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# Influence of Processing Parameters on Acrylamide Formation during Frying of Potatoes

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Consistent evidence suggests that the probable human carcinogen acrylamide is formed in starch-rich foodstuffs through heat-induced interaction of asparagine and reducing sugars during Maillard browning. However, information regarding the influence of processing parameters on acrylamide formation is scarce. We investigated the impact of temperature, heating time, browning level, and surface-to-volume ratio (SVR) on acrylamide generation in fried potatoes. Acrylamide content was determined by liquid chromatography (LC) and electrospray ionization tandem mass spectrometry (ESI-MS/MS). In potato shapes with low SVR, acrylamide content consistently increased with increasing temperature and processing times. By contrast, in shapes with intermediate to high SVR, maximal acrylamide formation occurred at 160−180 °C, while higher temperatures or prolonged processing times caused a decrease of acrylamide levels. Moreover, browning levels were not a reliable measure of acrylamide content in large-surface products.

**KEYWORDS: Acrylamide; potato; heating; browning; surface; carcinogen; mass spectrometry**

## INTRODUCTION

Detection of high concentrations of acrylamide in common heated starch-rich foodstuffs by the Swedish National Food Administration in April 2002 (*1*) attained considerable public concern, since acrylamide was found to be carcinogenic in rodents (*2*) and is classified as a probable human carcinogen (*3*). These findings gave rise to national health authorities (*4*, *5*), the European Community (*6*), and the WHO (*7*) to initiate concepts for minimization of acrylamide content in commercial as well as in homemade foods. Potato products (such as French fries, crisps, and hash browns) were among the food items containing highest amounts of acrylamide (*1*, *4*). Acrylamide formation was found to occur during the browning process by Maillard reaction of reducing sugars (glucose and fructose) with the amino acid asparagine at temperatures above 120 °C (*8*, *9*). The present study used a factorial design to investigate the impact of temperature, time of heat exposure, surface-to-volume ratio, and degree of browning on acrylamide formation in fried potatoes.

## MATERIALS AND METHODS

**Materials.** Potatoes of the cultivar Bintje were obtained from W. Kurth GmbH (Troisdorf-Spich, Germany). Corn starch oil for frying was from Unilever Bestfoods GmbH (Biskin Reines Pflanzenöl, Hamburg, Germany). Acrylamide (99.9% purity) was purchased from Bio-Rad Laboratories (Munich, Germany); methacrylamide (>98% purity) was from Fluka (Buchs, Switzerland). Analytical-grade *n*-hexane, perchloric acid, and formic acid were supplied by VWR (Darmstadt, Germany). HPLC-grade acetonitrile (Rotisolv) was from Roth (Karlsruhe, Germany). Potassium nitrite and copper sulfate were from Sigma (Deisenhofen, Germany)

**Samples and Sample Preparation.** Potatoes were harvested in September 2002 and stored in bulk piles at constant temperatures (8−10 °C) and humidity (95% relative humidity) for 6 months, at which time they had developed no sprouts or obvious signs of accelerated aging. Postharvest sprouting during storage was inhibited chemically by applying an aerosol of chlorpropham (CIPC) after the wound healing process was complete. Immediately prior to heat processing, the tubers were washed in water, peeled, and cut into three different shapes without further treatments. Cylindrical slices of 30 mm diameter and 15 mm height [surface-to-volume ratio (SVR) 0.27 $mm^{-1}$] and slices of 30 mm diameter and 3 mm height (SVR 0.80 $mm^{-1}$) were produced with a hole shaping device (maximal absolute deviation in each dimension 0.1 mm). Pieces of an average dimension of 16.8 × 6.0 × 1.2 mm (SVR 2.12 $mm^{-1}$) were produced by a manual grater and cut to the same length (maximal absolute deviation in length 0.5 mm, in widths and height 0.1 mm). Batches of five 15-mm slices, 25 3-mm slices, or 150 g of grated potatoes from five different middle-sized tubers were fried in 1500 mL of preheated corn oil at constant temperatures of 120, 140, 160, 180, 200, and 230 °C (maximal absolute deviation 2 °C) for indicated times, generating a total of 119 samples. The maximal processing time for each shape and at each temperature was evaluated in preliminary experiments as the time yielding a level of surface browning ≤3.5 (relative units) compared to the raw material (see below), which was the predetermined threshold for consumption ability. In some experiments the core temperature of the high-SVR shapes was assessed with a microsensor (ETS-D4, IKA Labortechnik, Staufen, Germany).

