

Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène
Herausgeber: Bundesamt für Gesundheit
Band: 93 (2002)
Heft: 6

Artikel: Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes
Autor: Biedermann, Maurus / Noti, Anja / Biedermann-Brem, Sandra
DOI: <https://doi.org/10.5169/seals-981746>

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Experiments on Acrylamide Formation and Possibilities to Decrease the Potential of Acrylamide Formation in Potatoes

Maurus Biedermann, Anja Noti, Sandra Biedermann-Brem, Valeria Mozzetti and Koni Grob, Official Food Control Authority of the Canton of Zürich

Received 3 October 2002, accepted 31 October 2002

Introduction

Acrylamide causes tumors in laboratory animals. As recently detected (1), it is formed in relatively large amounts in certain foods during baking, grilling, or frying. Potato chips, French fries, breakfast cereals, and crisp bread were identified as primary products of concern, as shown by the Swedish National Food Administration (1), the British Food Standard Agency (2), the Swiss Federal Office of Public Health (3), and the Norwegian Food Agency (4). With such surveys it is easily neglected that for a part of the population the main exposure concerns acrylamide from products prepared at home, such as roast potatoes or hash browns ("Rösti" in Switzerland). The World Health Organization (WHO, 5) and the Scientific Committee for Food (SCF) of the European Union (6) stated their concern about a "serious problem" and the SCF calls "for urgent research into measures to lower this formation".

A method was developed to determine the potential of acrylamide formation under standardized conditions (7): small amounts of the material to be tested, such as grated potato, are spread on a grid in an oven and heated to 120°C for 40 min. During the first about 10 min, the material is dried. A second sample is dried simultaneously and additionally heated to 160°C for 20 min. These potentials may be considered as worst case concentrations for real foods. Potato products, such as hash browns, roast potatoes, French fries, or potato chips, only contained a fraction of this amount, the concentrations strongly depending on the conditions of the preparation. The potentials may be approached, if the cooking process results in drying of the whole foodstuff. The determination of the potential of acrylamide for-

mation proved to be a useful tool for a first evaluation of different raw materials, such as potatoes of different cultivars or stored under different conditions.

A second analytical tool concerned the determination of acrylamide elimination through chemical reactions, which have not been identified so far: deuterated acrylamide (D_3 -acrylamide) is added before heat treatment and the residual concentration expressed in terms of percent reduction by the heating step. When D_3 -acrylamide was used for this purpose, methacrylamide was the internal standard for measuring acrylamide and D_3 -acrylamide (a GC-MS method described in ref. (8)).

Acrylamide concentrations found in heated food are the result of concurrent new formation and elimination, which is one of the reasons why concentrations do not increase exponentially with higher temperatures. Some samples contain massively more acrylamide after heating to 160°C than after 120°C , whereas some others even contain less. The chemistries running at the two temperatures seem to be quite different.

Acrylamide formation in wet samples, such as fresh potato or dough, is negligible. The experiments revealed that the acrylamide concentrations were similar whether the sample was dry or contained some 10 or 20% humidity (heating under pressure), which would be in contrast to the Maillard reaction, as the latter is most efficient at 12–18% humidity (9). A closer investigation showed, however, that it was the elimination being accelerated similarly as the new formation which held the acrylamide concentrations approximately constant. At the same time, this example demonstrated the importance of considering elimination in such a dynamic system.

This paper reports on further experiments about acrylamide formation, on the starting materials converted to acrylamide, and data suggesting possibilities of decreasing the potential of acrylamide formation in potato.

Experimental

The method for the analysis of acrylamide and D_3 -acrylamide was described in ref. (8). It involves swelling of the homogenated sample in water, extraction with 1-propanol, azeotropic evaporation of the propanol/water mixture, dissolution from the residue in acetonitrile, defatting with hexane, and GC-MS analysis with chemical ionization. A second internal standard, butyramide, enables to check the recovery over the sample preparation process for each analysis (verification).

Elimination of acrylamide was determined by addition of deuterated acrylamide (D_3 -acrylamide, Cambridge Isotope Laboratories, Andover, USA) before heat treatment (7). It has been shown that deuterium-hydrogen exchange does not interfere to a substantial extent, but $M^{13}\text{C}_3$ -acrylamide would completely rule out such artifacts.

The potential of acrylamide formation was determined by heating a small first sample of grated potato to 120°C for 40 min in a GC oven. During this step, a second sample of the same potato was dried and then additionally heated to 160°C for

20 min (7). Addition of D₃-acrylamide enabled to determine the elimination in the same experiment.

Potato was dried by spreading grated fresh potatoes (2×5 mm holes) on a towel and exposing it to the sun on a hot summer day during about 4 h (7).

Glucose and fructose were determined enzymatically, using the test kit from Scil Diagnostics, Martinsried, Germany. Potatoes were grated and homogenated. 20 g were weighed into a 100 ml measuring flask. After adding some 50 ml water, 5 ml each of Carrez I solution (85 mM) and Carrez-II solution (250 mM) were added and the flask intensively shaken. The pH was adjusted to 7.5–8.5 by addition of sodium hydroxide solution (10%) and the solution filled up to 100 ml with water. After 3 h, the sample was filtered (folded filters, Schleicher & Schuell, Basle, Switzerland, 595 ½), rejecting the first portion until the liquid dropped regularly. 100–500 ml of the solution were used for the enzymatic determination performed according to the instructions by the manufacturer. The detection limits for fructose and glucose were around 10 mg/kg of fresh potato.

Ammonium was determined by evaporation of ammonia from 30 g grated potato after addition of sodium hydroxide and water. A Kjeldahl apparatus (Büchi 323 distillation unit, Flawil, Switzerland) was used without prior heating of the sample in acid. The distillate was recovered in 30 ml of boric acid (3%). Ammonium was measured by titration with sulfuric acid (0.05 m), using a Titroprozessor 726 (Metrohm, Herisau, Switzerland).

