SPECIAL GUEST EDITOR SECTION

# Studies on the Stability of Acrylamide in Food During Storage

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**Acrylamide levels in a variety of food samples were analyzed before and after 3 months of storage at 10°–12°C. The analysis was performed by liquid chromatography tandem mass spectrometry (LC/MS/MS) using deuterium-labeled acrylamide as internal standard. Acrylamide was stable in most matrixes (cookies, cornflakes, crispbread, raw sugar, potato crisps, peanuts) over time. However, slight decreases were determined for dietary biscuits (83–89%) and for licorice confection (82%). For coffee and cacao powder, a significant decrease occurred during storage for 3 or 6 months, respectively. Acrylamide concentrations dropped from 305 to 210 μg/kg in coffee and from 265 to 180 μg/kg in cacao powder. On the contrary, acrylamide remained stable in soluble coffee as well as in coffee substitutes. Reactions of acrylamide with SH group-containing substances were assumed as the cause for acrylamide degradation in coffee and cacao. Spiking experiments with acrylamide revealed that acrylamide concentrations remained stable in baby food, cola, and beer; however, recovery levels dropped in milk powder (71%), sulfurized apricot (53%), and cacao powder (17%). These observations suggest that variations in the acrylamide content of food, especially in coffee and cacao, can vary depending on the storage time because special food constituents and/or reaction products can affect the levels.**

In April 2002, the Swedish National Food Administration reported the finding of alarmingly high levels of acrylamide in heat-treated potato products and other baked goods (1). Many researchers have confirmed the presence of acrylamide in different processed foods, and it was shown that its concentration might reach levels as high as several mg/kg depending on the composition and the way of processing (2–6). Numerous paths of formation have been discussed, predominantly the thermal degradation of the free amino acid asparagine via an Amadori product, which involves the reaction with reducing sugars, such as fructose and glucose (7–10).

Acrylamide is assigned as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence for carcinogenicity in experimental animals and mechanistic considerations (11). How high the risk of contracting cancer is in humans after the intake of acrylamide-containing foods cannot be reliably estimated at present. For reasons of consumer health protection, it is of great interest to reduce the concentration of this substance in food as much as possible, irrespective of the health assessment of acrylamide levels. Therefore, a worldwide monitoring of acrylamide in various products has started in the past 2 years. Many data have been published in several journals and magazines of diverse scientific quality, but to our knowledge practically no articles were concerned with the stability of acrylamide in processed foods. However, this might be of great interest since a minimizing concept was initiated in Germany. Thereby, the Federal Office of Consumer Protection and Food Safety (BVL) collects acrylamide data, which are provided by the official food surveillance laboratories of the federal states and by the Federal Institute of Risk Assessment (BfR), and the data are compiled to form product groups. In the individual product groups those products that count among the 10% group of foods with the highest contamination are being identified. The lowest level within this group in turn acts as signal level. For products belonging to the upper 10% of the respective data pool, the producers are urged by the food surveillance authority to reduce the concentration of acrylamide. However, if it cannot be excluded that acrylamide is not stable in the finished product (and therefore lower acrylamide levels in some products were a result of a longer storage period of the respective sample), this proceeding might not be justifiable without considering the production date.

This paper presents our first findings on the stability of acrylamide in different food matrixes.

## Experimental

### *Chemicals*

Acrylamide was supplied by Sigma (Deisenhofen, Germany) and deuterium-labeled $d_3$-acrylamide by Cambridge Isotope Laboratories, Inc. (CIL; Andover, MA). All other solvents and chemicals used were of analytical grade. Stock solution of standards (1 mg/mL) and working standard solutions (10 μg/mL) were prepared in methanol–water (20 + 80, v/v).

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### Sample Preparation

Depending on the sample matrix, the sample was ground by an Alexanderwerk mill, mesh size 2 mm (Alexanderwerk Inc., Horsham, PA). A 2 g amount of the homogenized sample was weighed into a filter, placed on a Witt'scher pot, equipped with a vacuum pump, and defatted by adding 80 mL iso-hexane (40–80 mL/min). A 200 µL volume of internal standard $d_3$-acrylamide (10 µg/mL) was added to the defatted sample, and after incubation of 30 min, 20 mL water was added. Acrylamide was extracted in an ultrasonic bath (Sonorex RK 510H, Bandelin Elecronic, Berlin, Germany) at 60°C for 30 min. The sample was purified by adding 20 mL acetonitrile and 500 µL Carrez I (potassium hexacyanoferrat, 150 g/L) and Carrez II (zinc sulfate, 300 g/L), respectively. The sample was subsequently centrifuged at 4500 rpm for 10 min (Hettich EBA 8 S, Hettich Zentrifugen, Tuttlingen, Germany), and the supernatant was filtered through a membrane filter (OPTI-Flow®, 0.45 µm NYL, 25 mm, Wicom, Heppenheim, Germany).

### Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) Analysis

The quantification of acrylamide was performed by LC/MS/MS with positive electrospray ionization using an Applied Biosystem API 2000 triple quadrupole mass spectrometer (Darmstadt, Germany) coupled to an Agilent 1100 LC system (Waldbronn, Germany) consisting of an 1100 binary pump, a 1100 thermostated autosampler, and an 1100 thermostated column compartment. The column used was a Merck LiChrospher® 100 CN column (250 × 4 mm id, 5 µm; Darmstadt, Germany) together with a Merck LiChrospher® 100 RP-18 guard column (4 × 4 mm id). The operating conditions were as follows: mobile phase, 50% acetonitrile in 1% acetic acid isocratic for 5 min, followed by rinsing with 100% acetonitrile for 5 min and reconditioning with 50% acetonitrile in 1% acetic acid for 10 min; flow rate, 0.7 mL/min, split ratio 1:5; injection volume, 40 µL; column temperature, 25°C; autosampler temperature, 20°C.

Acrylamide was identified by multiple reaction monitoring (MRM) set to record *m/z* 72 > 72, 72 > 55, and 72 > 44, using a collision energy of 18 eV. Monitored transitions for the internal standard $d_3$-acrylamide were *m/z* 75 > 75, 75 > 58, and 75 > 44. The dwell time for each MRM transition was 200 ms. The collisionally activated dissociation (CAD) operated with 4 mTorr pressure of nitrogen as collision gas. The electrospray voltage was set to 5500 V, the source temperature was 350°C. Quantification was performed by comparison of the peak area ratio of acrylamide with the internal standard $d_3$-acrylamide monitored by using the MRM transitions *m/z* 72 > 55 (acrylamide) and 75 > 58 ($d_3$-acrylamide).

## Results and Discussion

Acrylamide is a rather reactive component, as known from the fact that it reacts at body temperature with hemoglobin in the living organism. Therefore, it could be expected to react rather rapidly with food components.

### Stability of Acrylamide in Carbohydrate-Rich Food

A variety of different storable acrylamide-containing carbohydrate-rich foods, like cookies, crispbread, cornflakes, potato chips, peanuts, raw sugar, and licorice confection were analyzed in January 2004 and re-analyzed in April 2004. Analyses were performed in duplicate. Because earlier studies revealed that acrylamide concentrations in potato chips might not be stable over time, possibly caused by the formation of peroxide when stored fine-ground at ambient temperatures (12), original, not milled samples, were stored. The samples were divided into 2 parts; 1 part was homogenized and analyzed directly and the other was stored in a closed package at 10°–12°C in the dark. The respective losses of acrylamide are summarized in Table 1.

For most samples, acrylamide contents did not significantly differ after 3 months (Figure 1). The recovery was between 90 and 120% and was within the measurement uncertainty. However, a slight decrease was shown for the dietary products and for the licorice confection. For the dietary biscuit, acrylamide content decreased from 530 to 470 ± 12 µg/kg (11% decrease), for the dietary wholemeal biscuit from 2400 to 2000 ± 20 µg/kg (17% decrease), and for the licorice confection from 550 to 450 ± 6 µg/kg (18% decrease). In earlier studies the coefficient of variation (CV) for biscuits was determined to be 2.7% (12), i.e., the rate of decrease was higher than the analytical variability. However, it cannot be excluded that the lower acrylamide contents analyzed after storage were due to the inhomogeneity of the sample within the package, because the former homogenized sample was not re-analyzed but rather one part of the original sample.

A significant formation of acrylamide during storage can definitely be excluded. Slightly increased amounts were found for chocolate cookies, biscuit flour, cornflakes, and peanuts; however, this was within the analytical uncertainty. A possible formation of acrylamide in finished products was suspected by other authors (13) who noted that ammonium accelerates acrylamide formation from asparagine and fructose at low temperatures. In model experiments with wheat flour and corn starch, the addition of fructose (40%) and ammonium carbonate (1%) increased the acrylamide content to 1000 µg/kg during 6 weeks of storage, even at room temperature. Without the addition of ammonium only small amounts of acrylamide were formed (20 µg/kg). In order to investigate a possible formation caused by ammonium, a gingerbread (ammonium bicarbonate is used as raising agent) was re-analyzed after 6 months of storage at room temperature. However, in accordance with our results, acrylamide content did not increase but rather decreased from 850 to 500 µg/kg, i.e., elimination predominated formation. This effect was explained by the loss of ammonium during baking.

