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Potential of Acrylamide Formation, Sugars, and Free Asparagine in Potatoes: A Comparison of Cultivars and Farming Systems

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Glucose, fructose, sucrose, free asparagine, and free glutamine were analyzed in 74 potato samples from 17 potato cultivars grown in 2002 at various locations in Switzerland and different farming systems. The potential of these potatoes for acrylamide formation was measured with a standardized heat treatment. These potentials correlated well with the product of the concentrations of reducing sugars and asparagine. Glucose and fructose were found to determine acrylamide formation. The cultivars showed large differences in their potential of acrylamide formation which was primarily related to their sugar contents. Agricultural practice neither influenced sugars and free asparagine nor the potential of acrylamide formation. It is concluded that acrylamide contents in potato products can be substantially reduced primarily by selecting cultivars with low concentrations of reducing sugars.

KEYWORDS: Potato cultivar; farming systems; potential of acrylamide formation; sugars; asparagine

INTRODUCTION

Haemoglobin adducts of acrylamide and its metabolite glycidamide were observed in humans who had been occupationally exposed to acrylamide (*1*). A high background level of adducts and metabolites found in persons neither occupationally exposed to acrylamide nor being smokers (*2*) led to further research to identify the source of the exposure. Increased levels of haemoglobin adducts found in rats fed with a fried diet revealed that acrylamide may be formed during heating of foodstuffs (*3*).

In early 2002, acrylamide was detected in a range of foods heated during production or preparation (*4*, *5*). Concentrations often exceeded 1000 μg/kg, which caused a worldwide concern because acrylamide is classified as probably carcinogenic to humans (Group 2A) by the IARC (*6*). Particularly high concentrations were found in products of plant origin heated to high temperatures, such as potato chips (US terminology), french fries, pan-fried potato products, or crisp bread, whereas the contents in foods rich in protein were low (*4*). Particular attention was paid to potato products because of the high acrylamide concentrations and the consumption rate as a staple food.

Acrylamide is formed in the Maillard reaction (*7*−*12*). Heating of glucose with asparagine yielded acrylamide at a rate strongly increasing with temperature increasing from 120 to 170 °C. A pathway for the formation of acrylamide via Strecker degradation of asparagine with dicarbonyls was proposed (*7*). It was also shown that acrylamide is directly generated from N-glycosides formed from sugars and amino acids during an early stage of the Maillard reaction (*8*). Becalski et al. (*9*) found that ^{15}N-acrylamide is developed when ^{15}N-(amido)-labeled asparagine was heated with glucose. A proposed pathway starting from asparagine via a decarboxylated Amadori product pointed up the importance of reducing sugars in acrylamide formation (*12*). Consequently, asparagine, glucose and fructose are considered to be the main precursors, asparagine with its amide group delivering the backbone of the acrylamide molecule.

Potato tubers contain substantial amounts of the acrylamide precursors free asparagine, glucose, and fructose (*13*), which may explain the high concentrations of acrylamide in certain potato products. Reducing sugars and free amino acids are also the precursors of flavor components and of browning formed in the Maillard reaction (*14*, *15*), which means that acrylamide is generated parallel with flavors and browning.

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Storage of potatoes at temperatures below 8−10 °C induces a strong increase in sugar contents: The phenomenon is commonly known as "low-temperature sweetening" (*16*). Potatoes of the cultivar Erntestolz stored at 4 °C for 15 days showed an increase in reducing sugars from 80 to 2250 mg/kg (referring to fresh weight). As a consequence, the potential of acrylamide formation at 120 °C rose by a factor of 28 (*11*). Long-term storage at higher temperatures may, however, be a problem concerning sprouting in late spring/early summer, which usually requires the application of sprouting inhibitors (*17*).

Different potato cultivars grown in 2002 at various locations in Switzerland and different farming systems were analyzed for glucose, fructose, sucrose, free asparagine, and free glutamine. To confirm the interrelation of sugars and free asparagine with the formation of acrylamide, potatoes were subjected to a standardized heat treatment inducing formation of acrylamide in a controlled manner (*18*). The procedure for determining this "potential of acrylamide formation" turned out to be more reproducible than the analysis of, for example, potato chips or french fries produced under standardized conditions. The results of this test correlated with the acrylamide contents in products prepared from the same potatoes, such as french fries, pan-fried potato (hash browns), chips, and roast potatoes.

