Quality traits: Altered starch composition in potato

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INNOVATION
YIELDS
RESULTS

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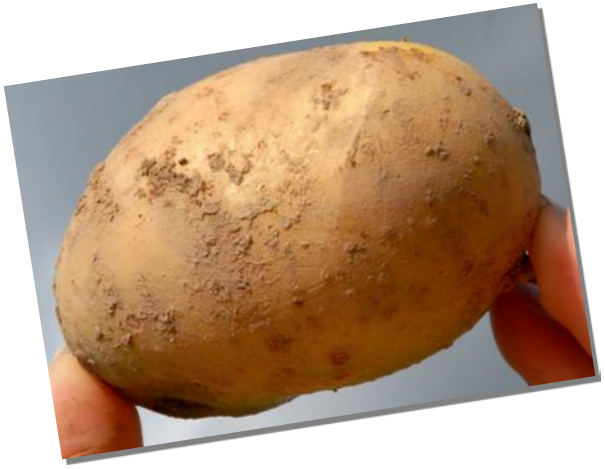
Agenda

- The trait, the crop and its uses
- Food and feed safety
- Environmental risk assessment aspects

The products (non-commercial)

Three potato events (varieties) as examples of a quality trait



- **Amflora, Amadea, Modena**
  - Modification of starch composition in the tuber
- **Approach**
  - Agrobacterium mediated transformation
  - With and without selectable marker gene
  - Vegetatively propagated crop (no breeding)
  - Thorough selection process in the greenhouse and the field for the event

The trait

Properties

» Pure amylopectin functionality

» Stable thickening without gelling

Advantages

» Improves product qualities

» Optimizes production processes

» Long shelf-life of solutions

Safety assessment

Guidance by international frameworks and national legislation to focus safety assessment

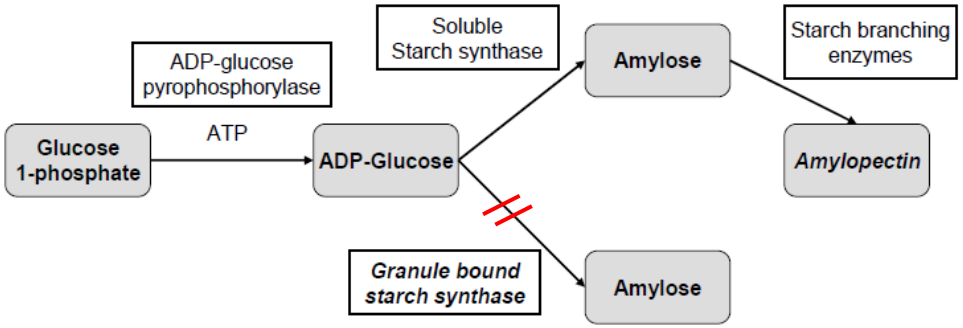
- Protection of human and animal health and the environment
- Comparative approach: assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart
- Unintended effects
  - Inherent and general phenomenon that can also occur in conventional breeding
  - Largely predictable based on knowledge of the inserted trait, its metabolic connections or of the site of insertion
  - Identify and detect and evaluate their biological relevance and potential impact on food safety
  - Take into account agronomic/phenotypic characteristics that are typically observed by breeders as first screen.