For each shape and temperature, replicate sample preparations were performed with different tubers and applying at least two processing times.

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**Acrylamide Extraction.** After the frying oil was allowed to drip off, potato samples were cut in small pieces and a homogeneous, micronized powder was obtained by freezing in liquid nitrogen and then crushing in a chilled mortar mill for 5 min. The powder (1.0 g) was extracted with 10 mL of 400 mmol/L perchloric acid in an overhead mixer (Heidolph Instruments, Munich, Germany) for 15 min at 40 rpm and 25 °C. Extracts were separated by centrifugation at 2000 rpm (=1600*g*) for 10 min at 25 °C. Supernatants were further cleaned up by ultracentrifugation at 45 000 rpm (=40000*g*) for 30 min at 20 °C. Methacrylamide was used as internal standard (50 $\mu$g/g, referring to the micronized powder).

**LC-MS/MS Analysis.** Analysis was carried out on a triple-quadrupole tandem mass spectrometer (TSQ Quantum, Thermo Electron, Dreieich, Germany) equipped with a thermostated Surveyor autosampler (Thermo Electron) coupled to a Surveyor HPLC system with a 5 $\mu$m Hypercarb column (50 × 2.1 mm) and a 5 $\mu$m Hypercarb Guard precolumn (10 × 2 mm) (Thermo Electron). Samples were determined in positive electrospray ionization mode ($ESI^+$). Spray voltage was set at 4.5 kV, and capillary temperature was kept at 300 °C. Nitrogen was used as nebulization gas (240 kPa) and as auxiliary gas (70 kPa). Argon was used as collision gas (0.2 Pa). Samples were kept at 10 °C; injection volume was 10 $\mu$L, and injections were made every 5 min. Samples were eluted isocratically. The mobile phase consisted of 98% (v/v) deionized water, 0.1% (v/v) formic acid, and 2% (v/v) acetonitrile at a flow rate of 0.3 mL/min. Only the HPLC eluate from 1 to 5 min was directed into the electrospray interface by means of a divert valve. To avoid analyte accumulation on the column, a water blank was run every seven injections for 5 min at 0.3 mL/min. After 100 matrix injections the precolumn was exchanged, and after 200 matrix injections the analytical column was washed with 100% acetonitrile for 12 h at a flow rate of 0.2 mL/min, followed by reconditioning with mobile phase for 2 h at 0.3 mL/min. Acrylamide and methacrylamide were identified by specific fragmentation spectra. Precursor ion $[M - H]^+ \rightarrow$ product ion transitions for acrylamide were *m*/*z* 72 → 55, *m*/*z* 72 → 54, and *m*/*z* 72 → 44 (collision energy 14 eV). Respective transitions for methacrylamide were *m*/*z* 86 → 69, *m*/*z* 86 → 58, and *m*/*z* 86 → 41 (collision energy 16 eV). For additional qualification, single reaction monitoring (SIM) of the acrylamide precursor ion *m*/*z* 72 was carried out. The scan time for each transition and SIM was 0.25 s; the scan width was 0.5 Da. Resolution settings were 0.7 (fwhm) on both mass filter quadrupoles. Ion transitions of highest intensity, *m*/*z* 72 → 55 for acrylamide and *m*/*z* 86 → 58 for methacrylamide, were used for quantification.