**Table 1. Average acrylamide levels (μg/kg) and recoveries (%) determined before and after a respective storage time (month) of different food products ($n$ = 2)**

| Matrix | Storage time | Acrylamide before storage | Acrylamide after storage | Recovery |
|---|---|---|---|---|
| Butter biscuits | 3 | 140 | 150 | 107 |
| Chocolate cookies | 3 | 250 | 275 | 110 |
| Baby biscuit | 3 | 150 | 140 | 93 |
| Wafers composition | 3 | 130 | 130 | 100 |
| Wholemeal spelt biscuit | 3 | 170 | 180 | 106 |
| Biscuit flour | 3 | 45 | 50 | 111 |
| Dietary biscuit | 3 | 530 | 470 | 89 |
| Dietary wholemeal biscuit | 3 | 2400 | 2000 | 83 |
| Crispbread | 3 | 760 | 770 | 101 |
| Wholemeal flakes | 3 | 250 | 260 | 104 |
| Cornflakes | 3 | 80 | 90 | 113 |
| Potato chips | 3 | 500 | 540 | 108 |
| Peanuts | 3 | 50 | 60 | 120 |
| Raw sugar | 3 | 130 | 120 | 92 |
| Licorice confect | 3 | 550 | 450 | 82 |
| Coffee, roasted and ground | 3 | 305 | 210 | 70 |
| Coffee, beans | 3 | 285 | 200 | 71 |
| Coffee, soluble | 3 | 840 | 850 | 101 |
| Coffee substitute | 12 | 1300 | 1200 | 92 |
| Cacao powder | 6 | 265 | 180 | 68 |

### *Stability of Acrylamide in Roasted Products (Coffee, Cacao)*

Coffee is known to contain acrylamide and various other reaction products responsible for the coffee aroma. In Germany, a signal level for roasted coffee exists that is actually 370 μg/kg. Reported values were between 82 and 650 μg/kg ($n$ = 60) with a median level of 260 μg/kg (14). This wide range might be explained by different processing techniques, e.g., the time of roasting. However, another explanation might be a different age of the analyzed samples. It could be possible that acrylamide levels decrease over time as a result of reactions with other constituents, e.g., sulfur-containing substances, even in the original vacuum-packed coffee samples.

In order to examine the stability of acrylamide in vacuum-packaged ground coffee, a storage trial was performed. Several original 250 g vacuum packs of ground coffee were stored at 10°–12°C. All packages were derived from 1 production batch, i.e., had the same date of expiry. To ensure homogeneity of acrylamide in all individual packages, we first analyzed 10 randomly selected packs in duplicate before storage. The data obtained showed sufficient homogeneity. The average acrylamide concentration was 305 μg/kg, and the standard deviation was 21 μg/kg. Ten unopened vacuum packages were then stored for 3 months and analyzed again. Significantly lower amounts were found for all packages. The average acrylamide concentration was 210 μg/kg and the standard deviation was 13 μg/kg, indicating a significant and uniform decrease of acrylamide over time even when vacuum-packaged (Figure 2). An evaporation of acrylamide during storage seems to be very improbable because the samples were stored closed. In addition, studies by Biedermann et al. (15) revealed that acrylamide does not normally evaporate from a test sample even when heated to 120°C.

In order check the stability of acrylamide in roasted coffee beans, we performed the same examination with whole beans packaged in 500 g original packages. A significant decrease of acrylamide levels was determined similar to that in the ground coffee (Figure 2). Average acrylamide concentrations were 285 ± 12 μg/kg before and 200 ± 8 μg/kg after storage. These results comply with findings reported by other authors (16) who noted a strong decrease of acrylamide levels in ground coffee that was stored at room temperature in its original containers but opened once. Re-analyses of selected samples after a 6-month storage revealed a 40–65% decrease. However, no acrylamide degradation was determined for frozen coffee stored at –40°C.