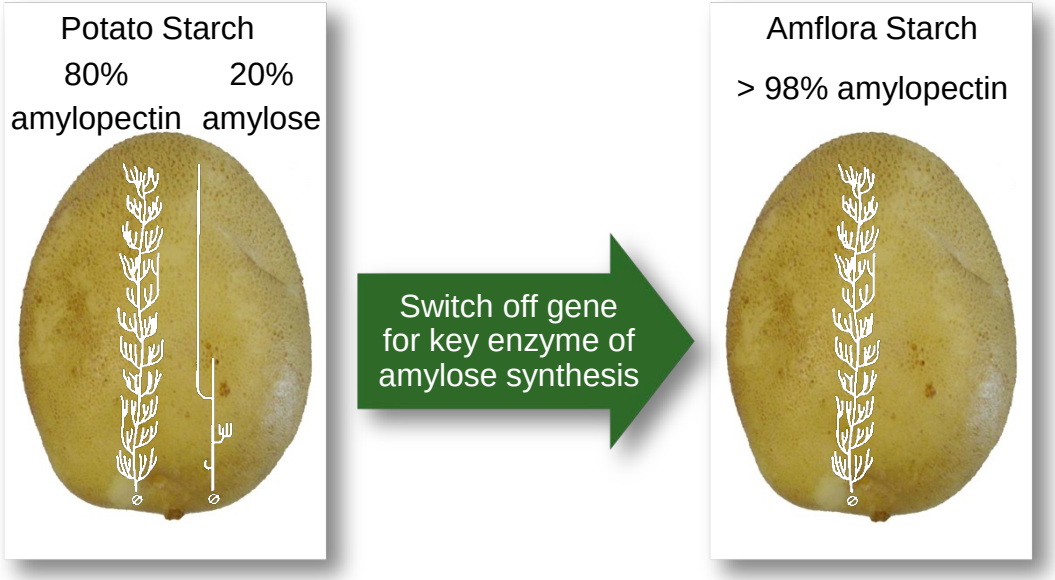
Source: Codex (2009) Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.

The genetic modification will not …

… lead to harmful effects on human and animal health or the environment –
Molecular aspects

- » Characterize insertion site (ORFs formed/disrupted…)
- » Antisense, RNAi (potato *gbss* gene)
- » Tuber-specific promoter
- » Inhibited expression of starch biosynthesis enzyme (GBSS) to be measured on protein and/or metabolite level (amylose)





Determine exposure

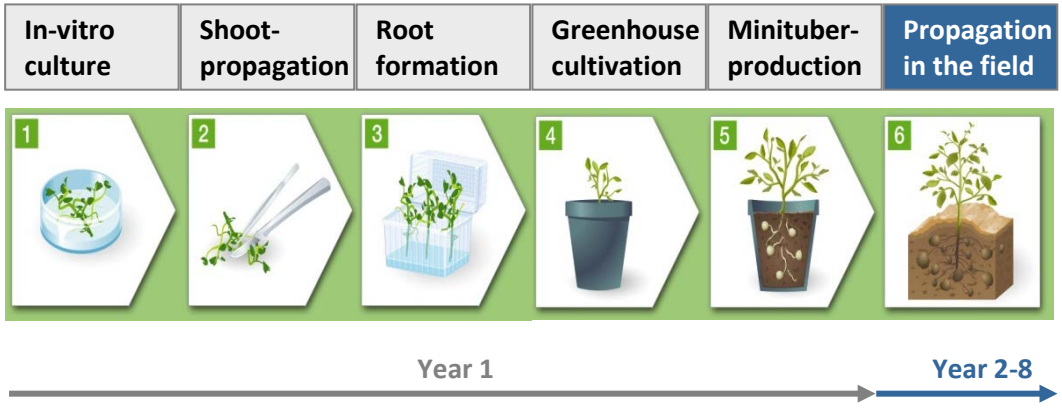
Intended use and exposure routes to be considered

- Cultivation, identity preserved, not a commodity
- Genetic background (mother variety): high tuber starch content, primary use for processing into starch
- Use of starch in the areas of e.g. paper, textiles, adhesives, food
- Use of processing by-product, pulp, as feed

Consider familiarity …

… with regard to the modification and the uses of the product

- » Amylose and amylopectin are components of all starchy foods or feeds in the diet
- » Ratio of amylose/amylopectin in known waxy starches (maize, potato, …) obtained by conventional breeding (mutagenesis, …)
- » Similar starch functionality obtained via chemical modification of plant-derived starches
- » Cultivation of conventional low-amylose potatoes in the EU
- » Potato propagation includes tissue culture process



Measurable endpoints to understand …

… if the use of the product as food or feed might cause harm when consumed

» Compositional analysis

- Focus on antinutrients and nutrients, vitamins, minerals (OECD recommended analytes)
- Focus on the pathway and possible changes in related metabolites
- Consider the magnitude of any effects
- Consider the biological relevance of any effects

