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Determination of Acrylamide during Roasting of Coffee

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In this study different Arabica and Robusta coffee beans from different regions of the world were analyzed for acrylamide after roasting in a laboratory roaster. Due to the complex matrix and the comparably low selectivity of the LC-MS at *m*/*z* 72, acrylamide was analyzed after derivatization with 2-mercaptobenzoic acid at *m*/*z* 226. Additionally, the potential precursors of acrylamide (3-aminopropionamide, carbohydrates, and amino acids) were studied. The highest amounts of acrylamide formed in coffee were found during the first minutes of the roasting process [3800 ng/g in Robusta (*Coffea canephora robusta*) and 500 ng/g in Arabica (*Coffea arabica*)]. When the roasting time was increased, the concentration of acrylamide decreased. It was shown that especially the roasting time and temperature, species of coffee, and amount of precursors in raw material had an influence on acrylamide formation. Robusta coffee contained significantly larger amounts of acrylamide (mean = 708 ng/g) than Arabica coffee (mean = 374 ng/g). Asparagine is the limiting factor for acrylamide formation in coffee. 3-Aminopropionamide formation was observed in a dry model system with mixtures of asparagine with sugars (sucrose, glucose). Thermal decarboxylation and elimination of the α-amino group of asparagine at high temperatures (>220 °C) led to a measurable but low formation of acrylamide.

KEYWORDS: Acrylamide; 3-aminopropionamide; roasted coffee; derivatization; HPLC; *Coffea arabica*; *Coffea canephora robusta*

INTRODUCTION

The roasting of coffee is done at high temperatures in the range of 240−300 °C (*1*). For laboratory roasting, 24 min at 220−230 °C was evaluated as optimal conditions for the acceptable sensory properties for the coffee beverage (*2*). During roasting of the green coffee the typical dark brown to almost black color develops. The "green baggy" aroma of the fresh green beans at the beginning turns into pleasant fresh roasted to even burned notes (*3*). Certain classes of substances are responsible for these changes in the beans. During the Maillard reaction, Strecker degradation, and pyrolysis reactions of carbohydrates and amino acids, a great variety of monomeric and polymeric compounds are formed that influence the aroma, taste, and other sensory properties of the beverage.

Acrylamide was mentioned by numerous researchers to be one of the hazardous compounds formed during the roasting, baking, and frying of foods. Acrylamide is carcinogenic to laboratory rodents and is described by the International Agency for Research of Cancer as a probable carcinogen to humans (*4*). In the human body acrylamide is oxidized to the epoxide glycidamide (2,3-epoxypropionamide) via an enzymatic reaction involving cytochrome P450 2E1 (*5*). Both acrylamide and glycidamide can form hemoglobin adducts (*6*), but only glycidamide has been shown to form adducts with amino groups of the DNA. It was shown that high levels of acrylamide can cause mutations and cellular transformation (*7*).

Coffee as a research object is important because of its high consumption in some countries and therefore possible hazardous influence on human health. The contribution of coffee to the dietary daily intake of acrylamide is high in countries with a high coffee consumption such as Norway and Sweden, where it can reach 30% (*8*), Denmark, where it can be 20% (*9*), and Switzerland, where it can reach 36% (*10*).

Although coffee beans are roasted at quite high temperatures, the amounts of acrylamide found in the roasted beans and ground coffee are reported to be low (*11*). There are no significant differences in acrylamide formation in normal or decaffeinated coffee. In coffee, acrylamide is formed in high concentrations during the first minutes of roasting, resulting in >7000 ng/g. The increase of roasting time leads to the degradation of acrylamide. Kinetic models and spiking experiments with isotope-labeled [^{14}C]acrylamide showed that >95% of the acrylamide is lost during roasting (*12, 13*). Similar results were reported by Lantz and co-workers (*14*). However, the roasting conditions have an important influence on the typical coffee aroma and taste that are desirable to consumers.

