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STUDY TITLE

Compositional Assessment of V11 Compared to Snowden

AUTHORS

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PERFORMING LABORATORIES

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STUDY NUMBERS

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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	8/30/16
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CERTIFICATION PAGE

This report is an accurate and complete representation of the study activities.

Signed	30 August 2016
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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

The purpose of this study was to evaluate the nutritional composition and trait efficacy of potato event V11 compared with its parental control, Snowden. Commercially available reference varieties with a history of safe use for food and feed were also grown as comparators. Field trials were conducted at a total of six sites during the 2012 and 2013 growing seasons. Plots of the test, control, and reference varieties were harvested, and tubers were assessed for those analytes important to potato nutrition as well as those related specifically to gene down-regulation and trait efficacy. The nutritional assessment evaluating proximates, vitamins, minerals, amino acids, and glycoalkaloids demonstrated that V11 is compositionally equivalent to Snowden. As expected, the efficacy assessment evaluating free amino acids and reducing sugars as well as acrylamide concentrations in chips demonstrated that V11 has lower levels of free asparagine, slightly lower levels of reducing sugars, and lower acrylamide potential than Snowden.

INTRODUCTION

Potato event V11 was generated by transforming the Snowden variety with plasmid pSIM1278 using *Agrobacterium* transformation. Traits conferred by the genetic elements of the insert are 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.

STUDY OBJECTIVES

The objectives of the study were to:

1. Compare the nutritional composition of V11 to the parental control and conventional potato varieties; and
2. Determine efficacy of V11 with respect to free asparagine, reducing sugars, and acrylamide.

KEY STUDY PERSONNEL

[personal information]

STUDY DATES

Field trials for tuber generation for compositional analysis were conducted during the 2012 and 2013 field seasons. Fresh tubers were analyzed immediately after harvest in the fall of 2012 and 2013. Stored tubers were analyzed after three, six, or nine months storage.

PERFORMING LABORATORIES

Covance Laboratories, Inc., Madison, Wisconsin
Covance Laboratories, Inc., Greenfield, Indiana

METHODS AND MATERIALS

Selection of Control and Reference Varieties

For V11, the most relevant comparator is Snowden, the parental variety. The only difference between V11 and Snowden is that V11 underwent transformation and contains a pSIM1278 insert.

Conventional non-transformed potato varieties with a history of safe use for food and feed were used as reference varieties. These varieties are commonly used in the chip, fry, dehydrated, and fresh markets. The following reference varieties were grown to provide a range of values common to conventional potatoes: Atlantic, Bintje, C0095051-7w, Gala, Golden Sunburst, Nicolet, Norkotah, Purple Majesty, Snowden, and TX278.

Field Trials

During the growing seasons of 2012 and 2013, V11 and its parental control were grown at a total of six locations in potato growing regions of the United States. Location, material planted, trial design, and row size are provided in Table 1.

In 2012, mini-tubers were used as planting material and in 2013, field-grown tuber 1 seed pieces were planted. In 2013, additional varieties were grown as references to provide a range of values common to conventional potatoes at a total of eight sites (Table 2).

The agronomic practices and pest control measures used were location-specific and were 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 the test, control, and reference varieties. References were included only in 2013 and not every reference variety was grown at every site. Every block (replicate) included a plot of each treatment. The experimental unit was the plot. All plots within each block were independently randomized so that the treatments were in random order.

In 2012, there were three replicates at each site and in 2013 there were four replicates. The increased number of replicates was a planned change and provided greater ability to detect statistically significant differences. Within each replicate, each potato variety was planted in plots arranged in random order. Each plot contained four rows. Rows were 20 feet long in all cases and the typical seed spacing was one tuber approximately every 12 inches. The seed tubers were placed by hand or machine to a depth appropriate for seed type and local agronomic practices.

Although some trials occurred in the same county over both years, they were not planted in the same location. Plots were in different fields, or in different locations on the farm due to crop rotation practices. Field conditions such as environment, field history, soil type, pest presence, and drainage can differ from year to year. Each county and year combination was considered a unique site. For each of the two trial years, plots were planted in the spring and harvested in the fall of the same year. Comparison of the effect of year was not part of the experimental design.