**Validation.** The analytical process was validated according to the EURACHEM/ CITAC Guide (*10*), consistent with the requirements of ISO 17025 (*11*) and SN ENV 13005 (*12*). Ten-point calibrations were performed, employing independently weighted triplicates of pure standard concentrations of 1, 5, 10, 50, 100, 250, 500, 1000, 5000, and 10 000 ng/mL acrylamide in 400 mmol/L perchloric acid, supplemented with 5000 ng/mL methacrylamide as internal standard. For determination of acrylamide concentrations, the peak area ratios of (*m*/*z* 55)/(*m*/*z* 58) were calculated against the calibration curves. Calibration curves were linear, producing correlation coefficients $r \geq 0.998$. Linearity was confirmed by the Mandel test according to DIN 38402 (*13*); variance homogeneity was shown by *F*-test. The limit of detection (LOD) was determined by the blank value method according to DIN 32645 (*14*) as 1 ng/mL (*y*-intercept of the calibration curve plus 3.3 times the repeated standard deviation of six blank samples), and the limit of quantification (LOQ) as 3 ng/mL (*y*-intercept of the calibration curve plus 10 times the repeated standard deviation of six blank samples). The working range reached from 30 $\mu$g/kg to 100 mg/kg acrylamide in a food sample. Process variation coefficients were below 1.5% and differed not significantly between different calibration plots, indicating robustness of the method. The overall run to run variation (intermediate precision) of the analytical process was assessed by six duplicate tests (same homogenized fried sample, complete extraction/determination procedure at different days). This gave a value for the standard uncertainty of the overall process, including run to run recovery variation (relative standard deviation of the differences divided by the square root of 2) of 0.036. The bias of the analytical procedure was investigated during in-house validation by use of spiked samples. Boiled mashed potatoes (1 g) were spiked with 100, 1000, and 5000 ng of acrylamide, allowed to stand for 45 min at room temperature, and then extracted in the same way as normal samples. Mean recovery of 12 samples was 1.007 with a standard uncertainty (SEM) of 0.021. A two-tailed *t*-test revealed that the mean recovery was not significantly different from unity at 95% confidence. Analysis of 34 pure standards of 20, 200, and 2000 ng/mL acrylamide yielded an almost equal mean recovery of 1.004 with a standard uncertainty of 0.009, demonstrating specifity of the method (i.e., the accuracy was independent of the presence of matrix components). The purity of internal standard with an estimated standard deviation of $0.01/\sqrt{3}$ (assuming rectangular distribution) was an additional source of uncertainty, while variation in purity of the acrylamide standard was negligible. Hence, the combined standard uncertainty $u_c(y)$ of the entire analytical process was calculated as 0.042. The expanded uncertainty *U* was calculated by multiplying the combined standard uncertainty with a coverage factor of 2 to give 0.084; i.e., at an approximate level of confidence of 95% the true value of the acrylamide concentration in a potato sample lies between 91.6% and 108.4%.

**Model Reactions and Acrylamide Determination in the Frying Oil.** To investigate acrylamide degradation at high temperatures, 1 mg of acrylamide in 20 $\mu$L of water was heated with 980 $\mu$L of frying oil in sealed glass tubes at indicated temperatures and for indicated times. The acrylamide was extracted with 400 mmol/L perchloric acid (3 × 1 mL) and the aqueous solution was defatted with *n*-hexane (3 × 1 mL). Completeness of extraction (recovery 95−105%) was determined from unheated samples. In some experiments equimolar concentrations of inhibitors of acrylamide polymerization ($KNO_2$ or $CuSO_4$) (*15*) were added. All experiments were performed in duplicate.

To assess a possible transition of acrylamide from the heated potatoes into the frying oil, samples of oil (1 mL) were withdrawn during and at the end of the frying time for each shape and temperature and extracted with 400 mmol/L perchloric acid/*n*-hexane.

**Analysis of Surface Browning.** For each fried potato sample, standardized images were created with a Rollei d41com digital camera and transformed in a digitized 8-bit monochrome grid of 640 × 480 pixels by use of SigmaScan Pro Image Analysis software (SPSS Inc., Richmond, CA). Actual intensity of each pixel (gray level) was represented by a value between 0 (black) and 255 (white) in fill measurement mode. The level of browning was defined by the ratio of average pixel intensity of the sample image before frying compared to average pixel intensity after frying.

