

# Quantification of 3-aminopropionamide in cocoa, coffee and cereal products

## Correlation with acrylamide concentrations determined by an improved clean-up method for complex matrices

Michael Granvogl · Peter Schieberle

Received: 3 August 2006 / Revised: 26 September 2006 / Accepted: 28 September 2006 / Published online: 5 December 2006  
© Springer-Verlag 2006

**Abstract** Based on recent results confirming 3-aminopropionamide (3-APA) as a very effective precursor of acrylamide in the absence of further “catalysts”, this compound was quantified for the first time in cocoa masses, cocoa beans, coffee and cereal products by LC–MS–MS after derivatisation with dansyl chloride. Cocoa masses contained  $> 3000 \mu\text{g}/\text{kg}$  of 3-APA, but varied significantly in its concentration. For the quantification of acrylamide (AA) in cocoa and coffee, an improved isolation procedure using charcoal was developed. In various samples of unroasted and roasted cocoa beans, the concentrations of AA were by a factor of  $> 5$  lower than those of 3-APA, but the concentrations of 3-APA and AA were more closely correlated as compared to the concentrations of AA and asparagine. Experiments on authentic cocoa beans from Ghana and Sulawesi indicated that the thermal generation of 3-APA during roasting was much more pronounced as compared to its biochemical formation. By administering fermented cocoa beans with [ $^{13}\text{C}_4^{15}\text{N}_2$ ]-asparagine before roasting, 3-APA was confirmed as transient intermediate in AA formation during cocoa roasting. Among the cereal products analysed, in particular popcorn contained quite high amounts of 3-APA, which were also well correlated with the AA concentration. Contrary, in coffee products, 3-APA was always lower than AA.

**Keywords** Cocoa · Coffee · Cereals · 3-Aminopropionamide · Acrylamide · Labelling studies

M. Granvogl · P. Schieberle (✉)  
Chair for Food Chemistry,  
Technical University of Munich,  
Lichtenbergstraße 4,  
85748 Garching, Germany  
e-mail: peter.schieberle@ch.tum.de

## Introduction

After it had been discovered by Swedish researchers that acrylamide (AA) is generated during food manufacturing involving a heat treatment [1], numerous studies were published aimed at determining the amounts of this food-borne toxicant in thermally processed foods [2–5]. For example, very high amounts of AA in the mg/kg range have been measured in potato chips [1, 3, 6], but also in gingerbread [7], crisp bread [8] and roasted nuts [9]. Shortly after the first report on the occurrence of AA in processed foods, the amino acid asparagine was identified as the key precursor of AA, when reacted in the presence of reducing carbohydrates [10], dicarbonyls [11, 12], but also 2-oxo acids [12], 2-oxoaldehydes [12] or simply aldehydes [13]. However, while a good correlation of the amount of free asparagine in the raw material with the AA concentrations formed was established for bakery products [7, 14–17], it is not yet possible to “predict” the amount of AA, e.g., formed in potato products [18–20] based on the available free asparagine.

With respect to the formation pathway, Stadler et al. have suggested that a decarboxylation of *N*-glycosides is the most important step leading to unstable ylides as intermediates [21], which should then directly release AA. However, although simply a decarboxylation/deamination reaction would yield AA from asparagine, the detailed formation pathway from asparagine is still under discussion.

3-Aminopropionamide (3-APA), which can be assigned as the biogenic amine of asparagine, has first been reported as a minor degradation product of asparagine, when reacted in the presence of glucose [13]. In a quantitative study, we could, however, show that 3-APA is not a minor product, but is formed in significant amounts during asparagine degradation in the presence of reducing carbohydrates [22]. In addition, a comparison of the time course of the formation

of 3-APA and AA from a binary mixture of asparagine and 2-oxopropionic acid revealed that the propionamide is formed earlier than AA [12, 23]. Furthermore, we could show that 3-APA is degraded into AA in very high yields and, also, no further reactant is necessary [12, 22]. These results suggested that 3-APA might be a transient intermediate in AA formation.

Recently, we could identify 3-APA for the first time as a food constituent in processed Gouda cheese and, furthermore, its role as transient intermediate in AA formation in foods based on an experiment using carbon-13 labelled asparagine was confirmed [23]. Because 3-APA very effectively generates AA in amounts above 50 mol% simply upon heating [22], its occurrence in foods, which might undergo an (additional) thermal processing step, such as cocoa or coffee, might be an additional source of AA formation.