Compositional analysis …

… and predictable effects

variety (year grown)	no. of locations	N	mean (range) starch (g/100 g)	glucose (g/kg)	fructose (g/kg)	sucrose (g/kg)	vitamin C (mg/100 g)	total glycoalkaloids (mg/kg)
Amflora (2004)	5	17	16.2 ± 2.4	3.1 ± 2.9	2.4 ± 2.3	3.1 ± 3.1	13.3 ± 4.0	138 ± 47
			(12.6–21.2)	(<0.1–9.7)	(<0.1–6.8)	(<2.0–11.7)	(7.1–20.2)	(72–247)
reference[b]	5	51	18.8 ± 2.5	1.9 ± 1.7	1.2 ± 1.3	<2.0	13.1 ± 4.8	272 ± 92
			(13.6–23.7)	(<0.1–5.2)	(<0.1–4.2)	(<2.0)	(3.6–25.4)	(70–474)
Amadea (2007/2008)	15	51	19.6	6.9[c]	6.8[c]	4.3[c]	10.8	371
			(16.5–23.7)	(2.9–12.4)	(3.0–12.4)	(0.5–9.0)	(6.4–18.6)	(163–713)
parental (Kuras)	15	51	20.3	6.0	5.9	1.8	9.3	553
			(17.2–25.4)	(2.0–11.9)	(2.0–11.7)	(0.5–4.8)	(6.6–14.2)	(341–1246)
reference[d]	15	367	17.6	5.0	4.0	1.9	9.9	185
			(8.8–24.5)	(1.3–11.4)	(1.2–9.2)	(0.5–10.2)	(5.2–14.3)	(29–609)
Modena (2011)	5	20	12.8	5	4	7	10.9	129
			(10.7–15.1)	(2–9)	(1–8)	(2–23)	(9.0–13.5)	(122–136)
parental (Karnico)	5	20	14.1	5	4	5	8.6	132
			(10.7–19.3)	(1–11)	(1–10)	(2–15)	(5.9–9.9)	(103–217)
reference[e]	5	128	15.8	5	4	5	10.5	160
			(11.0–20.8)	(1–12)	(1–11)	(2–26)	(5.8–16.2)	(30–599)

Source: Privalle et. al. (2013) J. Agric. Food Chem. 61, 8260-8266

Example for …

… variable parameters

» Protein, sugar and starch

» Variability for one variety depending on environment (location, climate)