Acrylamide seems to be stable both in soluble coffee (spray-dried coffee extracts) and in coffee substitutes (spray-dried extracts) over 3 months of storage (Table 1). For

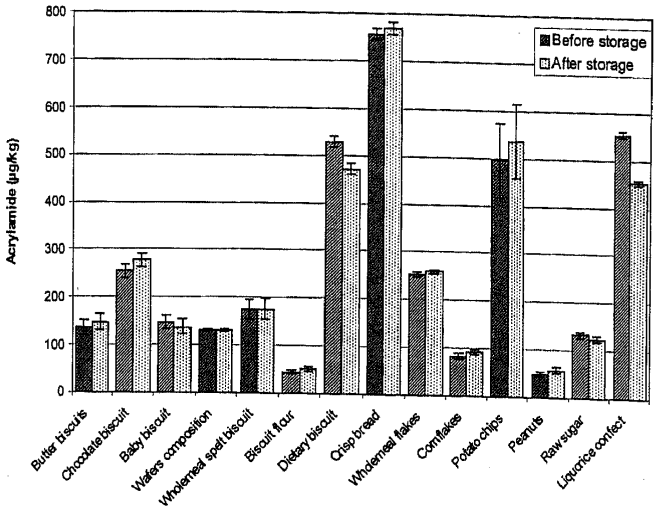


**Figure 1. Acrylamide levels analyzed in special carbohydrate-rich foods and respective concentrations determined after 3 months of storage at 10°–12°C in the dark. Analyses were performed in duplicate. Average levels and standard deviation are given.**

coffee substitutes, no significant decrease of acrylamide occurred even after 1 year of storage. These results suggest that acrylamide losses occur over time both in vacuum-packed ground coffee and in coffee beans, probably as a result of reactions with coffee-typical constituents. It can be supposed that these substances are present in coffee beans but are less (or not) available in the extract or in the spray-dried extract. In agreement with this, Andrzejewski et al. (16) proved that acrylamide is quite stable in brewed coffee, even over 5 h of heating. However, the degradation of acrylamide in soluble coffee has to be further investigated by increasing the storage time.

In addition to coffee and coffee substitutes, the stability of acrylamide was studied in cacao powder. Similar to formation in coffee, acrylamide is formed among numerous other Maillard products during the roasting process of the cacao beans. The cacao powder was stored in closed glass jars at 10°–12°C for 6 months. During this time, acrylamide concentrations significantly dropped from 265 ± 25 to 180 ± 13 µg/kg (Figure 2). This finding confirms the assumption that special substances (possibly formed during the roasting process) present in the sample are responsible for a decrease of acrylamide over time.

### *Stability of Acrylamide in Different Foods After Spiking*

In order to study the stability of acrylamide in special food products that normally contain no acrylamide, different samples (milk powder, baby food, sulfurized apricots, cola, and beer) were spiked with approximately 800 µg/kg acrylamide. For this, 25 g was added with 2 mL acrylamide (10 µg/mL). Apricots were first added with the same portion of water and homogenized to obtain a slurry. The samples were homogenized by intensive shaking and/or stirring. The stored cacao powder was also spiked. The samples were re-analyzed after 12 days storage at 4°C (baby food, sulfurized apricots, cola, and beer) or room temperature (milk and cacao powder). Acrylamide was stable in baby food (vegetable stew), cola, and beer and, to a lesser degree, in milk powder (71% recovery). In contrast, the recovery was only 53% in the sulfurized apricots and 17% in the cacao powder (Figure 3).

The finding that acrylamide degrades in sulfurized samples is in agreement with earlier findings (12). Here, a sugar solution was added with sodium sulfite and spiked with acrylamide. Acrylamide concentrations decreased rapidly within 7 days of storage, whereas concentrations remained stable without addition of sulfite.

The significant decrease of acrylamide concentrations in cacao powder was in accordance with the results obtained by the storage experiment and confirm a degradation of acrylamide by reaction with special cacao constituents. Because acrylamide remained more or less stable in the milk powder, a decrease exclusively as a result of evaporation can be excluded.

### *Causes for Acrylamide Decreases in Foods*

In general, different factors could cause the decrease of acrylamide in food samples. Friedman (17) questioned whether UV light might induce a polymerization of acrylamide in food. Because all samples were stored in the dark, decreasing rates in some samples as a result of acrylamide polymerization caused by UV light can be excluded.