Since comprehensive quantitative data on 3-APA in foods are not yet available, the purpose of this investigation was (i) to quantify 3-APA in several thermally processed foods and (ii) to clarify whether its occurrence shows a correlation with the amounts of AA. Because it is still open, whether in fermented foods, such as cocoa, 3-APA is formed enzymatically as “biogenic amine” or whether its formation is preferably induced by heat, a labelling experiment with cocoa beans was performed to indicate the influence of fermentation and roasting on 3-APA as well as on AA formation.

## Materials and methods

### Food samples

Cocoa masses processed from roasted cocoa beans were obtained from German suppliers. Roast and ground coffee, coffee surrogate and coffee extract (instant coffee) were obtained from the Institute for Reference Materials and Measurements (Geel, Belgium). Authentic samples of cocoa beans were obtained from on-site trials on cocoa bean fermentation, which were performed in Sulawesi (SU) and Ghana (GH). The fermentation and roasting was performed as reported earlier [24].

### Chemicals

3-Aminopropionamide ( $\beta$ -alaninamide) hydrochloride was obtained from Chemos (Regenstauf, Germany), [ $^2\text{H}_3$ ]- (98%) and [ $^{13}\text{C}_3$ ]-acrylamide (99%) were from CIL (Andover, MA), acrylamide (99.9%), asparagine monohydrate and glucose from VWR International (Darmstadt, Germany), activated charcoal (type Norit SA2, from peat, steam activated, powder), 5-(dimethylamino)-1-naphthalene sulfonyl chloride (dansyl chloride), glycinamide hydrochloride, norleucine, and [ $^{13}\text{C}_4^{15}\text{N}_2$ ]-asparagine monohydrate were from

Aldrich (Sigma–Aldrich, Steinheim, Germany). All other reagents were of analytical grade.

*Caution:* Acrylamide as well as [ $^2\text{H}_3$ ]- and [ $^{13}\text{C}_3$ ]-acrylamide and dansyl chloride are hazardous and must be handled carefully.

### Quantification of 3-aminopropionamide (3-APA)

For the quantification of 3-APA, a method recently developed for the quantification of the amide in potato samples [22] was modified:

To the food samples (2–5 g) powdered in liquid nitrogen by means of a laboratory mill (type A 10; Jahnke & Kunkel, IKA-Labortechnik, Staufen, Germany), tap water (150 ml) and defined amounts of the internal standard glycinamide hydrochloride (0.1–0.6  $\mu\text{g}$ ) were added, depending on the amounts of the analyte determined in a preliminary experiment to obtain analyte/standard ratios from 2:1 to 1:2. For equilibration, the sample was stirred for 60 s, then homogenised using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik) for 90 s and ultrasonified for another 2 min. After precipitation of the proteins with an aqueous solution of  $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$  (2.5 ml; 15 wt.%), followed by an aqueous solution of  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  (2.5 ml; 23 wt.%), the suspension was centrifuged twice (first run: 4000 rpm, 10 min at 10 °C; centrifuge GR 412, Jouan, Unterhaching, Germany; second run: 10,000 rpm, 10 min at 10 °C; Beckmann J2-HS, München, Germany). The sample (10 °C) was filtered and extracted with hexane (10 ml). To an aliquot of the aqueous phase (40 ml), sodium hydrogencarbonate (0.5 mol/l, 40 ml) was added and the pH was adjusted to  $10 \pm 0.2$  using sodium hydroxide (2.5 mol/l). After addition of dansyl chloride in acetone (10.8 mg in 20 ml), the reaction mixture was stirred for 3 h at room temperature in the dark. The solution was then extracted four times with dichloromethane (total volume = 100 ml), the organic phases were combined, centrifuged (4000 rpm, 5 min at 10 °C) to separate the water, and finally dried over anhydrous sodium sulfate. The solvent was removed at about 20 kPa and 35 °C, and the residue was dissolved in a mixture of acetonitrile and aqueous formic acid (0.1%; 1.5 ml; 1/5, v/v). After filtration (0.45  $\mu\text{m}$ ; Spartan<sup>®</sup> 13/0.45RC; Schleicher & Schuell, Dassel, Germany), the sample was analysed by LC–MS–MS [22]. To obtain a calibration curve, defined mixtures of 3-APA and glycinamide were worked-up as described above.

### Quantification of acrylamide (AA)

The quantification of acrylamide in the food samples, except the coffee and cocoa samples, was performed after derivatisation with 2-mercaptobenzoic acid as described recently [8].