## RESULTS AND DISCUSSION

We have developed and properly validated a method for rapid and reliable determination of acrylamide in foods over a wide concentration range. The simple slurry extraction procedure of micronized samples (particle size ≤ 50 $\mu$m) at low temperatures allowed complete extraction without loss of analyte (100% recovery) and thus provides advantages over more (instrumentally or timely) demanding extraction methods (*16*, *17*). In 46 replicate determinations (i.e., repeating the intire process of sample preparation, acrylamide extraction, and measurement), maximal deviations in acrylamide concentrations ≤12% were found, indicating the high reproducibility of the overall process and low variations between individual tubers.

**Temperature, Duration of Heating, and Surface-to-Volume Ratio.** Acrylamide was absent (below the detection limit of 10 $\mu$g/kg) in fresh potatoes. Acrylamide content in potatoes that were oven-dried for 72 h at 50 °C was only 50 $\mu$g/kg of dry weight, without differences between the tested shapes. In contrast, at all applied frying temperatures (120−230 °C) acrylamide concentrations above 1000 $\mu$g/kg were produced, with considerably higher levels in the grated potatoes (max. 18 000 $\mu$g/kg) and the 3-mm slices (max. 12 000 $\mu$g/kg) than in the 15-mm slices (max. 2500 $\mu$g/kg) (**Figure 1**). This finding is in accordance with previous studies (model reaction conditions) suggesting that the formation of acrylamide requires