Formation versus elimination of acrylamide

Onset of acrylamide formation in potato

At which temperature does acrylamide formation start to be effective? This was checked for potato of the cultivar Charlotte, harvest 2002. Since there is no relevant acrylamide formation in wet potato (7), the test material had to be dried before heat treatment in order to expose it to reacting conditions during a defined period of time. Drying had to occur at conditions ruling out acrylamide formation. D₃-acrylamide was added at a concentration of 5000 µg/kg referring to the dry mass of the sample. 5 g portions were evenly spread on a 6×10 cm grid and heated in a GC oven to a given temperature during 20 min. Methacrylamide, serving as internal standard for determining acrylamide and D₃-acrylamide, was added after heating.

Figure 1 plots concentrations of acrylamide and D₃-acrylamide against the temperatures applied. Before heating (40°C assumed to be reached during drying in the sun) and after heating to 80°C, acrylamide concentrations remained below the detection limit of 10 µg/kg. The apparent reduction of the D₃-acrylamide concentration at 80°C is within the experimental uncertainty. In the sample heated to 100°C, 150 µg/kg of acrylamide was determined and about 12% of the D₃-acrylamide was lost. Then the rates of formation and elimination increased rapidly. Heating to 120°C already caused the acrylamide concentration to exceed 1 mg/kg. For

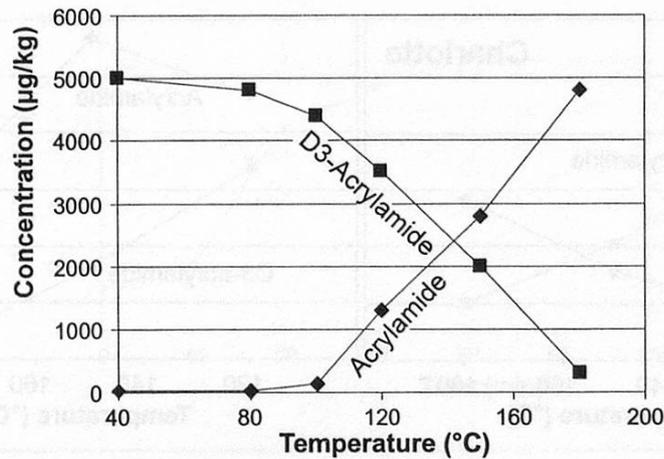


Figure 1 **Acrylamide and D₃-acrylamide in dried potato heated for 20 min to the temperatures indicated** (concentrations referring to dry weight)

this potato, acrylamide concentrations constantly rose up to 180°C, i.e. formation clearly predominated elimination over the whole temperature range. As shown below, there are samples showing different performance.

Formation versus degradation in potatoes

Dependence of acrylamide concentrations on temperature was determined for a number of different potatoes (same or different cultivars). Figure 2 reports the results of potatoes of the cultivars Charlotte (different lot than above) and Sirtema, both harvested shortly before the analysis, because they showed opposite behavior. The fresh potatoes were grated and sun-dried, i.e. heating started with a virtually dry material. The material was homogenized and samples of 5 g were heated for 20 min to the temperatures indicated. Uncertainty of the results is below 10% (7).

For the Charlotte, acrylamide concentrations increased from 2.7 mg/kg (referring to the dry mass) at 120°C to 8.5 mg/kg at 180°C. For the Sirtema, the concentration was higher at 150°C, but at 180°C it was substantially lower even than at 120°C. This is the result of different elimination rates, as shown by the D₃-acrylamide concentrations. In the Charlotte heated to 180°C, 80% of the D₃-acrylamide added (4.0 of 5.0 mg/kg) was lost; in the Sirtema it was 96%. This means that five times less D₃-acrylamide was left after heat treatment of the Sirtema or that the rate of elimination was five times higher. With a rate of elimination as observed for the Charlotte, the acrylamide concentration in the Sirtema after heating to 180°C had been around 35 mg/kg. The data suggests that the Sirtema potato was more active than the Charlotte in both senses: acrylamide formation was faster (as observed by the concentration determined at 120°C), but also its elimination. Incidentally this resulted in almost equal acrylamide concentrations after heating the Sirtema and the Charlotte at 180°C.

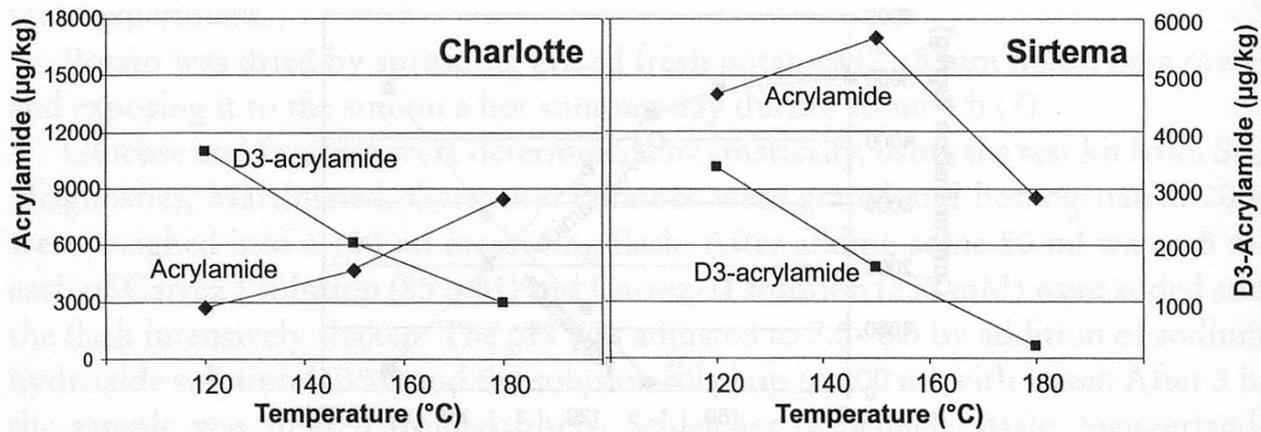


Figure 2 **Acrylamide concentration and elimination of D₃-acrylamide at various temperatures for potato of the cultivars Charlotte and Sirtema; concentrations referring to dry weight**