### Quantification of acrylamide (AA) in coffee or cocoa

To the food samples (10 g) powdered in liquid nitrogen by means of a laboratory mill (type A 10; Jahnke & Kunkel, IKA-Labortechnik), distilled water (100 ml) and defined amounts of the internal standard [ $^{13}\text{C}_3$ ]-acrylamide (2–10  $\mu\text{g}$ ) were added, depending on the amounts of the analyte determined in a preliminary experiment to obtain analyte/standard ratios from 2:1 to 1:2. For equilibration, the sample was stirred for 60 s, then homogenised using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik) for 2 min and ultrasonified for a further 3 min. The suspension was centrifuged (15,000 rpm, 10 min at 10 °C; Beckmann J2-HS), filtered (10 °C), and extracted with hexane (25 ml). To an aliquot (25 ml), distilled water (25 ml), methanol (21.4 ml) and activated charcoal (5 g; purified as described below) were added. The suspension was stirred for 15 min, ultrasonified for further 5 min, centrifuged (15,000 rpm, 10 min at 10 °C), and filtered (10 °C; 0.45  $\mu\text{m}$ ; Spartan<sup>®</sup> 13/0.45RC; Schleicher & Schuell). To an aliquot (50 ml), a solution of 2-mercaptobenzoic acid (0.5 ml; 154 mg in 10 ml of 0.25 mol/l sodium hydroxide) was added, the pH adjusted to  $10 \pm 0.2$  with sodium hydroxide (1 mol/l), and stirred for 3 h at room temperature in the dark. The excess of the reagent was removed by treatment with lead(II) acetate (5 ml of a saturated solution in water). After centrifugation (5000 rpm, 10 min), the supernatant was acidified to pH  $1.5 \pm 0.3$  using hydrochloric acid (5 mol/l) and finally extracted with dichloromethane (four times; total volume = 80 ml). The organic phases were combined, dried over anhydrous sodium sulfate, and evaporated to dryness at about 20 kPa and 40 °C. The residue was taken up in acetonitrile/0.1 wt.% formic acid (0.3 ml; 3/7, v/v), filtered (0.45  $\mu\text{m}$ ; Spartan<sup>®</sup> 13/0.45RC; Schleicher & Schuell) and, finally, injected for LC–MS–MS analysis as described recently [8]. To obtain a calibration curve, defined mixtures of AA and [ $^{13}\text{C}_3$ ]-AA were worked-up as described above.

Due to our observations, none of the charcoals tested in this study was free of acrylamide, and thus, the coal had to be purified prior to analysis as follows: To the charcoal (50 g), a mixture of methanol/distilled water (200 ml; 3/7, v/v) was added, stirred for 30 min, centrifuged (5000 rpm, 10 min, 10 °C), and filtered. Another 150 ml of the methanol/water mixture was added to the residue and the suspension was stirred for a further 30 min, centrifuged, and filtered. These steps were repeated six times. Finally, acetone (100 ml) was added to the charcoal, the suspension was stirred for 10 min, and centrifuged. After filtration, the charcoal was dried at 60 °C in an oven and stored in an exsiccator prior to use.

To avoid incorrect data for acrylamide, a blank value was run from every batch of purified charcoal: to the coal (5 g) a

mixture of methanol/distilled water (71.4 ml; 3/7, v/v) was added, stirred for 15 min, ultrasonified for a further 5 min, and worked-up as described above.

### Quantification of asparagine (Asn)

Aqueous buffer (20 ml; see below) containing the internal standard norleucine (30–500  $\mu\text{g}$ ) was added to the powdered, defatted (10 ml of hexane) food sample (2 g), depending on the amounts of asparagine determined in a preliminary experiment to obtain analyte/standard ratios from 2:1 to 1:2. The suspension was homogenised with an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik) for 3 min. After centrifugation (10,000 rpm, 30 min at 20 °C; Beckmann J2-HS), the supernatant was filtered (0.45  $\mu\text{m}$ ; Spartan<sup>®</sup> 13/0.45RC; Schleicher & Schuell) and diluted 1:10 with an aqueous buffer prepared as follows: Lithium acetate dihydrate (16.3 g), formic acid (7.5 ml), aqueous thiodiglycol (20 ml; 25%), and octanoic acid (0.1 ml) were dissolved in water (900 ml), the pH was adjusted to 2.20 with trifluoroacetic acid and made up to 1000 ml with water. The asparagine concentrations were analysed by means of an amino acid analyser LC 3000 (Onken, Gründau, Germany). For calibration, defined mixtures of Asn and norleucine were analysed (molar ratios 3 + 1 to 1 + 3).