MATERIALS AND METHODS

Collection of Potato Samples. Samples of the cultivars Agria, Appell, Bintje, Charlotte, Desirée, Eba, Naturella, Nicola, Panda, and Santana were collected in Switzerland by the Swiss College of Agriculture (Zollikofen, Switzerland) from end of August to end of September 2002. The potatoes were part of a three year on-farm experiment on 93 plots (20 organic, 31 integrated, 42 conventional farming system) focusing on quality aspects of Swiss potato production. Within this project, all relevant data concerning crop rotation, cultivation technique, and site parameters were collected and potatoes assessed for in terms of quality. According to a defined sampling plan, 55 tubers from 55 plants were taken from each field, making up a total of about 5 kg per sample. Storage conditions were 9 °C at 95−98% relative humidity for all cultivars except Charlotte, which was stored at 6 °C and 90% relative humidity to reduce sprouting.

Samples of the cultivars Erntestolz, Hermes, Lady Claire, Lady Rosetta, Markies, Marlene, and Panda were obtained from Zweifel Pomy-Chips AG (Spreitenbach, Switzerland), a producer of potato chips. These potatoes were harvested in September 2002, stored at 10−12 °C and 90% relative humidity without application of sprout inhibitors, and analyzed in November 2002.

Sample Preparation. Tubers (12−15) of a given sample were washed, and after the water was dripped off, cut lengthwise. From each tuber, one half was grated (holes of 2.5 mm × 7 mm, as typically used to prepare hash browns). The grated material was thoroughly mixed and used for all analyses.

Analysis of Acrylamide. For the determination of the potential of acrylamide formation (*18*), 20 g of grated potato was spread on a grid and placed in a preheated oven at 120 °C for 40 min. After measuring residual weight, water (to a total weight of 20 g) and methacrylamide (Fluka AG, Buchs, Switzerland) as internal standard (500 μg/kg, referring to fresh weight) were added. Acrylamide was then analyzed as described by Biedermann et al. (*19*); after swelling at 70 °C for 30 min, 10 g of sample was extracted with 40 mL of 1-propanol (Scharlau, Barcelona, Spain). The 1-propanol/water was removed by azeotropic evaporation. Acrylamide was extracted from the residue with 3 mL of acetonitrile (Merck, Darmstadt, Germany) and twice defatted with hexane (Merck). To determine the overall yield of sample preparation, 10 μL of butyramide solution (25 μg/mL, corresponding to 500 μg/kg fresh weight; Fluka) was added as a second internal standard to 1.5 mL of defatted acetonitrile extract. The solution was injected on-column onto a short GC capillary column coated in the laboratory with Carbowax 20 M (Fluka). Mass spectrometry with positive chemical ionization monitored the ions *m*/*z* 72 (acrylamide), *m*/*z* 86 (methacrylamide), and *m*/*z* 88 (butyramide). Results were calculated as acrylamide concentrations referring to fresh weight. If the overall yield was less than 40%, the analysis was repeated starting from the evaporation step.

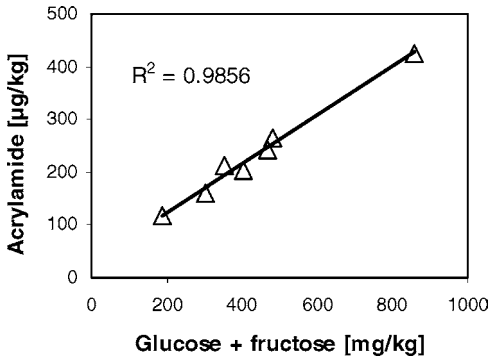

Figure 1. Concentrations of reducing sugars and potentials of acrylamide formation for seven tubers from the same lot of Agria potato.