Table 1. Field Trial Location and Study Design for V11 and Control Snowden

Year	USDA Notification #	State	County	Trial Design[1]	Rows x Seed Pieces Per Row	Material Planted
2012[2]	11-356-101n	Florida	St. John's	RCB, 3 reps	4x20	Mini-tubers
2012	12-066-102n	Michigan	Montcalm	RCB, 3 reps	4x20	Mini-tubers
2013	13-074-121n	Wisconsin	Adams	RCB, 4 reps	4x20	Field Grown Tubers 1
2013	13-079-108n	Michigan	Montcalm	RCB, 4 reps	4x20	Field Grown Tubers 1
2013	13-072-112n	Washington	Grant	RCB, 4 reps	4x20	Field Grown Tubers 1
2013	13-072-112n	Pennsylvania	Berks	RCB, 4 reps	4x20	Field Grown Tubers 1

[1]RCB= Randomized Complete Block.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.

Table 2. Field Trial List for Reference Varieties

Year	State	County	Reference Varieties[1]
2012	Florida	St. John's	Snowden
2012	Michigan	Montcalm	Snowden
2013	Maine	Aroostook	Atlantic, Bintje, Golden Sunburst, Nicolet, TX278
2013	Oregon	Washington	Atlantic, Bintje, Golden Sunburst, Nicolet, TX278
2013	Wisconsin	Adams	Snowden, Gala, Purple Majesty, C0095051-7w, Norkotah
2013	Michigan	Montcalm	Snowden, Gala, Purple Majesty, C0095051-7w, Norkotah
2013	Washington	Grant	Snowden, Gala, Purple Majesty, C0095051-7w, Norkotah
2013	Pennsylvania	Berks	Snowden, Gala, Purple Majesty, C0095051-7w, Norkotah

[1]Because Snowden is both the control and a conventional potato variety with history of safe use as food and feed, it was used as both the control and as a reference in calculating tolerance intervals. The inclusion of Snowden in the tolerance interval did not impact the statistical analysis, since it was calculated separately from the statistical comparisons.

Test, control, and reference tubers for the compositional assessment were collected from the same 2012 and 2013 field trial locations listed in Table 1 and Table 2. Each combination of year, site, material, and replicate represents one sample of six tubers in the compositional assessment.

For sugar and acrylamide analysis, samples consisted of three tubers and were collected from the following locations.

- Samples analyzed at the fresh time points were from all 2012 and 2013 field trial locations
- Samples analyzed after three month storage were from all 2012 field trial locations
- Samples analyzed after six and nine month storage were from Montcalm County, Michigan 2012

Post-harvest Analysis

Testing Facility. Analytical testing was completed by Covance Laboratories, Inc. Acrylamide testing was conducted in Greenfield, Indiana. All other analyses occurred in Madison, Wisconsin.

Storage Conditions and Transportation. Fresh tubers from V11 and Snowden were harvested from the field trials sites and transported to Covance Laboratories in Madison, Wisconsin for analytical testing. Tubers from 2012 sites 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 10 °C for storage intervals of three, six, and nine months.

After the predetermined storage interval, tubers were shipped at ambient temperatures to Covance 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 Covance 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 were prepared for acrylamide testing, frozen, and shipped on dry ice to Covance 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 the following tuber samples for acrylamide testing.

- Fresh tubers (not stored)
- Tubers stored at 10 °C at intervals of three, six, and nine months

Acrylamide: The acrylamide levels were determined by Covance Laboratories in Greenfield, IN using the Covance protocol ACMS_GRN_S:4 (FDA, 2003).

Ash. Ash levels were determined by Covance Laboratories using Covance protocol ASHM_S:5 (Method 923.03. AOAC, 2005).

Calories. Total calories were determined by Covance Laboratories using Covance protocol CALC:4 (Merrill and Watt, 1973).

Carbohydrates. Carbohydrate levels were determined by Covance Laboratories using Covance protocol CHO:6 (Merrill and Watt, 1973).

Crude Fiber. Crude fiber was determined by Covance Laboratories using Covance protocol CFIB_S:2 (Method 962.09. (AOAC, 2010).

Elements by ICP Emission Spectrometry. The minerals Copper (Cu), Magnesium (Mg), and Potassium (K) were determined by Covance Laboratories using Covance protocol (ICP_S:13) (Method 984.27 and Method 985.01. AOAC, 2005).