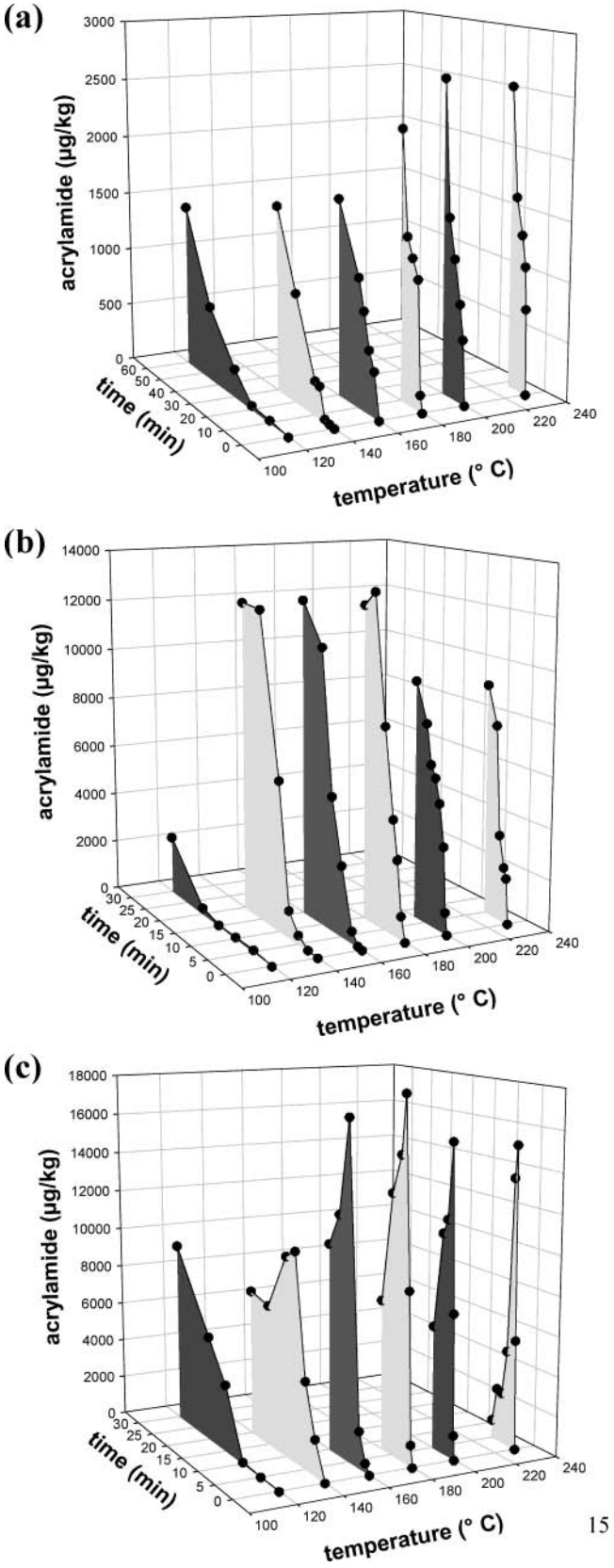


**Figure 1.** Effect of temperature and heating time on acrylamide formation (expressed in micrograms per kilogram of dry weight) in fried potatoes (cv. Bintje) of different shapes: (**a**) cylindrical slices of 30 mm diameter and 15 mm height (surface-to-volume ratio (SVR) 0.27 $mm^{-1}$); (**b**) cylindrical slices of 30 mm diameter and 3 mm height (SVR 0.80 $mm^{-1}$); (**c**) grated tubers with pieces of an average dimension of 16.8 × 6.0 × 1.2 mm (SVR 2.12 $mm^{-1}$).

temperatures ≥120 °C (*8*, *18*) and provides evidence that acrylamide synthesis is mainly limited to the surface of the heated material. However, our factorial investigation revealed that the maximum values of acrylamide produced in a defined shape were less influenced by the magnitude of the frying temperature but more by the time of exposure to a certain temperature. At lower temperatures, longer processing times were required to yield peak levels.

SVR-dependent differences in acrylamide formation may be more than merely a surface phenomenon. It is possible that heat transfer to the core may take longer in shapes with low SVR; thus maintenance of threshold temperatures in the core may be insufficient for significant acrylamide formation. This interpretation may apply especially to the initial stage of the heating process. However, after prolonged heating we measured an approximately equal temperature distribution across the samples with low SVR. Hence, the longer processing times of low-SVR shapes compared to high-SVR shapes (**Figure 1**) ensured that the total time of exposure of the core to the ambient oil temperature differed not significantly between low-SVR and high-SVR shapes.

A consistent increase of acrylamide formation with increasing temperatures was only observed in shapes with low SVR, while in the samples with higher SVR an absolute maximum of acrylamide levels was achieved at 160−180 °C. Interestingly, in these high-surface preparations higher temperatures produced lower maximal levels. Moreover, high temperatures and long processing times were associated with a decrease in acrylamide content. Consistently, in model reactions of asparagine and reducing sugars a maximum of acrylamide yield was reported at 175 °C, while acrylamide formation decreased at higher temperatures (*8*). A decrease of acrylamide content was also reported during prolonged oven heating of potato strips at 200 °C (*19*). This accords with the physical properties of acrylamide that was found to decompose and polymerize on melting above 175 °C (*20*). However, heating of acrylamide in sealed tubes at 120 °C for 30 min, at 180 °C for 10 min, and at 230 °C for 5 min produced a similar (40−50%) decrease of acrylamide content that was not altered in the presence of polymerization inhibitors, indicating that under the applied conditions (mimicking the frying process) the fall of acrylamide content already occurs below the melting point and is predominately due to degradation rather than polymerization. Moreover, it could be excluded that the processed potatoes lose acrylamide due to solution in the frying oil, since we detected no acrylamide in the withdrawn oil samples.

A recent study found that the potential for acrylamide formation in potato tubers of the cultivar Bintje linearly depended upon the content of the reducing sugars glucose and fructose (due to an about 5-fold excess of asparagine concentrations) (*21*). The lack of net acrylamide degradation with increasing time and temperatures of exposure in small-surface material, compared to large-surface shapes, may therefore be explained by different supply of the surface area with the acrylamide precursors, in particular reducing sugars. Thus, in low-SVR shapes, where saturating precursor concentrations are maintained by the large inner reservoir, the temperature-dependent enhancement of acrylamide formation rates appear to continuously exceed the concomitant increase in acrylamide degradation, whereas in high-SVR objects with progressive precursor impoverishment due to the small inner reservoir, degradation dominates synthesis during prolonged heating. The way in which the heat is transmitted to the material (frying, pan-roasting, oven-heating, microwave-heating) appears to have