### Labelling studies

To an aliquot of fermented cocoa beans (2 g) powdered in liquid nitrogen by means of a blender (Moulinex, Radolfzell, Germany), [ $^{13}\text{C}_4$   $^{15}\text{N}_2$ ]-asparagine monohydrate (430 mg dissolved in water) was added. After vortexing (10 s) and equilibration (30 min), the sample was heated as follows: the powdered material was kept for 15 min at 95 °C and then the temperature was raised within 20 min to 115 °C at a rate of 1 °C/min and kept for another 10 min at this temperature. After cooling down to room temperature (20 min), tap water (80 ml) and the internal standards glycynamide (2.0  $\mu\text{g}$ ) and [ $^{13}\text{C}_3$ ]-acrylamide (1.0  $\mu\text{g}$ ) were added. The sample was stirred for 60 s, homogenised using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik) for 90 s and ultrasonified for another 2 min. The suspension was centrifuged (10,000 rpm, 10 min at 10 °C; Beckmann J2-HS), the supernatant was defatted with hexane (15 ml), and divided into two parts. These were either used for the quantification of 3-aminopropionamide and [ $^{13}\text{C}_3$   $^{15}\text{N}_2$ ]-3-aminopropionamide by means of glycynamide as internal standard or for the quantification of acrylamide and [ $^{13}\text{C}_3$   $^{15}\text{N}_1$ ]-acrylamide via [ $^{13}\text{C}_3$ ]-acrylamide as internal standard, respectively.

The quantification of AA, [ $^{13}\text{C}_3$   $^{15}\text{N}_1$ ]-AA, 3-APA, [ $^{13}\text{C}_3$   $^{15}\text{N}_2$ ]-3-APA, and asparagine was performed as described previously [23].

**Table 1** Concentrations of 3-aminopropionamide (3-APA), acrylamide (AA) and asparagine (Asn) in different commercial cocoa masses

Cocoa mass	3-APA		AA		Asn	
	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>a</sup>	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>a</sup>	Conc. ( $\text{mg}/\text{kg}$ )	RSD (%) <sup>a</sup>
No. 1	217.2	4.4	62.8	8.2	24.7	7.7
No. 2	1835.0	0.2	329.4	5.9	277.1	2.6
No. 3	3013.7	2.8	643.3	1.4	271.4	1.9

<sup>a</sup>Relative standard deviation. Analyses were performed in triplicates.

## Results and discussion

In a previous study on the occurrence of amino acid related, biogenic amines in cocoa, we found that, besides 2-phenylethylamine, the previously unknown 2- and 3-methylbutylamines were present in roasted cocoa nibs in quite high concentrations [24]. But, systematic studies showed that the major part of the amines was not formed during fermentation of the beans, but during roasting [24]. Further experiments confirmed that obviously a new pathway of the *Strecker* reaction was involved in the formation of these amines. In model studies on the degradation of the amino acid asparagine in the presence of reducing carbohydrates, we could recently also identify significant amounts of 3-aminopropionamide [12, 22, 23], the biogenic amine of asparagine. These data inspired us to quantify 3-APA in cocoa, because cocoa undergoes a fermentation as well as a roasting process during manufacturing and, thus, 3-APA might be formed from free asparagine either as “biogenic” or as “thermogenic” amine.

A preliminary analysis of a dark chocolate indicated amounts of nearly 3000  $\mu\text{g}$  of 3-APA per kg chocolate (data not shown) suggesting cocoa as a potent source of the amine. To gain a further insight into the source of 3-APA in chocolates, first, three cocoa masses from different suppliers were analysed for their 3-APA content. The results revealed (Table 1) that all masses contained 3-APA, but significantly different amounts were present varying from 217 to 3014  $\mu\text{g}/\text{kg}$ .

Because 3-APA was recently established as a potent precursor of acrylamide in model reactions [12, 22, 23], also

the concentrations of AA were determined in the same cocoa samples. However, as already reported in the literature [25–27], the quantification of AA in cocoa products is not an easy task, because a lot of compounds interfering with the MS analysis are usually present in the extract. Thus, when the common isolation procedure was used (Fig. 1A), the quantification of AA was not possible. Therefore, a modified isolation procedure had to be developed, in which the new step was the use of activated charcoal to remove interfering substances. However, as outlined in the experimental part, prior to the use of charcoal, a sophisticated procedure was necessary to remove AA present as a contaminant in the coal. Application of the new isolation procedure on cocoa samples as well as on coffee samples showed a significant effect, because now an unequivocal quantification was possible (Fig. 1B).

The quantitative data revealed the presence of AA in all cocoa samples, but clear differences between the three samples varying from 63 to 643  $\mu\text{g}/\text{kg}$  were measured (Table 1).