There is not enough data to conclude whether this behavior is characteristic for the two cultivars or for other factors. The Charlotte characterized in figure 1 showed a similar increase of the acrylamide concentrations from 120 to 180°C, and the Sirtema used for the experiments described in table 5 of ref. (7) also showed a 30% decrease from 120 to 160°C. However, table 7 will show samples of both cultivars with opposite behavior.

Exhausted resources?

The increase in acrylamide concentration slows down when heat treatment is prolonged. Dry potato of the cultivar Agata (harvest 2002) was heated to 120°C for 10 to 60 min. As shown in figure 3, the concentration no longer increased after about 40 min. The decrease at 60 min is within the experimental uncertainty, but could mean that elimination became faster than formation. Similar results were shown in ref. (7).

As the rate of elimination does not increase with continuing heating (it tends to decrease (7)), this suggests a decreasing rate of formation. It could be the result of depleting starting materials, such as fructose and glucose, which are assumed to be involved in acrylamide formation (see below).

To rule out that the drying process (elimination of the last humidity) restricted acrylamide formation, fresh Agata potato was submitted to two consecutive heating cycles: 20 g of grated and homogenated potato were heated to 120°C for 40 min and to 160°C for 20 min. Then 10 g of water was added to the dried potato (3.4 g) and the latter allowed to swell at 60°C for 30 min. New D₃-acrylamide was added (on top of the negligible amount left from the first cycle) and the heat treatment repeated. As shown in table 1, the second heat treatment increased the acrylamide concentration by merely 10%, which corresponds to the above observation that

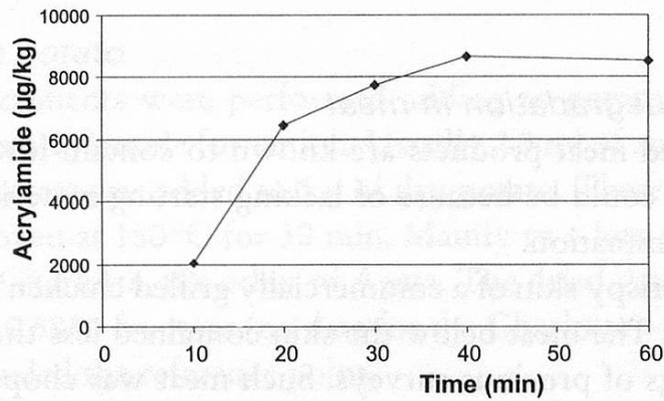


Figure 3 **Acrylamide concentrations (referring to dry weight) after heating at 120°C for different duration**

heat treatment beyond a certain duration no longer increases acrylamide concentrations. Hence drying was not the reason for the decreasing formation.

The more or less stable acrylamide concentration does not mean that no acrylamide was formed, since there was concurrent elimination. During the second heating, the elimination rate was reduced to about half (from 72 to 37% at 120°C and from 97 to 92% at 160°C). As the acrylamide concentration was about constant, the rate of its formation must have been similar to the elimination rate, i.e. about 400 µg/kg were formed during the second heating at 120°C and about 550 µg/kg at 160°C.

Table 1 also shows the concentration of the reducing sugars (sum of glucose and fructose): the first heat treatment reduced it by a factor of about 5, and after heating to 160°C, it fell below the detection limit. If the reducing sugars are, as assumed, indispensable starting material for acrylamide formation, this would suggest an even sharper decrease of formation. The continuing formation suggests that thermal

Table 1

Acrylamide concentration and elimination of D₃-acrylamide in an experiment involving a double heat treatment; contents of fructose and glucose (all concentrations referring to the fresh weight)

	Acrylamide µg/kg	Elimination (%)	Glucose + Fructose mg/kg
Fresh potatoes			2530
40 min 120°C	980	72	490
+20 min 160°C	1000	97	<10
<i>Addition of water and new D₃-acrylamide</i>			
40 min 120°C	1110	37	<10
+20 min 160°C	1100	92	<10

degradation of other potato components supplies material with suitable carbonyl functions.

Formation versus degradation in meat

Grilled or roasted meat products are known to contain low concentrations of acrylamide (1). This could be because of lacking starting material for the synthesis or because of fast elimination.

The brown and crispy skin of a commercially grilled chicken contained 17 µg/kg acrylamide (table 2). The meat below the skin contained less than 10 µg/kg, which confirms the findings of previous surveys. Such meat was chopped to small pieces and spiked with 1 mg/kg of D₃-acrylamide. Heating on a grid in a GC oven at 100°C for 30 min caused the sample to just about reach dryness; 92% of the D₃-acrylamide was eliminated. In the sample heated to 120°C, 98% was lost. In comparison, the heat treatment of potato at 120°C resulted in a loss of 50–60%, i.e. elimination is some 20 times slower (some 40% *versus* 2% D₃-acrylamide being left). In corn starch, the loss at a temperature of 150°C was merely 22% (table 4). At 140°C, in meat 99.7% of the D₃-acrylamide added was eliminated. The concentrations of acrylamide in these heated meat samples were below 10 µg/kg.

