	147	**0.0015**	17.4	16	124	192	46.2	234
	Snowden	127		11.4	16	114	153		
Aspartic Acid + Asparagine	Z6	308	**<.0001**	27.4	16	270	364	177	1548
	Snowden	502		50.0	16	408	594		
Cystine[3]	Z6	33.8	**0.0003**	3.97	16	26.6	38.9	10.0	41.6
	Snowden	27.6		3.71	16	20.5	33.3		
Glutamic Acid + Glutamine	Z6	510	**<.0001**	42.4	16	426	584	152	956
	Snowden	369		27.8	16	326	429		
Glycine	Z6	79.2	**<.0001**	8.63	16	67.8	91.9	30.7	372
	Snowden	66.1		5.94	16	57.4	76.2		
Histidine	Z6	38.9	0.0658	4.93	16	32.8	50.0	10.0	105
	Snowden	35.2		4.33	16	29.1	43.7		
Isoleucine	Z6	86.0	**0.0047**	9.53	16	73.1	103	21.3	137
	Snowden	74.7		6.43	16	65.4	85.4		
Leucine	Z6	148	**0.0004**	18.9	16	124	181	53.0	224
	Snowden	120		11.2	16	104	140		
Lysine	Z6	124	**0.0033**	15.2	16	103	147	44.4	495
	Snowden	107		9.38	16	92.9	120		
Methionine	Z6	39.5	**0.0009**	3.47	16	34.2	46.7	10.0	83.6
	Snowden	34.7		2.55	16	30.3	39.4		
Phenylalanine	Z6	101	**0.0065**	11.2	16	85.7	121	41.4	131
	Snowden	90.3		7.51	16	78.4	104		
Proline	Z6	80.9	**0.0005**	8.55	16	70.4	98.7	31.9	232
	Snowden	67.8		6.79	16	58.2	79.2		
Serine	Z6	87.9	**0.0007**	10.5	16	74.1	104	10.0	140
	Snowden	75.7		7.00	16	66	86.9		
Threonine	Z6	94.2	**0.0028**	10.6	16	80.2	111	19.8	133
	Snowden	79.2		7.63	16	69	90.9		
Tryptophan	Z6	23.1	0.0582	1.97	16	19.7	25.9	10.0	32.1
	Snowden	22.0		1.72	16	18.7	24.8		
Tyrosine	Z6	89.4	**0.0008**	10.3	16	76	109	27.5	237
	Snowden	73.7		6.34	16	63.5	83.1		
Valine	Z6	109	**0.0166**	9.85	16	97.7	131	24.6	259
	Snowden	99.0		9.86	16	86.5	122		

[1]P-values indicating significant differences are underlined and in bold.
[2]Combined literature ranges are from ILSI, 2019.
[3] Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid.

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Glycoalkaloids

Glycoalkaloids are toxins commonly found in solanaceous crops, including potato and 95% of the total glycoalkaloids in potato tubers consists of α-solanine and α-chaconine (OECD, 2002). The safety limit for total glycoalkaloids in tubers is 20 mg/100 g fresh weight (Smith et al., 1996).

The mean concentration of glycoalkaloids was significantly different between Z6 and Snowden. However, in both Z6 and Snowden, the mean concentrations were lower than the safety limit, and fell within the combined literature range (Table 5).

Table 5. Glycoalkaloids in Tubers of Z6 and Snowden

Variable	Variety	Mean (mg/100 g)	P-Value[1]	Standard Deviation	N	Range (mg/100 g)		Combined Literature Range (mg/100 g)[2]	
						Min	Max	Min	Max
Glycoalkaloids[3]	Z6	11.8	**0.0439**	3.16	16	7.26	19.0	3.20	210
	Snowden	13.9		3.70	16	9.08	23.5		

[1]P-values indicating significant differences are underlined and in bold.
[2]Combined literature ranges from Kozukue et al., 2008.
[3]Total of α-solanine and α-chaconine.

EFFICACY ANALYSIS RESULTS FOR SNOWDEN AND Z6

An assessment of Z6 for lower asparagine, lower reducing sugars, and reduced acrylamide potential consisted of the following analyses:

1. Free amino acids in tubers (Table 6)
2. Reducing sugars in tubers at harvest and after storage at 7 °C (Table 7)
3. Acrylamide in chips at harvest and after storage at 7 °C (Table 8)

Free Amino Acids

The results show that Z6 tubers contained significantly less free asparagine and significantly more free glutamine than Snowden tubers (Table 6). However, the mean concentrations of free asparagine and free glutamine for Z6 were within the combined literature range and therefore considered within the normal range for potatoes. Free amino acid analyses demonstrated that down regulation of asparagine synthetase was effective in reducing free asparagine in tubers.