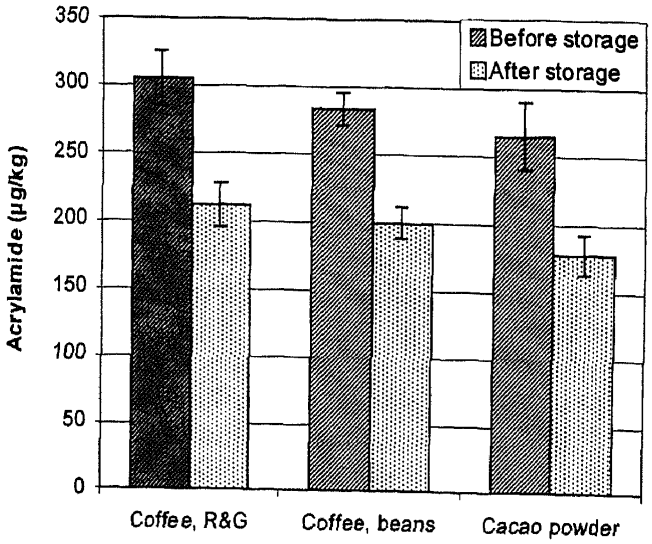


**Figure 2. Average acrylamide levels and standard deviations ($n$ = 10) of vacuum-packaged roasted and ground (R&G) coffee and packaged coffee beans determined before and after 3 months storage at 10°–12°C. Average acrylamide levels and standard deviations ($n$ = 10) for cacao powder were determined before and after 6 months storage at 10°–12°C in the dark.**

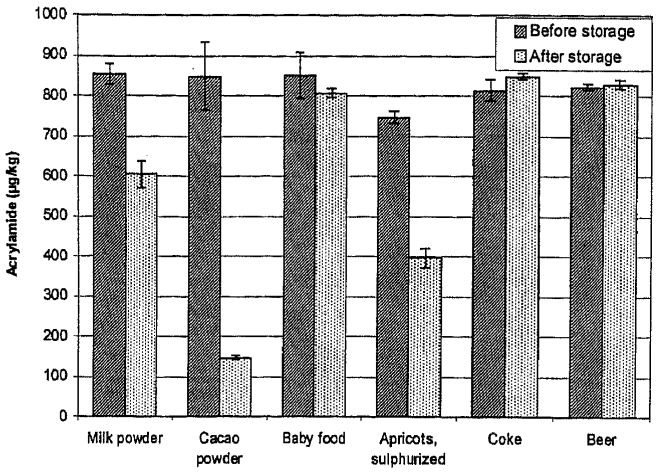


**Figure 3. Acrylamide concentrations of different food samples, spiked with about 800 µg/kg acrylamide, determined in duplicate before and after 12 days' storage. Average levels and standard deviations are given.**

In blood, hemoglobin adducts are formed as a result of the reaction of acrylamide with the ε-$NH_2$ group of the N-terminal valine of hemoglobin (18). Besides the ε-$NH_2$ group of amino acids, SH groups of cysteine residues have a strong affinity for the double bond of conjugated vinyl compound such as acrylamide (17). Several studies on nucleophilic addition reactions of amino and SH groups of amino acids, peptides, and proteins to the double bond of acrylamide were conducted by the working group of Friedman and are summarized in a review (17). They found that SH groups of bovine serum albumin and wheat gluten could selectively be alkylated by acrylamide at pH 7 and 30°C. SH groups were 100–300 times more reactive with conjugated vinyl compounds than were amino groups. The authors supposed that sulfur amino acids could reduce levels of acrylamide in heated food. According to other authors (19), acrylamide disappears in food at a rather high rate, presumably through bonding to reactive food constituents, such as thiols.

Studies of Biedermann et al. (15) have shown that the concentration of acrylamide in food depends on the interplay of formation and elimination, which plays an important role and is particularly fast in meat products. Easily 90% of the acrylamide formed is eliminated again when heated at 120° or 160°C. They supposed that the high elimination rates in meat might be due to reactions with nucleophiles, such as cysteine.

In our opinion, reactions of acrylamide with SH group-containing substances, which are, for example, part of the coffee and cacao aroma, can be assumed as a reason for the significant acrylamide degradation in coffee and cacao. In addition, ε-$NH_2$ and SH groups of amino acids might be responsible for the degradation of acrylamide in spiked milk powder. The decrease of acrylamide caused by the presence of sulfite in sugar solutions was shown earlier (12), and the fact that acrylamide, added to sulfurized apricots, significantly drops after a few days of storage, reinforces the assumption that acrylamide reacts with sulfur-containing substances in the food.

However, sulfur-containing substances might not be the cause of small acrylamide decreases determined in the dietary cookies as compared to conventional cookies. Dietary cookies mainly differ from conventional cookies by the exchange of sucrose by fructose. Biedermann and Grob (13) showed that at higher temperatures (120°–200°C) an elimination of acrylamide in food can occur through its reaction with sugars or their thermal degradation products. Although the addition of sucrose to wheat flour showed no effect on acrylamide elimination, fructose strongly increased the elimination rate. However, elimination rates at room temperature have not been studied so far. Therefore, it can only be suspected that higher rates of fructose were responsible for the lower stability of acrylamide in the dietary products.

In conclusion, these observations suggest that acrylamide concentrations in some foods can vary depending on the storage time, because the levels can be affected by special food constituents and/or reaction products.

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