Table 2
Acrylamide concentrations in grilled chicken and elimination of D₃-acrylamide in meat after heat treatment as indicated

	Acrylamide (µg/kg)	Elimination (%)
Chicken, grilled skin	17	
Chicken, meat	<10	
Meat, 30 min at 100°C	<10	92
Meat, 30 min at 120°C	<10	98
Meat, 30 min at 140°C	<10	99.7

This experiment does not enable a statement about the rate of acrylamide formation in meat, but the conclusion that fast elimination would result in low concentrations even if the rate of formation were rather high.

The current statement that acrylamide is primarily found in foods rich of starch gets an explanation: starch is not involved in acrylamide formation (hardly any is formed in purified starch, as shown below), but represents a relatively inert environment decreasing the rate of elimination. Other media, probably those with a higher content of protein, do not allow high concentrations to build up because elimination is too fast.

Starting substances for acrylamide formation

Experiments with potato

Numerous experiments were performed adding to potato components which could be involved in acrylamide formation. Usually 2.5 ml of an aqueous solution of the substance of interest was added to 5 g of dry potato. These sample were heated on a grid in a GC oven at 150°C for 30 min. Mainly two lots of potato were used, one of the cultivar Charlotte, the other of Agata. The dried Agata potato contained 0.73 % glucose and 0.58 % fructose (no data for the Charlotte). Addition of water to the dry potato provided the reference point.

Some results are listed in table 3 (many were confirmed by experiments not listed). They provided the basis for the following observations and speculations:

- As shown for both cultivars, addition of 5 % fructose increased the acrylamide concentration to 17 mg/kg, independent of the reference point (3 mg/kg for Charlotte and 6.5 mg/kg for Agata). Glucose was merely half as efficient in increasing acrylamide concentrations.

Table 3

Addition of components to dry potato before heating to 150°C for 30 min: resulting acrylamide concentration and elimination of D₃-acrylamide (additions and concentrations referring to dry weight)

<i>Charlotte</i>	<i>Acrylamide (µg/kg)</i>	<i>Elimination (%)</i>
+ water	3000	80
+ fructose (5 %)	17800	91
+ glucose (5 %)	9200	89
+ NH ₄ Cl (1 %)	2000	83
+ NH ₄ HCO ₃ (1 %)	2700	90
+ fructose (5 %) + NH ₄ HCO ₃ (1 %)	24000	93
+ fructose (5 %) + Na ₂ CO ₃ (1 %)	16500	92
+ fructose (5 %) + Na ₂ CO ₃ (5 %)	3400	96
+ fructose (5 %) + citric acid (5 %)	11050	77
+ fructose (5 %) + lysine (3 %)	6250	87
+ ascorbic acid (1 %)	2050	81
+ glycerol (5 %)	2900	88
+ olive oil (5 %)	2200	85
+ cysteine (1 %)	800	95
+ valine (1 %)	2200	90
<i>Agata</i>	<i>Acrylamide (µg/kg)</i>	<i>Elimination (%)</i>
+ water	6500	84
+ fructose (5 %)	17500	95
+ lactic acid (1 %)	6450	84
+ acrolein (0.1 %)	8600	98
+ hydroxy acetone (1 %)	25700	95
+ asparagine (1 %)	11000	85
+ asparagine (1 %) + NH ₄ ac (1 %)	9200	95
+ asparagine (1 %) + fructose (5 %)	42400	91

- Hydroxy acetone, a possible thermal degradation product from fructose, increased the concentration somewhat more strongly than fructose.
- Addition of ascorbic acid resulted in a weak decrease of the acrylamide concentration. It seems neither to be an efficient source, nor to inhibit acrylamide formation. It has no significant influence on elimination.
- The addition of sodium carbonate in combination with fructose decreased the acrylamide concentration, at least partly through increased elimination.
- Citric acid, again in combination with fructose, decreased the acrylamide concentration. Since elimination decreased rather sharply (77% compared to 91% without citric acid), it must have decreased acrylamide formation about five fold.
- The addition of edible oil had no effect.
- Addition of lactic acid, glycerol or acrolein (the last one in a closed flask, i.e. with a humid sample, explaining the high rate of elimination) had no relevant effect on the acrylamide concentration.
- Asparagine addition had a rather weak effect.
- Asparagine combined with fructose gave the strongest response.

Addition of fructose, glucose, hydroxy acetone and asparagine had a significant effect on acrylamide formation. For Agata, the added fructose increased the fructose concentration by a factor of almost 10. Referring to the sum of the two reducing sugars, the increase corresponded to a factor of 3.8. This resulted in an increase of the acrylamide concentration by a factor of almost 3. Taking into account that also the rate of elimination was about tripled (elimination increased from 84 to 95%), addition of fructose provided an approximately proportional increase in acrylamide formation. This supports that fructose is a key component in acrylamide synthesis. At least for this Agata potato, it was the limiting factor, i.e. the reaction partner was present in abundance. The experiment also showed that hydroxy acetone is an efficient starting material to substitute fructose and glucose.

Experiments with flour

Parallel experiments were conducted with wheat flour, since in potato the starting materials are present in such high concentrations that experimental additions only result in a weak response. Results are just shortly summarized here, as they finally contributed little to the conclusions.

The addition of fructose or glucose had hardly any effect, i.e. they are not limiting the formation of acrylamide as in potato. The addition of ammonium increased acrylamide formation several fold, but concentrations remained low. Even the combined addition of fructose and ammonium bicarbonate resulted in an acrylamide concentration which remained around 100 times lower than in potato. As elimination was also substantially lower (around 40% in dough compared to some 85% in potato, both referring to 150°C for 30 min), the difference in the rate of acrylamide

formation was even more pronounced. Glucose again gave about half of the response of fructose.