Measurement of Amino Acids. Free asparagine and glutamine were determined by the method of Arnold et al. (*20*); 10 g of grated potato was diluted with 60 g of bidistilled water, and 1 mL of glycine solution (10 mg/mL) was added as internal standard. After blending with a Polytron (Kinematica, Lucerne, Switzerland) 29 g of bidistilled water was added and the mixture thoroughly shaken. After addition of 50 μL of 1-octanol (Fluka) to break down foam, samples were left to settle for 1 h. If needed, samples were filtered (Schleicher & Schuell, Dassel, Germany). Prior to injection, amino acids were converted to their carbamates by reaction with 9-fluorenylmethylchloroformiate (FMOC-Cl, Fluka). Samples were separated on a 250 × 4.6 mm i.d. column with a C8 packing (MOS Hypersil 5 μm; Bischoff, Leonberg, Germany) with a gradient of acetate buffer/acetonitrile. Fluorescence detection was at 265/340 nm.

Determination of Sugars. Glucose, fructose, and sucrose were determined enzymatically using the test kit from Scil Diagnostics (Martinsried, Germany). A mixture of 20 g of grated potato and 60 g of bidistilled water was homogenized (Polytron). Solutions Carrez I (5 mL) (150 g of potassium hexacyanoferrate(II) trihydrate per liter, Merck) and Carrez II (5 mL) (300 g of zinc sulfate heptahydrate per liter, Fluka) were added. The mixture was thoroughly shaken, the pH adjusted to 7.0−7.5 with a few drops of KOH solution (4 mol/L; Fluka), foam broken by addition of 50 μL of 1-octanol (Fluka), and the volume adjusted to 250 mL with bidistilled water. Filtered samples (Schleicher&Schuell) were subjected to enzymatic analysis as described by the producer.

Statistical analysis. Univariate analysis of variance was performed using the software SPSS (SPSS Inc., Chicago, IL), version 11.0 for Windows. The level of significance α was set to 5%. Tukey-HSD and LSD were accomplished as Post Hoc tests.

RESULTS AND DISCUSSION

Precision of Results. The reproducibility of determining the potential of acrylamide formation was checked on a sample of grated and homogenated Sirtema potato that was exposed to the heat treatment and analyzed for acrylamide four times. At a mean result of 990 μg/kg, the relative standard deviation was 2.0%.

Seven tubers from a lot of Agria potatoes were analyzed individually. Sugar content and potential of acrylamide formation varied strongly (**Figure 1**) but correlated well with each other (R^2=0.9856). The size of the seven tubers varied from small to oversize. However, no correlation between size and sugars or acrylamide potential was found. Because of the observed strong variation between the tubers of the same potato lot, at least 12−15 tubers were analyzed.

Concentrations of Sugars and Amino Acids. The concentrations of the assumed precursors of acrylamide (i.e., glucose,

Table 1. Concentrations of Sugars and Free Amino Acids in Different Potato Cultivars in mg/kg (referring to fresh weight)[a]

	glucose		fructose		sucrose		asparagine		glutamine		
cultivar	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	n
Agria	335	218	143	99	616	205	2547	741	1017	414	22
Appell	1340		780		510		2030		1040		1
Bintje	441	197	239	128	759	129	3280	550	1431	365	12
Charlotte	1140	336	737	293	629	316	2942	597	1897	446	8
Desirée	1090		990		760		3750		1520		1
Eba	710	425	346	275	899	238	2771	799	1557	525	8
Erntestolz	153	76	67	89	1305	209	3214	161	nd		2
Hermes	232	186	163	163	1597	318	4030	16	nd		2
Lady Claire	100	86	44	61	882	331	4250	71	nd		2
Lady Rosetta	116	29	53	1	1443	397	2378	1241	nd		2
Markies	100		30		1020		2570		nd		1
Marlene	97	13	33	15	1123	262	3323	140	nd		2
Naturella	2550		1500		430		2010		990		1
Nicola	2147	571	1537	456	789	174	2849	347	1003	170	3
Panda	195	133	113	88	1471	229	3139	1179	1220	899[b]	4
Santana	690		540		1120		3520		2060		1
Saturna	198	105	95	68	1579	76	3870	1	nd		2
mean	684		435		996		3086		1372		

[a] SD = standard deviation, nd = not determined, no value = no standard deviation calculable. [b] $n = 2$.