Fat. Fat was determined by Acid Hydrolysis using Covance protocol FAAH_S:7(Method 922.06 and Method 954.02. AOAC, 2005).

Free Amino Acids. Free amino acid levels were determined by Covance Laboratories using the Covance protocol FAALC_S:6 (Barkholt and Jensen, 1989; Henderson et al., 2000; Schuster, 1988).

Glycoalkaloids. Glycoalkaloid levels were determined by Covance Laboratories using protocol COID_S:2 Method 997.13. AOAC, 2005).

Moisture Content. Moisture levels were determined by Covance Laboratories using Covance protocol M100_T100_S:4 (Method 925.09 and Method 926.08. (AOAC, 2008).

Protein. Protein was determined using the Kjeldahl method, approximating protein by multiplying Nitrogen by 6.25, as per Covance protocol PGEN_S:4 (Method 955.04 and Method 979.09. AOAC, 2005).

Sugars Analyzed in 2012 (SWET). Sugar levels were determined by Covance Laboratories using High Performance Anion Exchange Chromatograph (HPAEC) equipped with a Pulsed Amperometric Detector (PAD) and following Covance protocol SWET_S:9 (Lilla et al., 2005).

Sugars Analyzed in 2013 (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. Total amino acid levels were determined by Covance Laboratories using the Covance protocol TAALC_S:6 (Barkholt and Jensen, 1989; Henderson et al., 2000; Schuster, 1988).

Tryptophan. Tryptophan levels were determined by Covance Laboratories using the Covance protocol - TRPLC_S:3 (Method 988.15. AOAC, 2005).

Vitamin B3 (Niacin). Niacin was determined by Covance Laboratories using Covance protocol NIAP_S:11 (Method 944.13 and Method 960.46. AOAC, 2005).

Vitamin B6 (Pyridoxine): Pyridoxine was determined by Covance Laboratories using Covance protocol B6A_S:11 (Method 961.15. (AOAC, 2005; Atkin et al., 1943)

Vitamin C. Vitamin C levels were determined by Covance Laboratories using protocol VCF_S:5 (Method 967.22. AOAC, 2005).

Statistical Analysis. The statistical analysis was performed using SAS 9.3 (SAS Institute, Cary, North Carolina). All data were subjected to analysis of variance using the following linear mixed model.

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

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

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 differences were always interpreted in the context of variation observed in the conventional varieties.

The tolerance intervals were calculated using JMP 10.0 (SAS Institute, Cary, North Carolina) to contain, with 95% confidence, 99% of the values in the population. Tolerance intervals were used for compositional data to represent the natural variability among potatoes. The tolerance interval attempts to predict the range in which most values of a population will fall. Conventional potato varieties used in the tolerance interval are shown in Table 3 and include varieties suitable for fresh use, for frying, for chipping, and the V11 parental control, Snowden.

The parental control, Snowden, was used as a reference variety because of its widespread popularity and its history of safe use as food and feed. The inclusion of the control in the tolerance interval did not impact the statistical analysis because the tolerance interval was a separate calculation.

Table 3. Reference Variety Sample Size Used in Tolerance Interval

Variety	N Per Attribute
Atlantic	8
Bintje	8
C0095051-7W	16
Gala	16
Golden Sunburst	8
Nicolet	8
Norkotah	16
Purple Majesty	16
Snowden	21
TX278	8
Total N	125

A step-wise approach was used to interpret any differences between V11 and the control. First, statistical significance, $p < 0.05$, was determined for each attribute. If the p-value indicated no statistical significance, then V11 was considered equivalent to the control. Next, if the p-value indicated statistical significance, mean values were compared with the tolerance intervals and combined literature range. If the means were within either the tolerance interval or combine literature range, they were considered within the normal range for potatoes.

This tolerance interval and the combined range of values for each analyte available from the published literature were used to interpret the composition results. In interpreting the data, emphasis was placed on the analyte means; means that fell within the tolerance interval and/or combined literature range for the analyte were considered to be within the normal variability of commercial potato varieties.

RESULTS

A summary of analytes tested can be found in Table 4.

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. Tables 5 through 10 summarize data from across all sites and years. By-site data tables can be found in Appendix A.