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Table 6. Free Amino Acids in Tubers of Z6 and Snowden

Variable	Variety	Mean (mg/100 g)	P-Value[1]	Standard Deviation	N	Range (mg/100 g)		Combined Literature Range (mg/100 g)[2]	
						Min	Max	Min	Max
Asparagine	Z6	80.4	**<.0001**	14.8	16	55.4	104	31.4	456
	Snowden	309		44.1	16	237	397		
Aspartic Acid	Z6	45.1	0.5897	3.85	16	39	51.7	16.7	197
	Snowden	44.2		3.60	16	37.3	50.5		
Glutamic Acid	Z6	57.6	0.0610	8.28	16	44.6	71.7	12.5	136
	Snowden	54.5		7.83	16	44.1	68.7		
Glutamine	Z6	259	**<.0001**	40.1	16	186	314	33.6	411
	Snowden	162		22.1	16	118	193		

[1]P-values indicating significant differences are underlined and in bold.
[2]Combined literature ranges are from ILSI, 2019.

Reducing Sugars

Z6 showed significantly lower levels of reducing sugars, fructose and glucose, after six months of storage at 7 °C (Table 7). These results can be attributed to partial down regulation of R1 glucan water dikinase, and down regulation of phosphorylase L and vacuolar invertase. Down regulation of R1 glucan water dikinase and phosphorylase L slows the breakdown of starch into sugars in the amyloplast.

Sucrose levels were significantly higher in Z6 at harvest compared to Snowden (Table 7). This difference can be attributed to the down regulation of invertase, which slows the conversion of sucrose into fructose and glucose in the vacuole. However, mean sucrose content for Z6 was within the combined literature range for potatoes at harvest.

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Table 7. Sugars in Tubers of Z6 and Snowden at Harvest and after Storage at 7 °C

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Combined Literature Range[2]	
						Min	Max	Min	Max
Fructose and Glucose (mg/100 g)									
Fresh	Z6	6.76	0.0517	2.12	16	4.02	10.1	13.0	1,208
	Snowden	17.7		12.7	16	7.52	55.0		
6 Months Storage	Z6	6.32	**0.0299**	1.69	16	4.06	10.4	13.0	1,208
	Snowden	27.2		14.4	16	13.6	57.7		
Sucrose (mg/100 g)									
Fresh	Z6	133	**0.0036**	16.7	16	109	161	39.7	1,390
	Snowden	122		16.7	16	91.3	151		
6 Months Storage	Z6	137	0.8249	13.0	16	124	164	39.7	1,390
	Snowden	134		35.3	16	97.5	238		

[1]P-values indicating significant differences are underlined and in bold.
[2]Literature Ranges from Amrein et al., 2003 and Vivanti et al., 2006.

Acrylamide

At harvest, chips made with Z6 tubers contained 77.8% less acrylamide than chips made with Snowden (Table 8). Acrylamide concentrations in Z6 chips were significantly lower than Snowden chips at harvest and after six months storage. The significantly lower acrylamide levels were expected from down regulation of asparagine synthetase, R1 glucan water dikinase, phosphorylase L and vacuolar invertase, which reduced the free asparagine and reducing sugar reactants. Similar reductions in reducing sugars and acrylamide were reported by Zhu et al., 2014.

Table 8. Acrylamide in Chips from Z6 and Snowden at Harvest and after Storage at 7 °C

Variable	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
Fresh	Z6	334	**0.0168**	94.7	8	77.8	191	464
	Snowden	1,506		355	8		998	2,150
6 Months Storage	Z6	388	**<.0001**	95.0	8	74.1	302	571
	Snowden	1,593		366	8		1,160	2,130

[1]P-values indicating significant differences are underlined and in bold.

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CONCLUSION

A composition assessment was conducted on Z6 and Snowden. Two types of analytes were assessed:

- those important for potato nutrition;
- those related to trait efficacy.

The nutrition assessment evaluated proximates, vitamins, minerals, and total amino acids, and showed significant differences in carbohydrates, calories, moisture, Vitamin B3, Vitamin C, and 16 total amino acids. The glycoalkaloid mean concentrations in Z6 and Snowden were lower than the safety limit.

The efficacy assessment evaluated free amino acids and reducing sugars as well as acrylamide concentrations in chips. This demonstrated that Z6 has significantly lower levels of free asparagine and reducing sugars. Chips made out of Z6 tubers had significantly lower acrylamide than those made out of Snowden tubers.

All mean values for the nutrition assessment were within the combined literature range, which demonstrated that Z6 is compositionally equivalent to conventional potatoes and is as safe and nutritious for food and feed as potatoes that have a long history of safe consumption.

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APPENDIX A By-Site Results

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