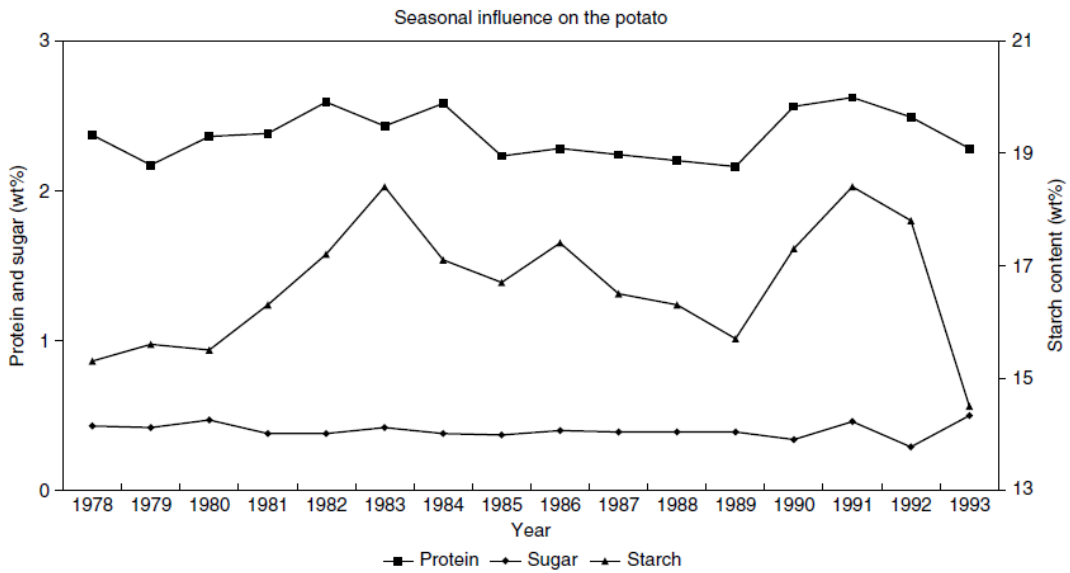
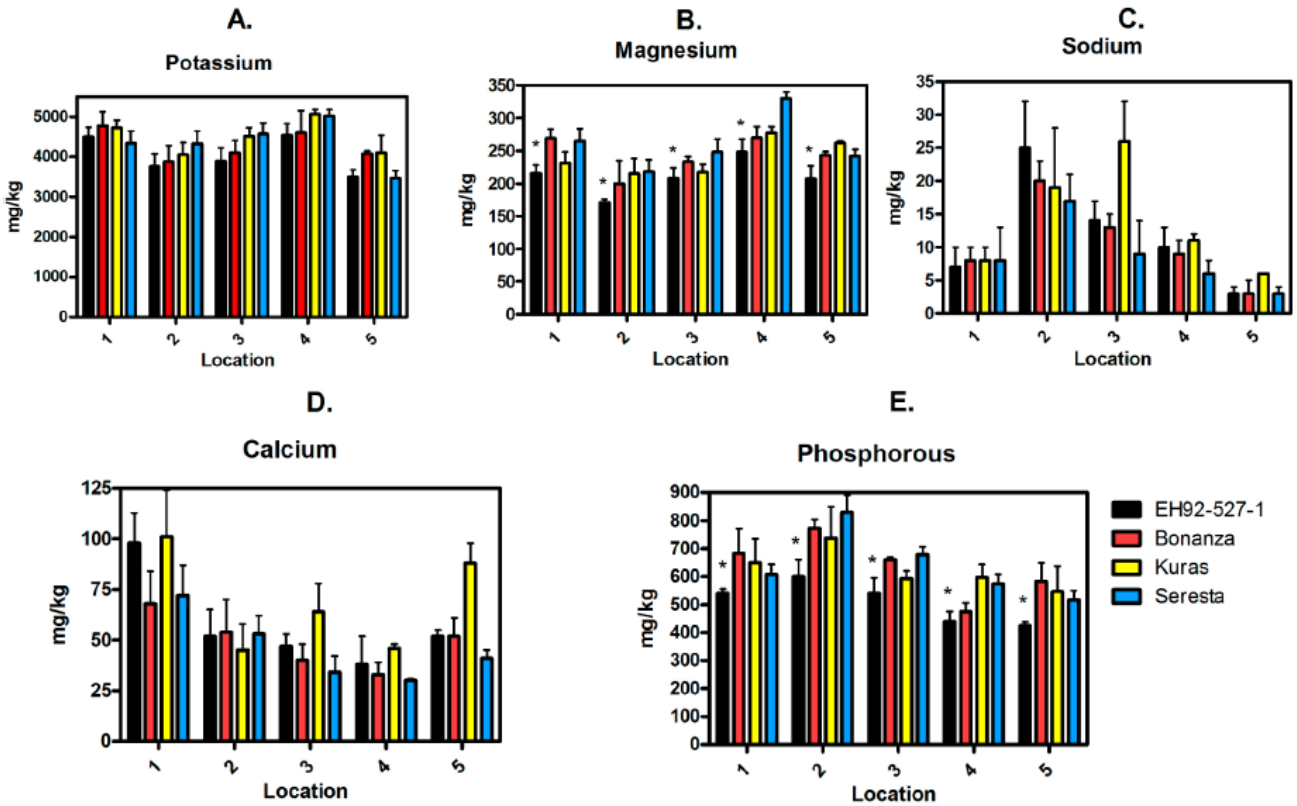


Figure 11.6 Variation in composition of the Ehud potato in The Netherlands.[11]

Source: Grommers and Krogt (2009) In Starch: Chemistry and Technology, 512-539

Compositional analysis …

… and environmental influences



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Agronomic and phenotypic parameters …