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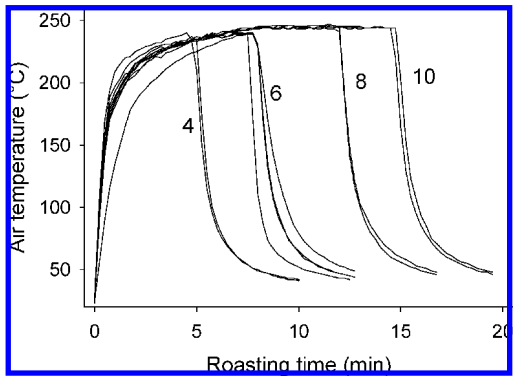

Figure 1. Air temperature profile in the small-scale convection roaster during the roasting experiments.

Therefore, the optimization of the roasting conditions with respect to a reduction of the acrylamide formation and maintaining the product quality has not been realized yet (*15*).

As it was reported in ref *16*, during the brewing step of the coffee beverage, almost all acrylamide present in the coffee powder is transferred to the liquid phase of the coffee drink, due to its high solubility in water. In analyzed grounds after brewing no acrylamide was detected. It seems that all acrylamide available in coffee powder is transferred to the water, where it is quite stable. No significant decrease in acrylamide levels was observed after several hours of heating (*14, 16*). In contrast to filtered coffee, the acrylamide is not transferred completely to the beverage when espresso coffee is prepared.

It has been noted that during storage the acrylamide content decreases (*5, 14, 16*). In instant coffee acrylamide content is reduced by 67% within 1 year of storage and in roasted coffee by 28% within 7 months (*5*). It was reported (*14*) that the storage temperature has a significant influence on the acrylamide degradation in vacuum-packed roasted and ground coffee.

The aim of this study was to investigate coffee for acrylamide formation influenced by time/temperature conditions, precursor concentrations in raw material, and type of coffee bean. Additionally, a model system of carbohydrates and asparagine was used to study the effects of precursors on acrylamide formation.

MATERIALS AND METHODS

Chemicals and Solvents. Water was distilled twice and further purified using a water purification system Simplicity 185 (Millipore, Billerica, MA). 3-Aminopropionamide hydrochloride 97% was purchased from ABCR (Karlsruhe, Germany). Other solvents and chemicals, such as dansyl chloride, approximately 95% TLC, and 2-mercaptobenzoic acid were purchased from Sigma-Aldrich (Steinheim, Germany), and $NaHCO_3$, NaOH, acrylamide >99%, L-asparagine anhydrous, D-(+)-glucose anhydrous, D-(+)-sucrose, glycine, and diethyl ether were purchased from Fluka (Buchs, Switzerland). $^{13}C_3$-Labeled acrylamide of 99+% purity was obtained from Merck-Schuchardt (Hohenbrunn, Germany). Acetonitrile and methanol, both of HPLC grade, acetone, and *n*-hexane were purchased from LGC Promochem (Wesel, Germany); formic acid of 98−100% purity was from Riedel-de-Haen (Seelze, Germany).

Coffee. The green coffee was purchased at Berliner Kaffeeroesterei (Berlin, Germany). The roasting experiments were done either in a small-scale coffee roaster (Heathware Precision Coffee Roaster, Wheeling, IL) or in a drying oven (Heraeus, Hanau, Germany). In the small-scale convection roaster 80.0 g of each type of green coffee bean was roasted using the programs 4, 6, 8, and 10. The temperature profile of the air in the roaster is shown in **Figure 1**.

Coffee Roasting in a Drying Oven. Ten grams of green Arabica or Robusta type of coffee beans was heated in a single layer in a drying oven at different time and temperature conditions. Before roasting, the glass dishes were preheated in the oven for 10 min. After roasting, the samples were immediately put on ice for 15 min. The cooled coffee was ground in an analytical mill (IKA, Staufen, Germany), and the samples were prepared for HPLC-MS analysis using a typical sample preparation procedure. In the drying oven the temperature for roasting was varied from 220 to 260 °C and the roasting time from 5 to 15 min.