Experiments with corn starch

Further experiments were performed with corn starch as a relatively pure matrix in order to eliminate active interference of endogenous material. As shown in table 4, the reference point (addition of water) was low. Addition of fructose, ammonium or a combination of both did not increase acrylamide formation at all. Some additional components were tested for a catalytic activity (citric acid, iron, calcium), but no effect was recorded. At this point, *Weisshaar* (10) suggested asparagine as the principal starting material, present in potato at 0.5–3 % (related to dry matter).

Table 4

Acrylamide concentrations and elimination of D₃-acrylamide in corn starch after addition of the components listed and heating at 150 °C for 30 min

Corn starch	Acrylamide (µg/kg)	Elimination (%)
+ water	53	22
+ NH ₄ HCO ₃ (1 %)	54	20
+ fructose (5 %)	42	30
+ fructose (5 %) + NH ₄ HCO ₃ (1 %)	52	18
+ fructose (5 %) + NH ₄ ac (1 %)		
+ citric acid (2.5 %, pH 5.7)	34	42
+ fructose (5 %) + NH ₄ ac (1 %) + Fe II + Fe III (20 ppm)	30	42
+ fructose (5 %) + NH ₄ ac (1 %) + CaCl ₂ (300 ppm)	20	40
+ asparagine (1 %)	170	18
+ asparagine (1 %) + NH ₄ ac (1 %)	1100	47
+ asparagine (1 %) + fructose (5 %)	27400	18
+ asparagine (1 %) + 1% NH ₄ ac + fructose (5 %)	114000	37
+ asparagine (1 %) + NH ₄ HCO ₃ (1 %) + fructose (5 %)	85000	20
+ asparagine (1 %) + NH ₄ ac (1 %) + hydroxy acetone (1 %)	33000	49
+ maleic acid diamide (1 %) + NH ₄ ac (1 %) + fructose (5 %)	630	40
+ glutamine (1 %) + NH ₄ ac (1 %) + fructose (5 %)	160	31

Addition of asparagine alone did not efficiently produce acrylamide, but in combination with fructose, about 1 % of the asparagine was converted to acrylamide. Hydroxy acetone again acted in the same way.

Addition of ammonium seemed to further enhance acrylamide formation, which gave some room for the hypothesis that the carbon source for acrylamide could be fructose or hydroxy acetone and asparagine/ammonium might participate as the source of nitrogen. However, little acrylamide was formed when asparagine was substituted by other amides, such as maleic acid diamide or glutamine. Ammonium might have an indirect influence on the reactions.

Conclusions on acrylamide formation

The above experiments suggest that asparagine is the starting material for the formation of acrylamide and that a carbonyl function, such as a reducing sugar or hydroxy acetone, is required to activate the fragmentation reaction resulting in acrylamide. This is in full agreement with the hypothesis of *Weisshaar and Gutsche* (12).

In the Agata potato, fructose and glucose were the limiting factors for acrylamide formation. The concentration of the reducing sugars before heating, 1.3 %, was in the same range as that of asparagine (not measured). It might be due to their rather rapid elimination by competing reactions that they remained the rate-determining factors.

Possibilities of reducing acrylamide formation in potato products

Potentials of acrylamide formation were measured to search for possibilities enabling a reduction of the acrylamide concentrations in baked, roasted, or fried potato products through more suitable raw material. The primary aim of this work was the identification of promising directions, rather than conclusions on practical measures to be taken.

To the data shown below, often concentrations of fructose and glucose are added because of the key role of these sugars at least in potato. It will point out that the potentials of acrylamide formation are directly related to the sugar contents, which also renders the reduction of the sugar contents the most promising route for decreasing the acrylamide concentrations in raw materials for foodstuffs.

Greenish potatoes

Within some lots, individual potatoes strongly differed in their potential of forming acrylamide. As an example, five pieces from a bag of Charlotte potatoes from a defined field near Zürich, harvested in June 2002, were analyzed individually by heating to 120°C for 40 min. As shown in table 5, four results were between 400 and 800 µg/kg, but a fifth (no. 4) reached 1900 µg/kg. It was a slightly greenish potato. A sixth, similarly greenish potato also gave a high result.

Table 5
Potentials of acrylamide formation and elimination of D₃-acrylamide at 120°C/40 min of 6 individual potatoes from the same lot

Sample	Acrylamide (µg/kg)	Elimination (%)
1	600	68
2	800	63
3	400	66
4 (greenish)	1900	66
5	550	66
6 (greenish)	1500	71

Freshly harvested potatoes of the cultivar Charlotte from the garden of one of the authors were divided in those without and with greenish parts (influence of light on potatoes grown near the surface). At 120°C, the homogenate of the greenish ones produced 3.7 times more acrylamide than that of the others (table 6). The greenish potatoes also contained 5.5 times more reducing sugar. Further determinations confirmed that greenish potatoes form 3–8 times more acrylamide, independently of whether they were exposed to light during growth or storage. Light seems to activate potatoes, initiating an increase in the concentration of reducing sugars and the potential of acrylamide formation. As a practical conclusion, the old rule is confirmed that potatoes should be stored in the dark.

Table 6
Potentials of acrylamide formation as well as reducing sugars of normal potatoes and potatoes with greenish parts

	<i>Acrylamide</i> ($\mu\text{g}/\text{kg}$)		<i>Glucose</i> (mg/kg)	<i>Fructose</i> (mg/kg)
	40 min 120°C	+ 20 min 160°C		
Normal	150	650	280	50
Greenish	560	1800	1150	680

Comparison of cultivars

It seems an obvious hypothesis that different cultivars of potato could have different potentials of acrylamide formation; cultivars are known to be characterized by differing contents of fructose and glucose (11). The results shown in table 7 usually represent mean values of around 5 potatoes from samples obtained on the local market in June/July 2002. They do not disprove an influence of the cultivar, but indicate that other factors are at least equally important.