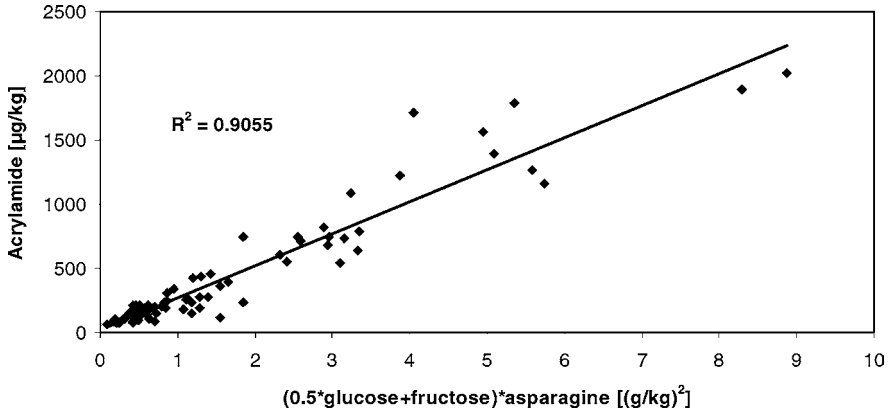

Figure 2. Correlation between the potential of acrylamide formation and the product of the concentrations of reducing sugars and asparagine.

fructose, and free asparagine) are listed in **Table 1** together with those of sucrose and glutamine. Glucose concentrations ranged from 40 to 2700 mg/kg, with the lowest values found in the samples of the cultivars Lady Claire and Marlene, and the highest in Naturella and Nicola. Concentrations of fructose varied similarly but were generally lower than those of glucose, again with the highest values in Naturella and Nicola. Concentrations of fructose were positively correlated with those of glucose ($R^2 = 0.9495$). Sucrose concentrations ranged from 160 to 1800 mg/kg and did not correlate with either glucose or fructose. The high sugar contents in the samples of the cultivar Charlotte were probably due to the lower storage temperature.

Free asparagine was found at concentrations between 1400 and 5170 mg/kg and therefore was generally more abundant than sugars. On a molar basis, the mean content of asparagine was 3.7 or 5.6 times higher than that of glucose or fructose, respectively. Concentrations of free glutamine were lower and ranged from 570 to 2520 mg/kg. Correlation between asparagine and glutamine was clearly weaker than that between glucose and fructose ($R^2 = 0.5930$). The two amino acids showed no correlation with the sugars.

Concentrations of glucose and fructose varied more strongly than those of asparagine: For the cultivar Agria (22 samples), the relative standard deviation was 28% for asparagine but 65 and 69% for glucose and fructose, respectively. In the cultivar Bintje, the variation was similar with a relative standard deviation of the concentrations of 17% for asparagine but 45 and 54% for glucose and fructose. Variations were broad also for the other two components analyzed, as well as the other cultivars (see standard deviations in **Table 1**).

Several authors reported widely varying contents of fructose and glucose within a given cultivar as well as between potato cultivars. Mean contents (ranges) of reducing sugars in the cultivars Trent and Onaway were reported as 340 mg/kg (180−460 mg/kg) and 1640 mg/kg (820−2470 mg/kg), respectively (*21*). For the cultivar Saturna, 1000 mg/kg, 800 mg/kg, and 1070 mg/kg were measured for glucose, fructose, and sucrose, respectively (*22*). Considerable variation was reported also for asparagine and glutamine: For the cultivar Pentland Dell, asparagine ranged from 2060 to 9310 mg/kg, glutamine concentrations from 1760 to 7660 mg/kg (*23*). In Bintje potatoes, free asparagine varied from 1370 to 7600 mg/kg and strongly depended on fertilization (*24*). Total reducing sugars and total free amino acids can vary considerably between different seasons, and storage temperature has a strong impact on the sugar content (*23*).