… rated as screen for unintended effects

- Agronomic
  - E.g. plant height, tuber yield, tuber starch content
- Phenotypic
  - E.g. growth habit, flower frequency, fruit frequency
- Plant development
  - E.g. days to emergence, crop vigor, plant stand uniformity, days to flowering
- Disease and pest
  - E.g. severity of infection (virus, Phytophthora, Erwinia, Alternaria), degree of infestation with Colorado potato beetle

Ratings …

… and assessment

» Same parameters used by breeders to assess new varieties (e.g. UPOV)

» Confidence in ratings (allows breeders to distinguish varieties)

» Variety characteristics generally available (common catalogue of varieties)

Parameter/Unit		AV43-6-G7	Karnico	Conventional
Days to Emergence (YDAYPE)	mean	22	23	25
number	min	16	18	18
	max	28	27	34
Uniformity of Emergence (EMUNIF)	mean	8	8	6
Ratings (1-9)	min	7	7	2
	max	9	9	8
Crop Vigor (CVIGOR)	mean	7	7	6
Ratings (1-9)	min	5	5	3
	max	8	9	8
Plant Stand Uniformity (PSUNIF)	mean	8	8	7
Ratings (1-9)	min	6	6	4
	max	9	9	9
Crop Cover (PFLADE)	mean	8	7	7
Ratings (1-9)	min	6	6	4
	max	9	9	9
Days to Flowering (YDAYFF)	mean	59	59	58
number	min	50	51	48
	max	65	66	68
Growth Stage (BBCH) at Canopy Burn Down (YESBBA)**	mean	94	94	95
number	min	91	91	91
	max	99	99	99

Parameter/Unit		AV43-6-G7	Karnico	Conventional
Number of Plants per Plot (YNRPLA)**	mean	28	28	28
number	min	27	28	24
	max	28	28	28
Plant Height (WUCHSH)	mean	66	68	69
cm	min	58	61	48
	max	74	79	88
Tuber Yield (ERTRFR)	mean	48.60	47.77	42.95
kg	min	30.14	30.10	22.5
	max	76.08	77.18	76.10
% Yield with Tuber Grade of SIB<30	mean	0.35	0.24	0.19
%	min	0	0	0
	max	0.71	0.65	1.28
% Yield with Tuber Grade of SIB 30-60	mean	39.35	34.71	35.98
%	min	20.54	13.27	9.37
	max	59.80	60.93	89.34
% Yield with Tuber Grade of SIB>60	mean	60.30	65.05	63.83
%	min	39.98	38.96	9.70
	max	79.19	86.64	90.63
% Starch Content	mean	17.9	19.4	21.2
%	min	13.7	16.2	15.2
	max	22.1	22.7	29.2

Source: Unpublished BASF Plant Science report

Environmental safety

Address the following areas of risks

» Persistence and invasiveness including gene flow
» Interactions with the GM plant and target organisms
» Interactions with the GM plant and non-target organisms
» Impacts of management practices
» Effects on biogeochemical processes

Effects on NTOs including soil fungal and microbial communities

Intensely investigated by a series of studies relating to the interaction of one potato, Modena, with non-target organisms as compared to the mother variety and a set of non-GM varieties

- Studies funded by the ERGO (Ecology Regarding Genetically Modified Organisms) program of the Dutch Research Organisation (NWO)
- Concluding that any observed differences between the GM potato and its conventional counterpart are minor when considered in the context of the variability exhibited by different conventional cultivars (genetic background) and magnitude of the environmental influences (location, climate, and correlated NTO presence/abundance).

Effects on NTOs including soil fungal and microbial communities

Related references

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Concluding remarks

A quality trait and the assessment of unintended effects

- Same assessment process as for agronomic traits applies
- Focus on what needs to be protected (provided by relevant legislation)
- Define what is harmful
- Guide risk assessment by hypothesis related to the crop and trait and how the trait was achieved (change in metabolic pathway, switch off gene) and apply a targeted approach
- Use weight-of-evidence approach and consider familiarity

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