Because the amounts of 3-APA and AA were very well correlated in the three samples analysed (Table 1), these data are a further hint that 3-APA can be regarded as a transient intermediate in AA formation. Furthermore, a much better correlation between the amounts of 3-APA and AA were found as compared to free asparagine and AA (Table 1). It can further be assumed that either the variety of the cocoa beans or, yet unknown, manufacturing steps in cocoa production obviously enhance/inhibit the formation of 3-APA/AA in cocoa masses.

To clarify the role of the fermentation and the roasting processes in the formation of 3-APA and AA, both compounds were then quantified in authentic, unfermented or fermented cocoa samples from Sulawesi and Ghana, respectively, as well as in the roasted samples prepared from the same batch.

The quantification of 3-APA in the unfermented beans from Ghana (Table 2) showed a comparatively low concentration of 313  $\mu\text{g}/\text{kg}$ , but the fermentation of the beans performed for 7 days led to an increase in the concentrations by a factor of about 2.5, which is undoubtedly caused by an enzymatic decarboxylation of asparagine as previously described by us for raw potatoes [22]. However, the roast-

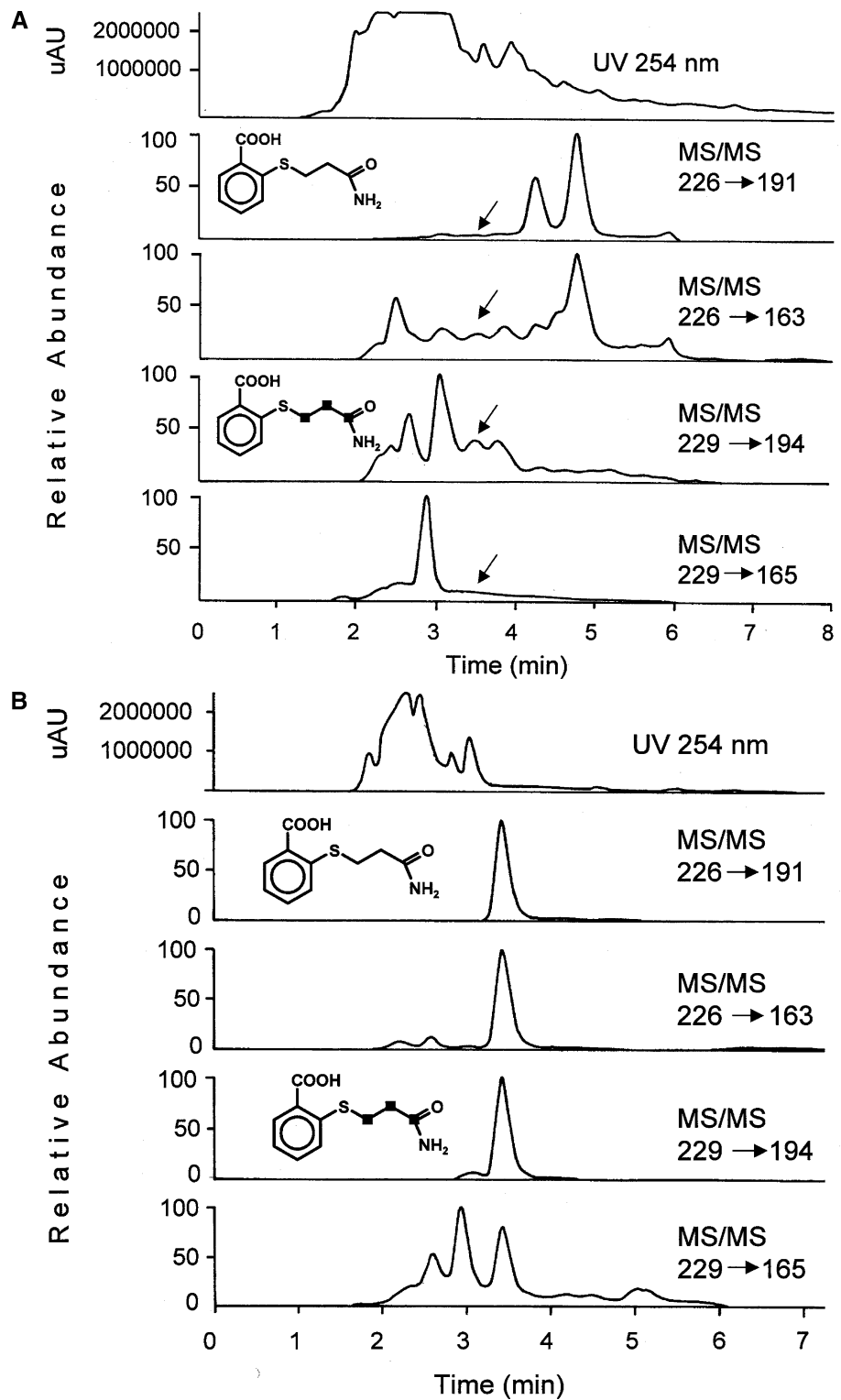
**Table 2** Influence of fermentation and roasting on the concentrations of 3-aminopropionamide (3-APA), acrylamide (AA) and asparagine (Asn) in cocoa beans from Ghana