Sample Preparation Procedure for Acrylamide Analysis. After roasting and cooling, coffee was ground immediately in a coffee grinder. Coffee (5.0 ± 0.1 g) was put into a 250 mL flask, and the nonpolar fraction (mainly triglycerides) was removed by a double extraction with 25 mL of *n*-hexane for 10 min. The residues of *n*-hexane were evaporated under a stream of nitrogen (purity = 5.0). For extraction of acrylamide 10 mL of cold water was added to 500 ± 0.1 mg of defatted coffee in a centrifuge tube. The values were corrected for a fat content of Arabica being 16% and Robusta being 10% (*3*). After extraction for 30 s in an ultrasonic bath, the samples were centrifuged at 3500*g* for 30 min and filtered (Acrodisc 0.45 μm, Pall Gelman Laboratory, Ann Arbor, MI). Then the supernatant (3 mL) was purified by SPE Bond Elut Accucat (Varian, Middleburg, The Netherlands). The SPE cartridge (200 mg) was conditioned with 3 mL of methanol and 3 mL of water. As acrylamide is not eluted with the first milliliter of eluate, this was discarded and the following 2 mL was collected. Until HPLC analysis the samples were stored at 4 °C.

For the validation of the LC-MS analysis the samples were prepared similarly with the addition of 100 μL of $^{13}C_3$-labeled acrylamide (10 μg/mL) as internal standard before the water extraction.

Derivatization of Acrylamide with 2-Mercaptobenzoic Acid. The derivatization of acrylamide using 2-mercaptobenzoic acid was done according to ref *17*. In brief, 3 mL of the supernatant (after ultrasonic treatment for 10 min) was collected in 4 mL vials, and pH ∼8 was achieved by adding 10 μL of 1 M NaOH. Two hundred microliters of 2-mercaptobenzoic acid (154 mg in 10 mL of 1 M NaOH) was added, and the mixture had to be stirred constantly for 3 h by shaking. The derivatized solutions were diluted 1:10 with purified water and then analyzed by LC-MS.

Preparation of Mixtures of Asparagine with Sucrose and Glucose. Asparagine and sugars in the molar ratios of 1:0.5, 1:1, and 1:1.5 were dissolved in 20 mL of water in a round-bottom flask. The samples were freeze-dried to obtain a fine, dry, and homogeneous powder. Approximately 50 mg of the freeze-dried asparagine, sucrose, or glucose mixtures was heated in 4 mL vials at 130, 150, 170, and 190 °C for 1−30 min in a temperature-controlled (Heidolph EKT 3001, Germany) aluminum heating block. After heating, the samples were cooled for 40 s in the air (20 °C) and for additional 15 min on ice. The heated samples were dissolved in 3 mL of 0.25 M $NaHCO_3$ (pH ∼8). After the aliquot had been sonicated for 10 min, 500 μL of the solution was centrifuged for 5 min, diluted 10 times if necessary, and then derivatized with dansyl chloride for the analysis of 3-aminopropionamide and with 2-mercaptobenzoic acid for the analysis of acrylamide.

Acrylamide Formation from Asparagine at High Temperatures. Fifty milligrams of anhydrous asparagine was heated in 4 mL vials at 210, 230, and 250 °C for 2, 5, and 10 min. After heating, the samples were cooled for 40 s in the air and for another 15 min on ice. After the heated asparagine was dissolved in 3 mL of purified water and sonicated for 10 min, the aliquots were derivatized with 2-mercaptobenzoic acid and analyzed for acrylamide by HPLC-MS.

Derivatization of 3-Aminopropionamide with Dansyl Chloride. To detect 3-aminopropionamide by HPLC with fluorescence detection, the samples were derivatized with dansyl chloride [(5-dimethylamino)naphthalene-1-sulfonylchloride] (according to ref *18*). For derivatization, 100 μL of the dansyl chloride solution (5 mg/mL in acetone) was added to 100 μL of sample in a test tube. The mixture was thoroughly mixed and left in the dark overnight. To eliminate excessive dansyl chloride, 20 μL of a glycine solution (100 mg/mL) was added after the reaction and left for another 15 min at ambient temperature. The reaction mixture was then extracted twice with 1 mL of diethyl ether. The combined extracts were dried under a stream of nitrogen, and the residue was redissolved in 700 μL of acetonitrile. The analysis was performed with LC-FLD.