Table 4. 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 and at selected monthly intervals.
[2]Analyzed in processed materials from fresh tissue and from tubers at selected monthly intervals.

NUTRITIONAL ANALYSIS RESULTS FOR SNOWDEN AND V11

These analyses were conducted to confirm that composition of V11 remained within the normal levels for potato when compared to its parental control, Snowden, and conventional potatoes. The compositional assessments determined the concentrations of the following.

1) Proximates, vitamins, and minerals (Table 5)
2) Total amino acids (Table 6)
3) Glycoalkaloids (Table 7)

Proximates, Vitamins, and Minerals

A statistically significant difference between V11 and the control was seen for vitamin C (Table 5). However, mean values for vitamin C were within the tolerance interval and combined literature range. These results indicate that V11 was equivalent to conventional potatoes. The remainder of the analytes presented in Table 5 showed no statistically significant difference between V11 and the control.

Table 5. Proximates, Vitamins, and Minerals in Tubers from V11 and Control Snowden

Compound	Variety	Mean	P-value[1]	N[2]	Standard Deviation	Range		Tolerance Interval[3]		Combined Literature Range[4]	
						Min	Max	Min	Max	Min	Max
Moisture (%)	V11	78.5	0.1064	22	1.89	76.0	83.0	71.7	87.0	63.2	86.9
	Control	79.2		21	1.83	76.3	83.2				
Protein (%)	V11	2.34	0.9048	22	0.259	1.99	2.91	0.830	3.48	0.700	4.60
	Control	2.33		21	0.240	2.01	2.82				
Fat (%)	V11	0.166	0.8899	22	0.0530	0.100	0.300	0.100	0.500	0.0200	0.200
	Control	0.162		21	0.0610	0.100	0.330				
Ash (%)	V11	1.03	0.6646	22	0.105	0.820	1.20	0.500	1.37	0.440	1.90
	Control	1.01		21	0.107	0.803	1.20				
Crude Fiber (%)	V11	0.475	0.3731	22	0.0860	0.340	0.630	0.197	0.830	0.170	3.50
	Control	0.503		21	0.102	0.353	0.700				
Carbohydrates (%)	V11	17.9	0.1296	22	1.87	13.5	20.5	9.30	25.4	13.3	30.5
	Control	17.3		21	1.81	13.4	20.4				
Total Calories (kcal/100 g)	V11	82.5	0.1161	22	7.70	64.0	93.2	48.8	111	80.0	110
	Control	79.9		21	7.29	64.2	92.1				
Vitamin B_3 (Niacin) (mg/100 g)	V11	2.19	0.0984	22	0.259	1.62	2.64	0.794	2.68	0.0900	3.10
	Control	2.05		21	0.201	1.68	2.32				
Vitamin B_6 (mg/100 g)	V11	0.110	0.9855	22	0.0110	0.0970	0.140	0.0640	0.190	0.110	0.340
	Control	0.110		21	0.0110	0.0960	0.140				
Vitamin C (mg/100 g)	V11	26.9	**0.0050**	22	2.45	22.1	32.0	12.1	34.4	1.00	54.0
	Control	24.1		21	4.10	15.2	30.4				
Copper (mg/100 g)	V11	0.0800	0.9679	22	0.0230	0.0500	0.120	0.0500	0.160	0.0200	0.700
	Control	0.0800		21	0.0240	0.0500	0.120				
Magnesium (mg/100 g)	V11	22.6	0.2320	22	3.77	17.9	31.0	11.3	31.0	11.3	55.0
	Control	21.8		21	3.51	17.4	29.4				
Potassium (mg/100 g)	V11	488	0.1021	22	43.0	426	605	240	587	350	625
	Control	473		21	39.2	405	557				

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.
[3]99% Tolerance Interval, 95% confidence.
[4]Literature ranges are from Horton and Anderson, 1992; Lisinska and Leszczynski, 1989; Rogan et al., 2000; Talburt et al., 1987).

Total Amino Acids

Significantly lower aspartic acid + asparagine and significantly higher glutamic acid + glutamine were noted between V11 and Snowden. These results were expected because of the down-regulation of the *Asn1* gene in V11.