Sample	3-APA		AA		Asn	
	Conc. ( $\mu\text{g}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>	Conc. ( $\mu\text{g}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>	Conc. ( $\text{mg}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>
Unfermented	312.5	8.1	57.2	5.1	721.0	2.9
7 days fermented	792.1	5.9	64.3	4.8	654.8	0.8
Unfermented, roasted	2078.3	3.1	222.5	8.4	584.1	7.7
7 days fermented, roasted	3476.0	3.1	922.2	1.3	471.9	4.8

<sup>a</sup>Based on dry weight

<sup>b</sup>Relative standard deviation. Analyses were performed in triplicates.

**Fig. 1** Mass traces monitored for acrylamide and [<sup>13</sup>C<sub>3</sub>]-acrylamide derivatised with 2-mercaptobenzoic acid in isolates from cocoa beans before **A** and after **B** treatment of the extract with charcoal



ing process was much more effective in generating 3-APA, because simply roasting of the unfermented cocoa beans increased the amounts of 3-APA by a factor of 6.5. In comparison, roasting of the fermented sample was, however, less effective, because only by a factor of about 4.5 more 3-APA was formed as compared to the fermented, unroasted cocoa

beans (Table 2). These data clearly indicated that the unfermented cocoa beans already contain significant amounts of the precursor(s) for 3-APA formation. Because the concentrations of asparagine were lowered either by the fermentation as well as by the roasting process (Table 2), it can be assumed that 3-APA is formed partly as “biogenic”,

**Table 3** Influence of fermentation and roasting on the concentrations of 3-aminopropionamide (3-APA), acrylamide (AA) and asparagine (Asn) in cocoa beans from Sulawesi

Sample	3-APA		AA		Asn	
	Conc. ( $\mu\text{g}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>	Conc. ( $\mu\text{g}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>	Con. ( $\text{mg}/\text{kg}$ ) <sup>a</sup>	RSD (%) <sup>b</sup>
Unfermented	114.7	9.2	66.3	10.1	501.6	3.5
7 days fermented	154.7	6.9	79.2	4.5	459.3	0.1
Unfermented, roasted	1498.0	6.0	122.1	1.3	439.9	2.2
7 days fermented, roasted	1818.1	3.0	337.2	6.9	337.0	6.1

<sup>a</sup>Based on dry weight.<sup>b</sup>Relative standard deviation. Analyses were performed in triplicates.

but more effective as “thermogenic” amine from this amino acid.

Quantification of acrylamide in the four cocoa samples revealed quite low amounts in the unfermented as well as the 7 days fermented cocoa beans (Table 2), but a clear increase was measured in both roasted samples. Surprisingly, the amounts of AA were much higher in the fermented, roasted sample as compared to the unfermented, roasted sample. These data suggest that the fermentation process obviously generates compounds or conditions favouring the formation of AA during roasting. For example, the lower pH of the fermented beans caused by high amounts of acetic acid might favour the deamination of 3-APA into AA. As observed for the cocoa masses, the amounts of 3-APA were more closely correlated with AA than the amounts of free asparagine.

To corroborate the results of these experiments, the same study was performed, but using cocoa samples from another provenance, namely Sulawesi. Also in these samples, the amounts of 3-APA in the unfermented beans as well as in the fermented beans were quite low (Table 3), but, as found for the Ghana samples, its concentrations were significantly increased during roasting of both, the unfermented and the fermented sample. Also, the amounts of acrylamide were low in both unroasted samples, but clearly increased during roasting (Table 3). Interestingly, although the amounts of AA were higher in the fermented, roasted than in the unfermented, roasted cocoa, the difference between the samples was not as pronounced as found for the respective sample from Ghana (cf. Tables 2 and 3).