HPLC-FLD Operating Conditions. For HPLC analysis 10 μL of the sample was injected into a HP 1100 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) with a fluorescence detector, equipped with a cooling autosampler set at 6 °C, a quaternary pump, and a degasser. The HPLC column Merck Lichrosphere 60 Select B 125 × 4 mm i.d., 5 μm particle size (Merck, Darmstadt, Germany), with a precolumn was used. Analysis was performed at ambient temperature. As a mobile phase 10% of acetonitrile and 90% water were used. The gradient elution program was held to 30% of acetonitrile for 2 min, increased to 70% at 12 min, and held until the end of the run (20 min) with a flow rate of 1 mL/min. Dansyl chloride derivatives were detected with a fluorescence detector, set at $\lambda_{Ex} = 320$ nm and $\lambda_{Em} = 500$ nm. Quantification was done by external calibration using standard solutions of 3-aminopropionamide in 0.25 M $NaHCO_3$ (50−1000 ng/mL), which have been derivatized with dansyl chloride in the same manner. The limit of detection (LOD) was determined as 17 ng/mL, the limit of quantification (LOQ) as 30 ng/mL, and the RSD as 3.2%.

HPLC-MSD Operating Conditions for Acrylamide Analysis. LC-MS analysis was carried out by using an Agilent HP 1100-MSD equipped with a vacuum degasser, a quarternary pump, an autosampler, a temperature-controlled column oven, and a diode array detector, which was coupled to a MSD equipped with an electrospray source. For the chromatography a phenyl-hexyl 150 × 3 mm i.d was used. A 3 μm particle size analytical column was used. The eluent composition was 30% acetonitrile and 70% acetic acid (0.1%) aqueous solution. The analytical column temperature was 25 °C. A flow rate 0.4 mL/min was performed in isocratic elution. The sample injection volume was 3 μL. Detection was performed at a MS positive (API-ES) mode, SIM at *m/z* 226 (for protonated acrylamide derivative) and 248 (for Na^+ adducts of derivatized acrylamide) and at *m/z* 229 and 251 for labeled derivatives, respectively. The acrylamide concentration of the samples was calculated by external calibration. The LOD in coffee was determined as 157 ng/g, the LOQ as 270 ng/g, and the RSD as 5.6% using software Validata (version 1.01). For determination of the recovery, experiments were performed using $^{13}C_3$-labeled acrylamide. The recovery was determined as 45%.

Carbohydrate Analysis. The analysis of the carbohydrates was described earlier (*19*). The method is described briefly: 100 mg of the ground green coffee was extracted with 2 mL of water in an ultrasonic bath and centrifuged for 10 min at 16000*g*. After centrifugation, the pellet was reextracted twice with 500 μL of water. The pooled supernatants were diluted to 10 mL with water, passed through a 0.45 μm membrane, and diluted further with water according to the concentrations needed. All analyses were carried out in duplicate.

The chromatographic analysis was carried out on a HPLC HP1100 (Agilent, Waldbronn, Germany) using a CarboPac PA20 (150 × 3 mm, 6.5 μm, Dionex, Sunnyvale, CA) and a precolumn of the same material (30 × 3 mm). For electrochemical detection a HP 1049A (Hewlett-Packard) equipped with a gold electrode was used in pulsed amperometric mode [$P = 0.10$ V (800 ms), $P_1 = +0.60$ V (300 ms), $P_2 = -2.00$ V (300 ms)]. The separation of the mono- and disaccharides was carried out isocratically with water (18.2 MΩ cm^{-1}) at a flow rate of 0.45 mL/min. For detection 100 mM NaOH was added postcolumn at a flow rate of 1 mL/min. After each analysis, the column was washed with 300 mM NaOH for 15 min. For the separation the column was thermostated to 31 °C. The injection volume was 20 μL. For identification the retention times were compared with those of authentic standards (mannitol, arabinose, galactose, sucrose, glucose, mannose, and fructose). The quantification was done by external calibration. Due to easy overload of the column by sucrose, two different dilutions had to be analyzed: one was used for sucrose and the higher concentration of the extract for the minor carbohydrates.