Potentials of Acrylamide Formation and Correlations. The potentials of acrylamide formation were related to contents of reducing sugars and free amino acids. Thereby the sugar and asparagine concentrations were combined into the product of reducing sugars and asparagine. **Figure 2** shows a strong correlation between this measure and the potential of acrylamide formation (data set including all 74 samples from 17 different cultivars). The formula (0.5 · glucose + fructose) · asparagine

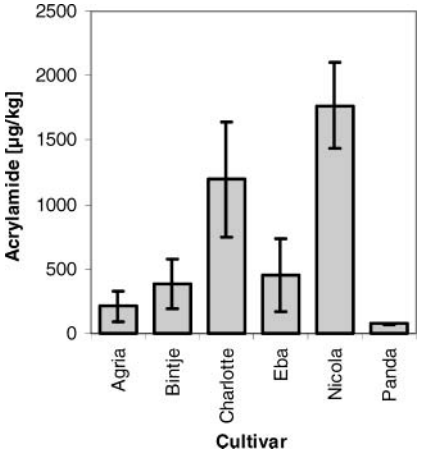

Figure 3. Potential of acrylamide formation in different potato cultivars (mean values, error bars are ± standard deviation).

assumes a bimolecular reaction between sugar and asparagine as the rate-determining step for acrylamide formation. Glucose was weighted half because fructose was about twice as effective as glucose in supporting acrylamide formation (*11*).

The validity of the assumed bimolecular reaction was checked by considering the following alternative ways of calculation: (A) The analogous correlation based on moles instead of weight resulted in virtually the same coefficient ($R^2 = 0.9026$), (B) concentrations (weight or moles) referring to dry matter turned correlations to be slightly weaker ($R^2 = 0.8813$), (C) the correlation of the potentials of acrylamide formation merely with the reducing sugars (i.e., 0.5 · glucose + fructose) was slightly weaker ($R^2 = 0.8768$), and (D) asparagine alone showed no correlation with the potential of acrylamide formation ($R^2 = 0.0062$).

The high correlation between the potentials and the reducing sugars is primarily explained by the stronger variation and the lower values of the concentrations of the reducing sugars, while the asparagine contents were more stable and substantially higher. The small improvement of the correlation when considering the asparagine concentrations confirms this interpretation. It means that, in practice, fructose and glucose determine acrylamide formation, even though they just act as a mediator. This corresponds to the experience that the reducing sugars determine the browning and the flavor formation by the Maillard reaction (*14*).

The asparagine content did not correlate with acrylamide formation in french fries from the cultivar Eba (*25*). Virtually the same strong correlation between the formula (0.5 · glucose + fructose) · asparagine and the potential of acrylamide formation was also observed in potato chips prepared from different cultivars (*26*).

Although sucrose may form acrylamide with asparagine (*8*), no correlation with the potential of acrylamide formation was observed ($R^2 = 0.0402$). Sucrose can contribute to nonenzymatic browning in model systems as well as in potato chips (*27*). However, the far more efficient fructose and glucose seem to completely surpass the activity of sucrose. No correlation between the potentials of acrylamide formation and glutamine ($R^2 = 0.0375$) or dry matter ($R^2 = 0.0470$) was observed.

Influence of Cultivars. For cultivars of which at least three samples have been analyzed, potentials of acrylamide formation are shown in **Figure 3**.

The cultivar Nicola had the highest potential of acrylamide formation (maximum, 2020 µg/kg), followed by Charlotte (maximum, 1700 µg/kg), while the cultivar Panda exhibited the lowest mean potential (80 µg/kg). The difference between the extremes corresponds to a factor of 28, reflecting the large differences in contents of reducing sugars.