To study the role of 3-APA as precursor of AA in a complex food system, the following labelling experiment was performed: to samples of ground, 7 days fermented raw cocoa beans, [<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub>]-asparagine was added. The amounts were adjusted exactly to a 1.5:1 ratio for the Ghanaan beans and to a 1:1 ratio for the Sulawesian beans as compared to the natural concentration of Asn. The samples were then roasted mimicking the commercial procedure and, finally, the amounts of the unlabelled 3-APA and AA as well as the two labelled isotopologs were quantified. If the formation of AA from Asn should proceed via 3-APA, the ratios of 3-APA to [<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>2</sub>]-3-APA as well as

**Table 4** Comparison of the concentrations of 3-aminopropionamide (3-APA), [<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>2</sub>]-3-aminopropionamide ([<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>2</sub>]-3-APA), acrylamide (AA) and [<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>1</sub>]-acrylamide ([<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>1</sub>]-AA) formed during roasting of fermented, unroasted cocoa beans administered with 430 mg/kg of [<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub>]-asparagine<sup>a</sup>

Compound	Sulawesi		Ghana	
	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>b</sup>	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>b</sup>
3-APA	1095.4	5.1	1682.8	0.7
[ <sup>13</sup> C <sub>3</sub> <sup>15</sup> N <sub>2</sub> ]-3-APA	660.2	7.1	650.5	5.0
AA	346.4	2.4	543.5	3.7
[ <sup>13</sup> C <sub>3</sub> <sup>15</sup> N <sub>1</sub> ]-AA	180.9	2.0	184.4	2.6

<sup>a</sup> The ratio of Asn to [<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub>]-Asn was 1:1 for the Sulawesian beans and 1.5:1 for the Ghanaan beans, respectively.<sup>b</sup>Relative standard deviation. Analyses were performed in triplicates.

of AA to [<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>1</sub>]-AA should be similar to 1.5:1 and 1:1, respectively.

The results summarised in Table 4 showed a ratio of 2.6:1 for 3-APA/[<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>2</sub>]-3-APA and 2.9:1 for AA/[<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>1</sub>]-AA for the Ghanaan beans and a ratio of 1.6:1 for 3-APA/[<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>2</sub>]-3-APA and 1.9:1 for AA/[<sup>13</sup>C<sub>3</sub><sup>15</sup>N<sub>1</sub>]-AA for the Sulawesian beans. These results clearly support the idea that 3-APA is an important transient intermediate of AA also under the conditions in a real food system. Although the yields of both target compounds were lower from the labelled Asn as compared to the natural, unlabelled Asn, this result might be attributed to the fact that the administered, labelled Asn was not in close contact to other precursors, e.g. present inside the intact cocoa cells.

In a next series of experiments, several cereal products were analysed for their concentrations of 3-APA and AA (Table 5). The data revealed quite high concentrations of 3-APA in popcorn, while the other processed cereals were much lower in this amine. However, because popcorn was also highest in AA, also for these foods a correlation between both compounds is obvious.

In a last series of experiments, 3-APA and AA were determined in roasted ground coffee and coffee products (Table 6). By contrast to cocoa and the cereal products, all coffee samples showed lower concentrations of 3-APA as compared to those of AA. This is obviously due to the higher temper-

**Table 5** Concentrations of 3-aminopropionamide (3-APA) and acrylamide (AA) in processed cereal products

Product	3-APA		AA	
	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>a</sup>	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>a</sup>
Popcorn	1877.7	4.2	320.0	11.0
Wheat snack	287.5	11.8	161.3	3.5
Wheat bread snack	288.9	10.1	52.9	2.8
Rice-cocoa snack	199.1	7.8	43.4	8.1
Corn flakes	171.2	7.9	78.2	5.6
Rusk	33.5	4.2	54.9	14.0

<sup>a</sup>Relative standard deviation. Analyses were performed in triplicates.

**Table 6** Concentrations of 3-aminopropionamide (3-APA) and acrylamide (AA) in different coffee products

Product	3-APA		AA		z-score
	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>a</sup>	Conc. ( $\mu\text{g}/\text{kg}$ )	RSD (%) <sup>b</sup>	
Coffee surrogate	453.3	3.4	1492.4	6.1	0.8
Coffee extract	240.8	9.2	806.9	2.9	-0.4
Ground coffee	122.1	1.9	267.7	0.5	0.2

<sup>a</sup>Relative standard deviation. Analyses were performed in triplicates.

<sup>b</sup>Relative standard deviation. Data are mean values of duplicates.

ature used in coffee roasting leading to a more pronounced degradation of 3-APA as compared to, e.g., cocoa or cereals.