Amino Acid Analysis. The analysis of the amino acids was described earlier (*19*). The method is described briefly: 200 mg of the fine ground green coffee was extracted in 10 mL of 0.1 N HCl in an ultrasonic bath for 15 min, filtered, and diluted appropriately. α-Aminobutyric acid was used as internal standard. For derivatization, 10 μL of the sample and 10 μL of the internal standard (2.5 mM) were buffered to pH 8.8 with borate buffer to a total volume of 80 μL. The derivatization was initiated by the addition of 20 μL of reagent solution (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, AccQ.Fluor, 3 mg/mL in acetonitrile). The samples were heated to 55 °C for 10 min and then analyzed by HPLC with fluorescence detection. The HPLC system (HP 1100, Agilent, Waldbronn, Germany) consisted of a quaternary pump, a vacuum degasser, a thermostated autosampler, a thermostated column department, and a fluorescence detector. Separation of the derivatized amino acids was performed on an AccQ.Tag amino acid analysis column (150 × 3.9 mm, 4 μm, RP18; Waters, Milford, MA) by gradient elution. Three eluents were used: A [100 mL of the concentrate (148 g of sodium acetate·$3H_2O$ and 7.06 g of triethylamine were added to 1 L of water, the pH was set to 5.70 with 50% phosphoric acid and then diluted to 1100 mL)], B (similar composition but pH set to 6.80), C (acetonitrile), and D (water). During the separation the flow rate was set to 1 mL/min. The linear gradient was as follows: 0 min, 90% A, 90% B; 0.5 min, 89% A, 10% B, 1% C; isocratic until 17 min; 20 min, 88% A, 10% B, 2% C; 30 min, 86% A, 9% B, 5% C; 36 min, 63% A, 25% B, 12% B; 36.5 min, 0% A, 87.5% B, 12.5% C; 36.8 min, 22% A, 65.5% B, 12.5% C (flow set to 1.3 mL/min); 40 min, 22% A, 65% B, 13% C; 50 min, 22% A, 63% B, 15% C; then the column was washed without buffer for 7 min and reconditioned for another 9 min (60% C, 40% D). The column temperature was maintained at 39 °C, and the injection volume was 5 μL. For detection of the derivatized amino acids the fluorescence of the eluent was monitored at $\lambda_{Ex} = 250$ nm and $\lambda_{Em} = 395$ nm at a photomultiplier gain of 12. The amino acids were identified by comparison of the retention times with those of authentic standards.

The described methods were fully validated, including the determination of the LODs and LOQs for all investigated compounds. Calibration curves were obtained from five different concentrations (in duplicate or 4-fold, respectively, for the lowest and highest concentrations used) versus the peak areas. The linearity of the calibration curves was checked by linearity tests according to the method of Mandel. The suitability of the linear model was verified by the analysis of the residuals (*20*) software supported (MS-Excel macro VALIDATA) (*21*). Furthermore, for the validation of the methods the variances were tested for their homogeneity based on the 95 and 99% confidence intervals.

Calculations of LODs and LOQs are based on the calibration function. LOD is defined as the lowest concentration of the analyte that can be determined with a SD = 5%.

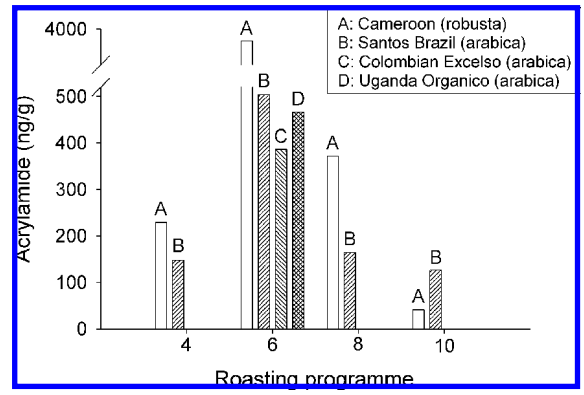

Figure 2. Formation of acrylamide during roasting of different types of coffee at 240 °C.