For the cultivar Charlotte, for instance, results obtained after heating at 120°C varied between 120 and 2350 $\mu\text{g}/\text{kg}$, i.e. by a factor of about 20. At the higher temperature, the differences were reduced to a factor of well 3 owing to a stronger increase of the low values – in fact, for the first sample the concentration determined after heating to 160°C was not even higher than that from heating to 120°C (approaching the behavior observed for Sirtema potato shown in fig. 2). An even more drastic discrepancy is observed for the two samples of the cultivar Nicola.

As a first conclusion, the comparison of cultivars presupposes the strict elimination of interfering factors. The main conclusion, however, is the need for an investigation of these interfering factors, as they seem to play at least as important a role in reality as the cultivar. From the point of view of acrylamide formation, the potatoes with a high potential are undesirable and it should be determined what causes the potential to be far higher for some lots than for others of the same cultivar. As the samples with the extremely high potentials were from commercial sources, it cannot be ruled out that they have been submitted to cooling to a low temperature, perhaps in a cooling room together with other fresh food (see below).

Table 7

Comparison of the potential of acrylamide formation for samples of various cultivars

Cultivar	Harvest	120 °C, 40 min		+ 160 °C, 20 min	
		Acrylamide (µg/kg)	Elimination (%)	Acrylamide (µg/kg)	Elimination (%)
Sirtema	2002	1400	66	1500	93
	2002	750	58	1200	95
Charlotte	2002	2350	70	2300	93
	2002	600	70	1400	92
	2002	150	57	650	91
	2002	120	45	700	83
	2002	500	57	1300	95
Agria	2001	1800	65	2200	92
	2001	2200	71	3100	94
	2001	500	77	1200	94
	2002	140	56	990	81
Bintje	2001	1150	78	1400	95
	2001	1300	72	2000	94
	2001	750	64	1600	91
	2001	1000	69	1150	96
	2001	700	50	1450	93
Urgenta	2001	6600	79	5750	96
	2001	3300	74	3200	97
	2002	308	42	1100	71
	2002	160	65	600	96
Ostara	2001	3150	82	3250	98
	2001	1000	50	2000	89
	2002	180	52	830	87
Nicola	2002	70	74	260	96
	2002	2260	74	3500	97
Amandine	2002	2050	69	1420	96
Agata	2002	980	72	1000	97

Table 8 shows the correlation between the fructose/glucose contents and the potentials for acrylamide formation for 5 types of potato. These potatoes have been used for the comparison of the potentials of acrylamide formation with the real concentrations in chips, hash browns, baked potatoes, and French fries (7). Particularly the data concerning the potential at 120 °C confirm the important role of fructose and glucose and suggest that the low concentrations of fructose and glucose keep the acrylamide concentrations low for the products prepared from Erntestolz and Lady Rosetta.

The data also correlates the drastic increase in acrylamide concentrations found in products from the Erntestolz potatoes cooled to 4 °C for 15 days (factors

Table 8

Comparison of fructose and glucose contents with the potentials of acrylamide formation (concentrations referring to fresh weight)

<i>Cultivar</i>	<i>Reducing sugars (mg/kg)</i>		<i>Acrylamide µg/kg</i>	
	<i>Fructose</i>	<i>Glucose</i>	<i>120 °C</i>	<i>160 °C</i>
Erntestolz	20	60	100	1550
Erntestolz 4 °C	1250	1300	2800	5100
Lady Rosetta	20	40	70	700
Panda 2001	100	170	440	880
Sirtema	1050	1850	1750	1250

between 6 and 50) with the increased concentrations of fructose and glucose (factors of 60 and 20, respectively).

Storage

Potatoes are stored during a relatively long period, and this is a possible reason for strongly increased acrylamide formation. Cooling helps to keep potatoes fresh and to slow down germination. In fact, even text books suggest "ideal" storage temperatures of 4–6 °C (12). On the other hand, it is known that cooling below about 8 °C drastically increases the concentration of fructose and glucose (see, e.g., ref. (13)). The strong increase is reversible only to a minor extent when the potato is later reconditioned at higher temperature. This is the reason why producers of potato chips never cool their raw material below 8 °C: reducing sugars would have caused undesired browning of the chips (13). If cooling causes a strong increase in reducing sugars, it probably also enhances the potential of acrylamide formation.

The experiment with the Erntestolz potatoes cooled to 4 °C for 15 days confirmed this hypothesis (table 8; table 5 in (7)). The effect of cooling by far exceeded the differences between cultivars.

Results of an experiment with Charlotte potatoes from the garden of one of the authors lead to similar conclusions (table 9). The freshly harvested potatoes (July 4) had a low potential of acrylamide formation (compare with table 7). In the potatoes analyzed after keeping them in the laboratory for 25 d, the potentials were slightly higher, perhaps influenced by the fact that they were not kept in complete dark. A sample kept in a cellar for the same duration had a similar potential of acrylamide formation. The substantially lower results obtained after 60 days in the same cellar might be an indication of the difference between individual potatoes rather than a significant trend.

Storage during 17 days at 4 °C (cooling room) increased the potential of acrylamide formation by more than a factor of 10. The concentrations of fructose and glucose were determined only 8 days later for a sample cooled for 25 days. Compared to those in the potatoes stored in the cellar, they had increased by factors of 116 and 33, respectively. Reconditioning at 25 °C for a week reduced the sugar con-

Table 9

Potentials of acrylamide formation and concentrations of fructose and glucose for Charlotte potato after different storage (data referring to fresh weight)

	Acrylamide ($\mu\text{g}/\text{kg}$)		Fructose mg/kg	Glucose mg/kg
	120°C	160°C		
Fresh	150	650		
25 d at 25°C	300	800	70	230
25 d in cellar (ca. 15°C)	290	1150	25	105
60 d in cellar	120	470	40	85
17 d at 4°C	3250	3950		
25 d at 4°C	2250	3000	2900	3450
17 d at 4°C+7 d at 25°C	2500	2750	1350	2050

centrations, as to be expected according to experiments reported in literature. Corresponding results for the potential of acrylamide formation are little indicative, but rule out a strong decrease.