Statistically significant differences between V11 and Snowden were also noted for alanine, arginine, cystine (including cysteine), glycine, isoleucine, leucine, serine, threonine, tyrosine, and valine (Table 6). In all cases, the mean values for V11 were within the tolerance interval and/or the combined literature range, and therefore considered equivalent to conventional potatoes.

Table 6. Total Amino Acids in Tubers from V11 and Control Snowden

Compound	Variety	Mean (mg/100 g)	P-value[1]	N[2]	Standard Deviation	Range		Tolerance Interval[3]		Combined Literature Range[4]	
						Min	Max	Min	Max	Min	Max
Alanine	V11	70.9	**0.0067**	22	5.62	60.4	82.9	22.4	105	39.2	95.2
	Control	64.2		21	4.99	56.7	76.1				
Arginine	V11	142	**0.0056**	22	29.4	109	204	15.8	188	70.0	138
	Control	123		21	21.6	89.4	169				
Aspartic Acid + Asparagine[5]	V11	300	**<.0001**	22	35.0	249	377	44.2	799	339	738
	Control	519		21	62.9	414	627				
Cystine	V11	30.2	**0.0221**	22	5.12	23.5	41.8	10.0	49.5	48.0	92.5
	Control	26.6		21	3.97	22.7	35.7				
Glutamic Acid + Glutamine[6]	V11	495	**<.0001**	22	79.3	327	653	128	581	292	604
	Control	350		21	44.4	283	428				
Glycine	V11	72.7	**0.0103**	22	7.89	59.3	89.3	10.0	110	1.00	97.5
	Control	65.4		21	6.67	56.8	81.7				
Histidine	V11	36.0	0.1944	22	5.74	30.1	49.1	11.5	52.5	13.3	46.9
	Control	34.3		21	5.14	27.5	45.7				
Isoleucine	V11	82.2	**0.0085**	22	9.05	67.7	101	20.0	123	52.5	95.3
	Control	75.5		21	8.37	63.8	94.5				
Leucine	V11	138	**0.0026**	22	13.0	114	167	10.0	225	68.5	138
	Control	124		21	11.5	109	153				
Lysine	V11	118	0.0534	22	11.4	99.8	143	36.6	173	68.7	137
	Control	111		21	8.76	102	132				
Methionine	V11	39.2	0.1648	22	4.03	31.8	46.6	11.3	59.7	9.00	128
	Control	36.9		21	3.52	30.2	42.9				
Phenylalanine	V11	96.6	0.0638	22	10.7	75.9	121	11.7	154	55.2	109
	Control	91.2		21	9.73	76.6	114				
Proline	V11	78.9	0.3559	22	16.1	55.8	111	10.0	155	35.5	146
	Control	72.3		21	13.7	51.9	95.3				
Serine	V11	82.7	**0.0049**	22	10.2	63.2	103	10.0	130	50.0	102
	Control	74.7		21	7.30	62.0	90.9				
Threonine	V11	85.6	**0.0027**	22	8.91	70.3	105	11.5	129	43.6	85.5
	Control	77.7		21	7.53	68.6	97.1				
Tryptophan	V11	20.9	0.2731	22	4.66	13.9	32.2	10.0	36.3	11.4	28.2
	Control	20.1		21	4.47	11.5	27.6				
Tyrosine	V11	85.9	**0.0020**	22	10.2	72.0	108	17.3	124	45.7	94.2
	Control	76.1		21	8.83	66.1	94.3				
Valine	V11	109	**0.0225**	22	13.0	90.0	133	43.3	159	75.2	145
	Control	102		21	12.2	82.6	123				

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site omitted because of an error at harvest.
[3]99% Tolerance Interval, 95% confidence. Negative values or values below the limit of detection, arising from variability measured in the samples, were adjusted to the limit of detection (10 mg/100 g).
[4]Combined literature ranges are from (OECD, 2002; Rogan et al., 2000; Talley et al., 1984).
[5,6]Reported as total aspartic acid plus asparagine and total glutamic acid plus glutamine. During analysis, an acid hydrolysis step converts asparagine to aspartic acid and glutamine to glutamic acid, respectively.

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 mean concentration of glycoalkaloids was not significantly different between V11 and the control. In both varieties the mean concentration was lower than the safety limit, and fell within the tolerance interval and the combined literature range (Table 7). The safety limit for total glycoalkaloids in tubers is 20 mg/100 g fresh weight (Smith et al., 1996).