RESULTS AND DISCUSSION

Four different types of coffee were roasted in a laboratory roaster: Cameroon Robusta (low quality), Santos Brazil NY 2 17/18 TOP Italian preparation, Arabica (low quality), Colombian Excelso Centrals mild, Arabica and Uganda Organico Biocoffee, and Arabica (high quality), analyzed for its acrylamide content. **Figure 2** shows acrylamide content in Cameroon Robusta and Santos Brazil Arabica coffees after roasting according to programs 4, 6, 8, and 10 (the roasting times were 6, 7.5, 12, and 14.5 min, respectively). The different roasting programs differed in only roasting time, having a repeatable maximum temperature between 240 and 250 °C. Both Arabica coffees

Table 1. Acrylamide Content in Coffee Roasted in the Small-Scale Convection Roaster at 240 °C

coffee	sucrose (mg/g)	asparagine (μg/g)	acrylamide formed (ng/g)
Robusta Indian Parchment	56.3	780	762
Robusta Vietnam	41.9	813	653
mean	49.1	797	708
SD	10.2	23	77
Kenya AA	90.1	395	299
Indonesian Sumatra Lintong	82.5	289	301
Costa Rica Tarazzu	79.9	418	310
Nicaragua Talia Extra SHG	78.1	559	433
Mexico Maragogype	76.4	532	363
Ethiopian Sidamo Virgamo grade 2	71.3	505	425
Papua New Guinea Sigri C	70.9	475	352
Guatemala SHB	65.6	617	374
Honduras SHG	65	499	307
Indian Monsooned Aspinwalls Malabar AA	63.8	575	575
mean	74.4	486	374
SD	8.5	97	86

Colombian Excelso and Uganda Organico were roasted using only program 6, which was done as control experiments. The standard roasting procedure that results in an acceptable product is 6 (heating time = 7.5 min, **Figure 1**). However, the roasting time to obtain an optimal product depends mainly on the type of bean (composition, humidity, size) and has to be optimized for each green coffee. The experiments carried out here were all done by applying constant energy input and did not relate roasting to color development or aroma formation. The results showed that in Cameroon Robusta the acrylamide content reached nearly 3800 ng/g with a quick degradation after the maximum. The maximum that was obtained from the Arabica beans was only 13% (500 ng/g) of that of Robusta. Prolonged heating of coffee resulted in a reduction of acrylamide.

The influence of the precursor content in green coffee on the formed acrylamide roasted at 240 °C using program 6, which is 7.5 min of roasting, is collated in **Table 1**. The main precursors are sucrose and asparagine. The concentration of sucrose in green coffee was 49.1 ± 10.2 mg/g in Robusta varieties and 74.4 ± 8.5 mg/g in Arabica varieties, and the concentration of free asparagine in Robusta was 797 ± 23 μg/g and in Arabica, 486 ± 97 μg/g (*19*). The other carbohydrates might not contribute significantly to the reaction due to the low concentration in the case of monomeric carbohydrates. The polymeric carbohydrates present (*22*) might not contribute to the acrylamide formation as well. Statistical analysis of the correlation of the concentration of carbohydrates in green coffee to the formed acrylamide showed only for sucrose a significant correlation. The correlation of acrylamide formation with the content of either sucrose or asparagine is shown in **Figure 3**. The different types of coffee from different origins were selected with the aim to have the highest possible variation in the natural precursor concentration. The correlation showed that higher sucrose content leads to a reduced acrylamide formation and that an increased content of asparagine resulted in a higher formation of acrylamide. As the maximum of acrylamide was formed at a molar ratio of around 1:1 (*23*) and a surplus of either of the compounds resulted in lower formation, it can be concluded that in coffee asparagine is the limiting compound in the acrylamide formation. This means that the investigated Robusta coffees having less sucrose and more asparagine form significantly more acrylamide than the Arabica coffees. The Indian Monsooned having intermediate concentrations of the precursors formed also intermediate amounts of