An experiment performed with potatoes of the cultivar Amandine from a commercial source (unknown previous storage) showed that a few days are sufficient to increase the potential of acrylamide formation (table 10). As the potential was high to begin with, the effects were less drastic.

Table 10

Storage experiment with commercial Amandine potatoes

	Acrylamide ($\mu\text{g}/\text{kg}$)	
	120°C	160°C
As received	1950	1450
3 days 4°C	2600	2350
7 days 4°C	3250	2400
21 days 4°C	3300	2300

Influence of nitrogenous fertilizer

The concentration of ammonium or asparagine (storage of nitrogen) in potato might depend on the availability of nitrogen during cultivation, i.e. on the use of nitrogen fertilizer. Some clues on this aspect were obtained from experiments with three cultivars of potato grown at the same place without and with two different additions of ammonium nitrate (agricultural research station Reckenholz, Zürich). Ammonium concentrations in strongly fertilized potato were increased, indeed, and for two of the cultivars (Fontane and Lady Claire) acrylamide formation at 120°C was also somewhat higher (table 11). Acrylamide formation at 160°C did not show a relationship.

The effect of fertilization was superposed by stronger other influences. In particular, the large differences in acrylamide concentrations determined at 120°C are

Table 11

Potentials of acrylamide formation, ammonium and reducing sugars in potato grown with differing nitrogenous fertilization

Cultivar	Fertilizer N (kg/ha)	Acrylamide ($\mu\text{g}/\text{kg}$)		Ammonium (mg/kg)	Fructose (mg/kg)	Glucose (mg/kg)
		120°C	+ 160°C			
Fontane	0	70	820	120	30	150
	120	200	1100	200	120	270
	200	240	920	250	30	230
Lady Claire	0	65	1000	110	33	116
	120	90	1300	200	35	111
	200	85	1300	260	24	97
Naturella	0	550	1400	130	100	1800
	120	390	1200	190	70	1200
	200	690	1400	200	330	1100

rather well correlated with the contents of reducing sugars (fig. 4). Correlation was better for the sum of glucose and fructose than for just one of them. Correlation with the acrylamide formed during additional heating to 160°C was (once more) poor, presumably because the sugar contents had been strongly reduced before (table 1), other materials supported acrylamide formation, and elimination gained a predominant role.

Regarding fertilization, it is concluded that "organic" potato (grown without mineral fertilizer) cannot be claimed to generally form less acrylamide than that from conventional production. There may be some influence, but characteristics of the cultivar, storage conditions and other, unknown factors usually play a more

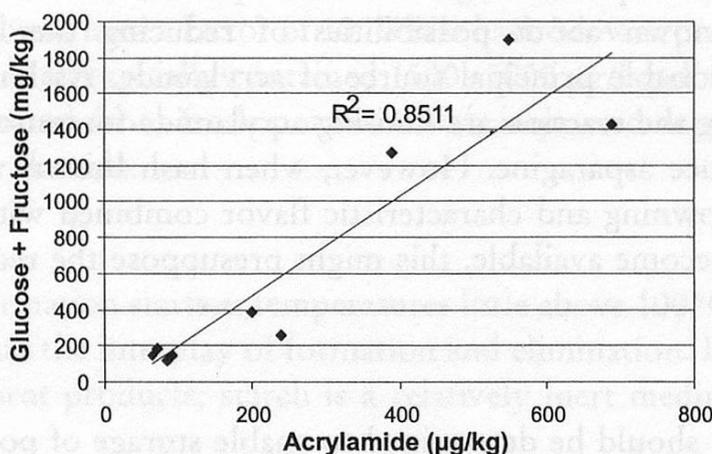


Figure 4 **Acrylamide concentrations after heating to 120°C for 40 min plotted against the reducing sugars present before heating; samples listed in table 11**

important role. In fact, at 120°C the non-fertilized Naturella potato formed some 7 times more acrylamide than the strongly fertilized Lady Claire.

Discussion

The results suggest that the optimization of potatoes should enable a substantial reduction of acrylamide formation and indicate the directions to pay attention to. Institutes with competence in the particular fields should check these as well as possible measures for improvement.

Potatoes

The potential of potatoes to form acrylamide can be reduced many fold. In August 2002, potatoes of the harvest 2002 with a potential at 120°C of merely 70 µg/kg acrylamide were marketed, but also others with a potential of 3000 µg/kg acrylamide – about 40 times more! Of a given cultivar, some lots formed up to 30 times more acrylamide than others (e.g. Nicola, table 7). As the potential of freshly harvested potatoes was always at the low end of the range, it is assumed that the massive increase resulted from some inadequate handling which should be identified with first priority. Optimization of the cultivars is of second priority, promising to provide substantial improvement once the main factors are under control.

Results show that potatoes should be trimmed for low contents of reducing sugar. Fructose and glucose seem to largely determine the rate of acrylamide formation. This connects the new problem to work performed over many years aiming at low sugar contents, e.g. to obtain potato chips and French fries with a minimum of browning. However, this connection also creates a dilemma: the reduction of the potential of acrylamide formation will also reduce the browning for potato products for which the darker color and the flavor are desired, since it is the same reducing sugars which are responsible for the Maillard reaction.

Nothing is known about possibilities of reducing the concentrations of asparagine, the probable principal source of acrylamide. As long as the reaction partners catalyzing the reaction are limiting acrylamide formation, it may even be ineffective to reduce asparagine. However, when hash browns or roast potatoes with attractive browning and characteristic flavor combined with low acrylamide contents should become available, this might presuppose the reduction of asparagine.