Table 7. Glycoalkaloids in Tubers from V11 and Control Snowden

Compound	Variety	Mean (mg/100 g)	P-value[1]	N[2]	Standard Deviation	Range		Tolerance Interval[3]		Combined Literature Range[4]	
						Min	Max	Min	Max	Min	Max
Glycoalkaloids[5]	V11	9.70	0.3878	22	4.10	5.00	19.4	5.00	20.4	3.20	210.4
	Control	10.8		21	7.21	5.04	38.9				

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.
[3]99% Tolerance Interval, 95% confidence.
[4]Combined literature ranges from Kozukue et al., 2008.
[5]Total of α-solanine and α-chaconine.

EFFICACY ANALYSIS RESULTS FOR SNOWDEN AND V11

An assessment of V11 for low acrylamide potential and lowered reducing sugars consisted of the following analyses.

1) Free amino acids in tubers (Table 8)
2) Reducing sugars in tubers (Table 9)
3) Acrylamide in chips (Table 10)

Free Amino Acids

The free amino acid analysis demonstrated that down-regulation of *Asn1* was effective in reducing free asparagine in tubers. The results show that V11 tubers contained significantly less free asparagine and significantly more free glutamine than Snowden tubers (Table 8). However, the mean concentrations of free asparagine and free glutamine for V11 were within the tolerance interval and the combined literature range and therefore considered within the normal range for potatoes.

Table 8. Free Amino Acids in Tubers from V11 and Control Snowden

Compound	Variety	Mean (mg/100 g)	P-value[1]	N[2]	Standard Deviation	Range		Tolerance Interval[3]		Combined Literature Range[4]	
						Min	Max	Min	Max	Min	Max
Asparagine	V11	79.4	**<u><.0001</u>**	22	21.6	35.5	128	10.0	520	31.2	689
	Control	312		21	51.4	212	407				
Aspartic Acid	V11	53.7	0.3054	22	35.0	33.8	77.8	4.20	71.4	6.4	75.2
	Control	51.5		21	62.9	35.8	74.0				
Glutamine	V11	222	**<u><.0001</u>**	22	62.2	71.2	322	10.0	298	44	539[5]
	Control	125		21	36.0	55.9	181				
Glutamic Acid	V11	66.5	0.2872	22	13.5	37.9	90.2	4.40	96.4	45	74.2
	Control	61.8		21	11.5	41.9	78.4				

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.
[3]99% Tolerance Interval, 95% confidence. Negative values or values below the limit of detection, arising from variability measured in the samples, were adjusted to the limit of detection (100 mg/100 g).
[4]Combined literature ranges are from Davies et al., 1977; Lisinska and Leszczynski, 1989; Shepherd et al., 2010.
[5]A value of 1,824mg/100 g from a single site in the combined literature range was not included because it appeared to be an outlier.

Reducing Sugars

V11 showed a trend for lower levels of the reducing sugars, fructose and glucose, although the differences were not statistically significant (Table 9). V11 was designed to lower levels of reducing sugars in tubers by slowing the breakdown of starch into sugars in the amyloplast. Mean results for fructose plus glucose and sucrose in V11 were within the tolerance interval and the combined literature range for both fresh and stored conditions.

Table 9. Sugars in Tubers from V11 and Control Snowden at Harvest and After Storage at 10 °C

Timing	Variety	Mean	P-value[1]	N[2]	Standard Deviation	Range		Tolerance Interval[3]		Combined Literature Range[4]	
						Min	Max	Min	Max	Min	Max
Fructose + Glucose (mg/100 g)											
Fresh[5]	V11	26.7	0.7689	22	31.1	5.50	108	1.00	435	18	803
	Control	35.1		21	46.2	5.20	145				
Month 3[6]	V11	53.5	0.2127	6	74.2	11.5	204	1.00	435	18	803
	Control	151		5	137	26.7	319				
Month 6[7]	V11	39.4	0.9450	3	0.212	11.5	95.0	1.00	435	18	803
	Control	14.7		3	4.06	11.1	19.1				
Month 9[7]	V11	92.3	0.9970	3	9.93	80.9	99.1	1.00	435	18	803
	Control	105		3	20.2	84.2	125				
Sucrose (mg/100 g)											
Fresh[5]	V11	197	0.8569	22	90.5	114	424	1.00	443	39.7	1,390
	Control	194		21	97.4	116	432				
Month 3[6]	V11	147	0.4911	6	13.4	131	170	1.00	443	39.7	1,390
	Control	179		5	62.1	127	262				
Month 6[7]	V11	98.0	0.7371	3	62.0	55.0	169	1.00	443	39.7	1,390
	Control	74.2		3	10.4	62.4	82.1				
Month 9[7]	V11	171	0.9867	3	33.5	146	209	1.00	443	39.7	1,390
	Control	145		3	2.65	143	148				