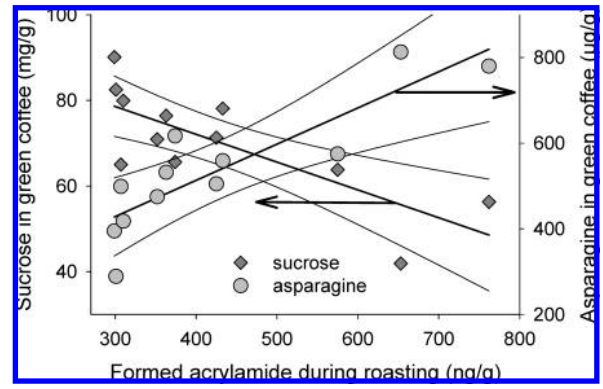

Figure 3. Correlation of asparagine and sucrose contents with acrylamide formation.

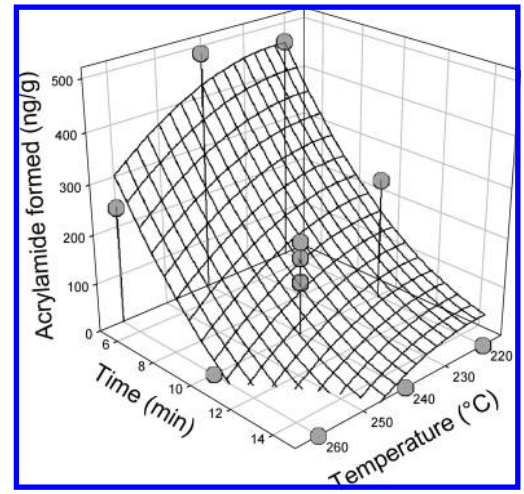

Figure 4. Formation of acrylamide during roasting of coffee.

acrylamide. The results are in accordance with other studies (*14*) in which it was reported that asparagine is a limiting factor in acrylamide formation during the roasting process.

The influence of roasting time and roasting temperature can be seen in **Figure 4**. The experiment shown is based on a three-level factorial design 3^2 with four repetitions of the central point (Statgraphics plus for Windows, 2.0). This also reflects the data as discussed in **Figure 2**. The experiment was carried out in a drying oven, which allowed different temperatures from 220 to 260 °C to be applied. The roasting time was varied from 5 to 15 min. To reduce the influence of limited heat transfer, the coffee beans were put as a single layer in preheated glass dishes of 12 cm diameter. In this experiment the highest concentrations of acrylamide were obtained at low temperatures (220 °C) and a short roasting time (5 min). During more intense roasting the acrylamide was degraded until it could no longer be detected. After 15 min, there was no more acrylamide present at any temperature tested. At 260 °C the acrylamide was completely degraded already after 10 min.

Following the degradation of asparagine during roasting (**Figure 5**) it can be seen that—although asparagine is not a very strongly reacting amino acid in the Maillard reaction—the amino acid reacted quickly with other food components at 240 °C or was thermally degraded without any other reaction partner. In <5 min the asparagine could not be found any more. This result is interesting as acrylamide can also be formed from asparagine by thermal decarboxylation and elimination of the amino group. The formation of acrylamide from asparagine without any other reaction partner is shown in **Table 2**. The highest amounts of acrylamide were found after asparagine had

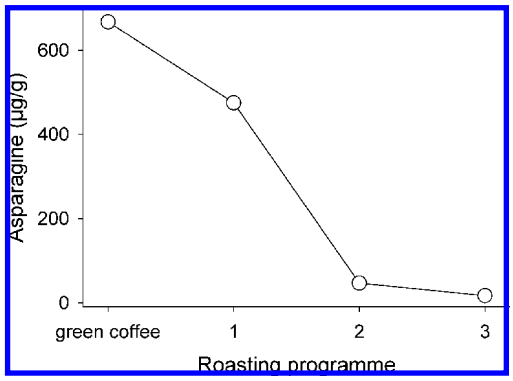

Figure 5. Degradation of asparagine during roasting of coffee at 240 °C.