Storage

The best ways should be determined to enable storage of potatoes up to early summer of the following year in good quality (e.g. suppressing germination) without building up a high potential of acrylamide formation. Cooling below 8°C must be avoided: as potatoes react to low temperatures by strongly increasing concentrations of reducing sugars, the potential of acrylamide formation multiplies. Recondi-

tioning at higher temperatures does not efficiently revert the situation. Also unwanted cooling owing to influences of cold weather should be avoided.

The practical consequences are:

1. Cooling below about 8°C (limit established for the formation of sugars) cannot be used to hinder germination during long term storage.
2. Potatoes must not be strongly cooled in cooling rooms together with other fresh foods (shops and restaurants).
3. In households, potatoes must not be stored in the refrigerator.
4. Farmers should avoid that potatoes are exposed to cold weather, as it may occur in the soil when harvest is late or for pallets standing around in late autumn waiting for delivery.

Cooking methods

Temperatures and duration of heating during the manufacture of potato products should be revisited: acrylamide formation only starts after crust formation, and a minor extra heating may increase the acrylamide content many fold (table 5 in (7)). Perhaps extraction could reduce the contents of sugar and asparagine in the surface layer which is most strongly exposed to heating.

Pre-fabricated products for the preparation of French fries or hash browns must be optimized such that only a minimum of acrylamide is formed during heating in the kitchen. This means a careful selection of the potatoes and perhaps an extraction in hot water.

In the kitchen (professional or private), acrylamide formation can be massively reduced when certain rules are observed.

Conclusion

Acrylamide formation can be reduced at modest costs. For potatoes, a combination of better raw material and improved cooking practices should result in an average improvement by at least a factor of 10. While hash browns prepared by conventional methods in May typically contained 1500–5000 µg/kg of acrylamide, crispy hash browns prepared from fresh potatoes with improved methods contain less than 100 µg/kg acrylamide.

Summary

Acrylamide formation starts at temperatures little above 100°C. Concentrations observed depend on the interplay of formation and elimination. Elimination is particularly fast in meat products; starch is a relatively inert medium slowing down elimination.

The finding of Weisshaar and Gutsche is confirmed that acrylamide is primarily formed by degradation of asparagine. The reaction presupposes a partner with a carbonyl group, fructose and glucose being the important ones at least in the beginning

of the heating process. Particularly in potato with low contents of reducing sugars, acrylamide formation is limited by these sugars.

Cultivars of potato with low contents of reducing sugar also have a low potential of forming acrylamide. However, strong variations among potatoes of the same cultivar suggest that other factors, such as storage, have an even stronger influence. Cooling to temperatures below 8°C causes a drastic increase of the potential of acrylamide formation.

With carefully selected potatoes and improved methods of preparation in the kitchen, as massive reduction of acrylamide concentrations can be achieved.

Zusammenfassung

Die Bildung von Acrylamid setzt bei Temperaturen wenig über 100°C ein. Die beobachteten Konzentrationen hängen vom Wechselspiel von Neubildung und Elimination ab. Elimination ist besonders schnell in Fleischprodukten; Stärke ist ein relativ inertes Medium, das die Elimination verlangsamt.

Der Befund von Weisshaar und Gutsche wird bestätigt, dass Acrylamid vor allem durch Zersetzung von Asparagin gebildet wird. Die Reaktion benötigt eine Carbonylverbindung als Partner. Zumindest am Anfang der Erhitzung scheinen dies vor allem Fructose und Glucose zu sein. Besonders in Kartoffeln mit geringem Zuckergehalt wird die Acrylamidbildung durch Fructose und Glucose gesteuert.

Kartoffelsorten mit tiefen Gehalten an reduzierenden Zuckern haben auch ein geringes Potential zur Acrylamidbildung. Allerdings zeigen starke Schwankungen bei Knollen der gleichen Sorte, dass andere Faktoren, wie z.B. Lagerung, einen noch stärkeren Einfluss ausüben. Kühlung auf Temperaturen unter 8°C bewirken einen drastischen Anstieg des Potentials für Acrylamidbildung.

Mit der sorgfältigen Wahl der Kartoffeln und verbesserten Zubereitungsmethoden in der Küche können die Acrylamidgehalte massiv gesenkt werden.

Résumé

La formation de l'acrylamide commence à des températures peu au-dessus de 100°C. Les concentrations observées dépendent de l'interaction entre la formation et l'élimination. L'élimination est particulièrement rapide dans les produits à base de viande; l'amidon est une matrice relativement inerte qui ralentit l'élimination.

La conclusion de Weisshaar et Gutsche selon laquelle l'acrylamide se forme principalement par dégradation d'asparagine est confirmée. La réaction nécessite un partenaire avec un composé carbonylé. Au moins au commencement de l'échauffement il s'agit surtout du fructose et du glucose. Particulièrement pour les pommes de terre à basses teneurs en sucres réducteurs, la formation d'acrylamide est limitée par ces sucres.

Les sortes de pomme de terre avec une basse teneur en sucre réducteur ont un potentiel plus limité pour former de l'acrylamide. Cependant les fortes variations parmi des pommes de terre de la même sorte suggèrent que d'autres facteurs, tels

que le stockage, ont une influence plus forte encore. Le refroidissement aux températures au-dessous de 8 °C augmente fortement le potentiel pour former de l'acrylamide.

Avec des matières premières soigneusement choisies et des méthodes de préparation culinaires optimisées, une réduction massive de concentrations en acrylamide peut être réalisée.

Key words

Acrylamide in heated food, Storage of potatoes, Cultivars of potato, Asparagine, Fructose and glucose in potato

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Corresponding author: Koni Grob, Official Food Control Authority of the Canton of Zurich, P.O. Box, CH-8030 Zurich