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.
[3]99% Tolerance Interval, 95% confidence.
[4]Literature Ranges from Amrein et al., 2003; Vivanti et al., 2006.
[5]Samples analyzed at the fresh time points were from all 2012 and 2013 field trial locations.
[6]Samples analyzed after three month storage were from all 2012 field trial locations.
[7]Samples analyzed after six and nine month storage were from Montcalm County, Michigan 2012.

Acrylamide

At the time of harvest, chips made with V11 tubers contained 64.3% less acrylamide than chips made with Snowden. When potatoes were stored for three, six, and nine months at 10 °C, acrylamide concentrations in chips made from V11 were reduced by 48.9, 47.9, and 15.6%, respectively (Table 10).

Acrylamide concentrations in V11 chips were significantly lower than Snowden at the time of harvest and after three months storage. The significantly lower acrylamide levels after storage were expected from down-regulation of the *Asn1, R1 and PhL* genes, which lowered free asparagine and reducing sugars. Similar reductions in reducing sugars and acrylamide were reported by Zhu et al., 2014.

Lowered free asparagine, fructose and glucose levels lead to an overall reduction of acrylamide in processed potato products because they are reactants in the formation of acrylamide.

Table 10. Acrylamide in Chips from V11 and Control Snowden at Harvest and After Storage at 10 °C

Timing	Variety	Mean (ppb)	P-value[1]	Percent Reduction	N[2]	Standard Deviation	Range	
							Min	Max
Fresh[3]	V11	262	**<.0001**	64.3	22	127	112	540
	Control	734			21	414	239	1,540
Month 3[4]	V11	289	**0.0066**	48.9	6	186	125	582
	Control	566			5	206	399	857
Month 6[5]	V11	306	0.6386	47.9	3	28.1	279	335
	Control	587			3	217	337	717
Month 9[5]	V11	708	0.9839	15.6	3	323	499	1,080
	Control	839			3	270	530	1,030

[1]P-values indicating significant differences are underlined and in bold.
[2]Data from one replicate of the Snowden control from the Florida 2012 site were omitted because of an error at harvest.
[3]Samples analyzed at the fresh time points were from all 2012 and 2013 field trial locations.
[4]Samples analyzed after three month storage were from all 2012 field trial locations.
[5]Samples analyzed after six and nine month storage were from Montcalm County, Michigan 2012.

CONCLUSION

A compositional assessment was conducted on V11 and its parental control, Snowden. Two types of analyses were conducted.

- Compositional nutritional assessment, for those analytes important to potato nutrition
- Traits affecting composition, for those analytes related specifically to gene down-regulation and trait efficacy

The nutritional assessment evaluating proximates, vitamins, and minerals noted a significant difference between V11 and Snowden for vitamin C, although the mean was within the range seen in conventional varieties. The glycoalkaloids assessment did not identify any significant differences between V11 and Snowden. The analysis for total amino acids noted significant differences between V11 and Snowden for alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, isoleucine, leucine, serine, threonine, tyrosine, and valine, although all mean values were within the tolerance interval and/or combined literature range.

As expected, the efficacy assessment evaluating free amino acids and reducing sugars as well as acrylamide concentrations in chips demonstrated that V11 has significantly lower levels of free asparagine, slightly lower levels of reducing sugars, and significantly lower acrylamide potential in chips than Snowden.

These analyses demonstrated that V11 is compositionally equivalent to Snowden and is as safe and nutritious for food and feed as conventional potatoes that have a long history of safe consumption.

APPENDIX A By-Site Results
[CCI]

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