Table 2. Formation of Acrylamide (Micrograms per Gram) from Asparagine

	heating time		
heating temperature (°C)	2 min	5 min	10 min
210	0	0	0
230	0	2.4	4.0
250	6.0	6.2	0

been heated to 260 °C for 2 and 5 min. Longer heating resulted in a complete loss of acrylamide. However, the amounts of acrylamide formed were comparably low (2−6 ng/g). Additionally, this pathway might not be relevant in coffee roasting because at the time needed for this reaction the asparagine has already reacted completely with other food components and is therefore no longer available for this reaction.

It was recently reported that 3-aminopropionamide (3-APA) might be an important precursor for acrylamide formation (*18*). In this study mixtures of asparagine with carbohydrates (sucrose, glucose) of different molar ratios were heated and analyzed for acrylamide as well as 3-APA. The heating experiment was carried out at 130, 150, and 170 °C for 7 min. Both of the substances, 3-APA (**Figure 6**) and acrylamide, were detected. At 130 °C no acrylamide was formed in the model systems either from glucose or from sucrose. At 150 °C the formation of acrylamide began, but only in the model system containing glucose. Increasing the temperature to 170 °C resulted in acrylamide formation in both model systems. The concentration of acrylamide in the sucrose asparagine model system was 360 µg/g, whereas in the glucose model 416 µg/g was formed. There was no clear dependence of acrylamide formation on the molar ratio of carbohydrate to amino acid.

The asparagine mixture with glucose had a higher capacity even at lower temperatures (130 °C) to form 3-APA. The highest amount of 3-APA was detected in the mixture of glucose with asparagine heated to 150 °C. The molar ratio of asparagine to glucose does not strongly influence the 3-APA formation in this model system. Using sucrose instead of glucose and higher temperatures (170 °C), a reduced formation of 3-APA was observed at higher molar ratios of sucrose to asparagine. Using sucrose there was no 3-APA formed at 130 °C. This might indicate that sucrose itself is not reactive. 3-APA could not be detected in any experiments when coffee was roasted. This might be due to the high reactivity of this compound, which results in a low concentration of the intermediate, or, as 3-APA was not detected in model experiments using higher temperatures, a different pathway of acrylamide formation might occur.

The results of this study show that the acrylamide levels in roasted coffee beans decreased with increased time of roasting.

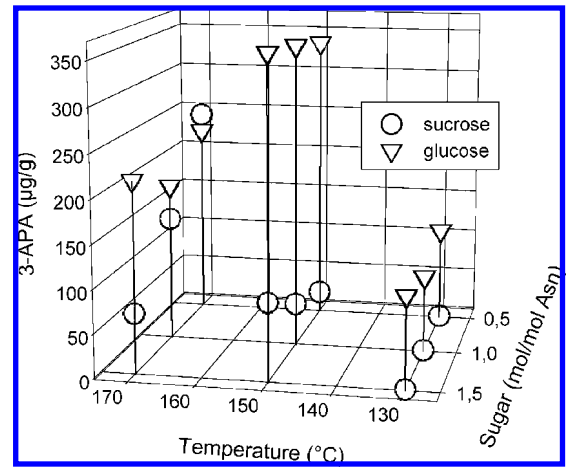

Figure 6. 3-Aminopropionamide formation in a model system.

The main factors affecting the acrylamide amount formed in coffee were type of coffee (Arabica/Robusta) and time and temperature conditions during roasting. Asparagine amount in the raw material seems to be a limiting factor for the acrylamide formation in coffee. 3-Aminopropionamide could be a potential precursor for acrylamide. In the model system with asparagine and sugars (sucrose, glucose) this substance was formed at rather low temperatures compared to the ones when coffee roasting took place.

Because coffee is a product with very stringent quality attributes, it seems that there are not many options currently available for mitigation of acrylamide during the roasting process. As the aroma is a result of the roasting process, which is related to the chemical composition of the raw material, every change in the raw material or the roasting process leads to totally different product characteristics. However, for future developments a selection of coffee varieties with a lower content of asparagine might be a possibility to reduce the acrylamide content.

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