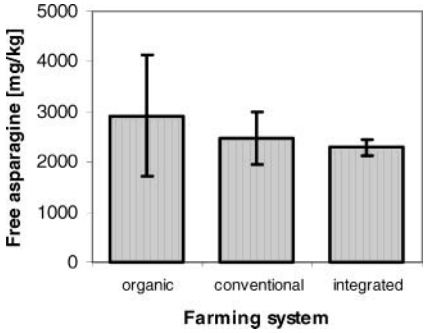

Figure 4. Asparagine concentrations in potatoes of the cultivar Agria from different farming systems (mean values, error bars are ± standard deviation).

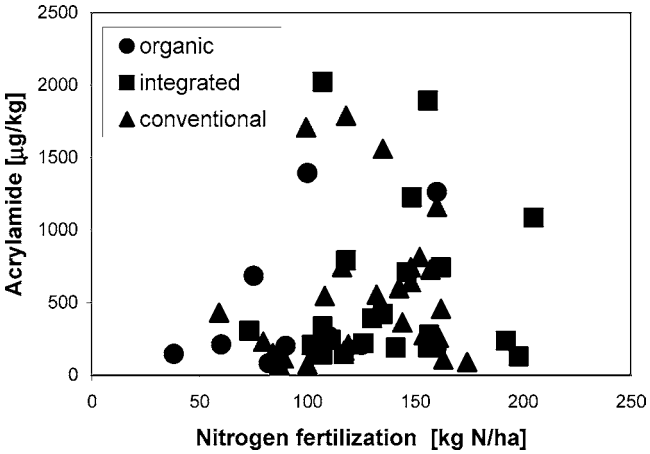

Figure 5. Potentials of acrylamide formation in potatoes depending on total nitrogen fertilization (total N = mineral N + available N in manure) in different farming systems.

The differences between cultivars Panda, Agria, Bintje, and Eba did not turn out to be significant in univariate analysis of variance ($\alpha = 5\%$) and Tukey-HSD test. In contrast, Charlotte and Nicola were each significantly different from all other five cultivars. The glucose and fructose contents in Charlotte and Nicola were also significantly higher from the other four cultivars and significantly different compared to each other. Tukey-HSD test revealed no significant differences between Agria, Bintje, and Panda in terms of glucose and fructose contents. Asparagine concentrations did not differ significantly in any cultivars tested.

The average age of the potatoes, defined as the time from harvest to analysis, was 97 days (72 to 113 days). The age neither correlated with sugars, nor with asparagine, glutamine, or the potential of acrylamide formation.

Influence of the Farming System. No influence of the farming system on glucose, fructose, and asparagine concentrations was observed. **Figure 4** shows asparagine concentrations of the cultivar Agria (data set with 21 samples) grown according to three different farming systems, organic ($n = 6$), conventional ($n = 10$), and integrated ($n = 5$). Statistical analysis revealed no significant differences.

Nitrogen fertilization or the farming system did not significantly influence the potential of acrylamide formation, as shown in **Figure 5** for the three farming systems and 57 samples of potato from 10 different cultivars. This is in agreement with the observation that the farming system did not significantly

influence the contents of reducing sugars and asparagine. Thus the potential of acrylamide formation strongly depends on the cultivar, while cultivation technique only seems to have a marginal influence.

CONCLUSIONS

Data obtained from 74 different samples enable a first classification of 17 potato cultivars that are important in Switzerland with respect to their potential for forming acrylamide. Acrylamide formation in potatoes, determined as potentials at 120 °C, is proportional to the product of the concentrations of reducing sugars and asparagine. Sugar contents vary by a factor of 118 (single values) or 32 (average values for cultivars). The contents of asparagine are higher and vary far less, which explains why glucose and fructose were found to present the determining factor for acrylamide formation in potatoes.

Because there seems to be little possibility for varying the asparagine content, the reducing sugars are considered to be the components through which acrylamide formation can be reduced most efficiently. Neither the farming system nor the extent of nitrogen fertilization influenced the measured components and the potential of acrylamide formation which means that future efforts should focus on cultivar selection.

However, selection of cultivars only achieves this goal if at the same time storage temperatures below 8−10 °C are avoided to prevent substantial release of reducing sugars. In practice, optimization of cultivars and storage conditions are interdependent and many further criteria have to be met, which will need further research.

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