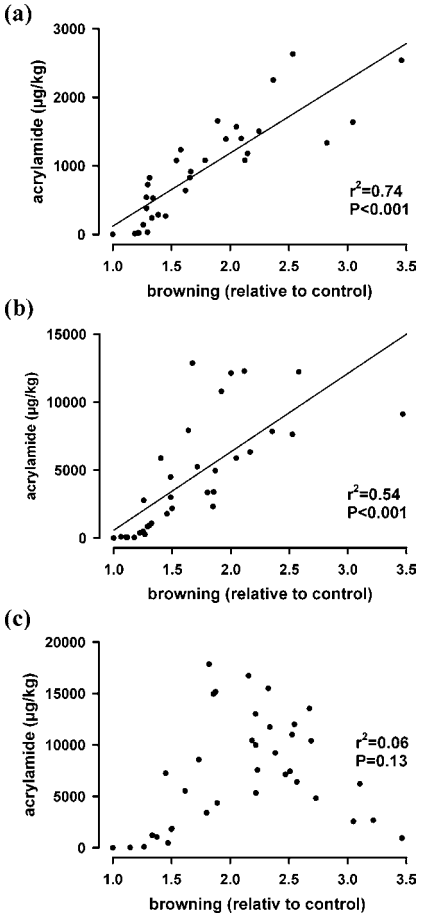


**Figure 2.** Correlation of acrylamide content with surface browning (expressed as the ratio of average pixel intensity of the sample image before frying to average pixel intensity after frying) in potatoes (cv. Bintje) of different shapes: (**a**) cylindrical slices of 30 mm diameter and 15 mm height [surface-to-volume ratio (SVR) 0.27 $mm^{-1}$]; (**b**) cylindrical slices of 30 mm diameter and 3 mm height (SVR 0.80 $mm^{-1}$); (**c**) grated tubers with pieces of an average dimension of 16.8 × 6.0 × 1.2 mm (SVR 2.12 $mm^{-1}$). Conformity with linear correlation was assessed by Pearson's test. $P < 0.05$ was considered statistically significant.

negligible impact on the rate of acrylamide formation. For example, the recently determined acrylamide yield of 400−500 µg/kg after oven-heating of Bintje tubers of standardized small-surface shapes at 120 °C for 40 min (*21*) is consistent with our finding of the 600 µg/kg acrylamide yield in 15-mm slices after frying at 120 °C for 45 min. Additionally, the type of the frying oil did not markedly influence acrylamide content (*17*).

**Browning.** To investigate whether the level of surface browning adequately reflects acrylamide content, we performed a linear regression analysis. A close linear correlation between browning levels and acrylamide concentration could be shown only for small-surface material ($r^2 = 0.74$, $P < 0.001$). A somewhat less close correlation was observed for intermediate-surface material ($r^2 = 0.54$, $P < 0.001$), while for large-surface material no correlation was observed ($r^2 = 0.06$, $P = 0.13$) (**Figure 2**). The correlation plots further reveal marked intra- and interindividual differences in the distribution of acrylamide concentrations over shapes of different SVR at a given degree of surface browning. For example, at a relative browning level of 2 (corresponding to the usual "golden brown" surface color of many fried potato products), the acrylamide content of the 15-mm slices varied only between 1000 and 1500 µg/kg; however, in the 3-mm slices acrylamide content ranged from 2500 to 13 000 µg/kg, and in the grated high-surface potatoes, from 4000 to 18 000 µg/kg (**Figure 2**). Although acrylamide appears to be generated during Maillard browning, i.e., the complex process involving nonenzymatic, heat-induced reactions of amines, amino acids, peptides, and proteins with reducing sugars and vitamin C (*22*), the lack of correlation between surface browning and acrylamide content in material with high SVR is due to net degradation of acrylamide at long processing times, which is not accompanied by a decrease but rather a further increase in browning. However, it should be considered that the degradation of acrylamide was observed at a more severe processing than usually carried out in domestic or commercial practice.

Nevertheless, variations in the data suggest that the browning level alone is not a reliable predictor of acrylamide concentration.

**Conclusions.** Our results indicate that surface area and processing time are important determinants of acrylamide generation during frying of raw potato products. Considerable amounts of acrylamide can be already formed at temperatures of only 120 °C. Under certain preparation conditions (high-SVR products, 2−5 min processing time, 160−180 °C) maximal acrylamide concentrations approximating 20 mg/kg are possible. A high surface browning level does not generally indicate a high acrylamide content.

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