For the quantification of AA the improved clean-up method was used again. The performance of the method was proven by analyses of certified reference materials within an interlaboratory test organised by the Institute for Reference Materials and Measurements (IRMM; Geel, Belgium). The obtained z-scores reflect the very good accuracy of the method (Table 6) combined with a very low limit of detection (LOD) varying from 1.0 to 3.0  $\mu\text{g}/\text{kg}$  depending on the food matrix.

In conclusion, the results show that 3-aminopropionamide, the biogenic amine of asparagine, is a natural constituent of raw as well as of various thermally processed foods. However, as clearly shown for cocoa, the thermo-conversion of asparagine into 3-APA seems to be much more effective than its biochemical formation. Because 3-APA was confirmed as an effective intermediate in acrylamide formation, its presence in all food products, which might undergo a thermal processing, should be considered as additional source of AA formation.

**Acknowledgments** The authors gratefully acknowledge the skillful assistance by Jörg Stein and S. Kaviani-Nejad. Thanks are also to Ines Otte for performing the LC-MS-MS measurements and Kätthe Schiesser for performing the amino acid analyses.

## References

- Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M (2002) *J Agric Food Chem* 50:4998–5006
- Rosén J, Hellenäs K-E (2002) *Analyst* 127:880–882
- Becalski A, Lau BP-Y, Lewis D, Seaman SW (2003) *J Agric Food Chem* 51:802–808
- Ono H, Chuda Y, Ohnishi-Kameyama M, Yada H, Ishizaka M, Kobayashi H, Yoshida M (2003) *Food Addit Contam* 20:215–220
- Ahn JS, Castle L, Clarke DB, Lloyd AS, Philo MR, Speck DR (2002) *Food Addit Contam* 19:1116–1124
- Weisshaar R (2004) *Eur J Lipid Sci Technol* 106:786–792
- Amrein TM, Schoenbaechler B, Escher F, Amado R (2004) *J Agric Food Chem* 52:4282–4288
- Jezussek M, Schieberle P (2003) *J Agric Food Chem* 51:7866–7871
- Amrein TM, Lukac H, Andres L, Perren R, Escher F, Amado R (2005) *J Agric Food Chem* 53:7819–7825
- Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert M-C, Riediker S (2002) *Nature* 419:449–450
- Mottram DS, Wedzicha BL, Dodson AT (2002) *Nature* 419:448–449
- Schieberle P, Koehler P, Granvogl M (2005) New aspects on the formation and analysis of acrylamide. In: Friedman M, Mottram D (eds) *Advances in experimental medicine and biology*, vol 561. Springer Verlag, New York, pp 205–222
- Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, Gruber DC, Morsch TR, Strothers MA, Rizzi GP, Villagran MD (2003) *J Agric Food Chem* 51:4782–4787
- Vass M, Amrein TM, Schönbaechler B, Escher F, Amado R (2004) *Czech J Food Sci* 22:19–21
- Levine RA, Smith RE (2005) *J Agric Food Chem* 53:4410–4416
- Surdyk N, Rosén J, Andersson R, Aman P (2004) *J Agric Food Chem* 52:2047–2051
- Mustafa A, Andersson R, Rosén J, Kamal-Eldin A, Aman P (2005) *J Agric Food Chem* 53:5985–5989
- Becalski A, Lau BP-Y, Lewis D, Seaman SW, Hayward S, Sahagian M, Ramesh M, Leclerc Y (2004) *J Agric Food Chem* 52:3801–3806
- Williams JSE (2005) *Food Chem* 90:875–881
- De Wilde T, De Meulenaer B, Mestdagh F, Govaert Y, Vandeburrie S, Ooghe W, Fraselle S, Demeulemeester K, Van Peteghem C, Calus A, Degroot J-M, Verhé R (2005) *J Agric Food Chem* 53:6550–6557
- Stadler RH, Robert F, Riediker S, Varga N, Davidek T, Devaud S, Goldmann T, Hau J, Blank I (2004) *J Agric Food Chem* 52:5550–5558
- Granvogl M, Jezussek M, Koehler P, Schieberle P (2004) *J Agric Food Chem* 52:4751–4757
- Granvogl M, Schieberle P (2006) *J Agric Food Chem* 54:5933–5938
- Granvogl M, Bugan S, Schieberle P (2006) *J Agric Food Chem* 54:1730–1739
- Riediker S, Stadler RH (2003) *J Chromatogr A* 1020:121–130
- Delatour T, Perisset A, Goldmann T, Riediker S, Stadler RH (2004) *J Agric Food Chem* 52:4625–4631
- Aguas PC, Fitzhenry MJ, Giannikopoulos G, Varelis P (2006) *Anal Bioanal Chem* 385:1526–1531