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Quantitation of 3-Aminopropionamide in Potatoes—A Minor but Potent Precursor in Acrylamide Formation

MICHAEL GRANVOGL,† MAGNUS JEZUSSEK,† PETER KOEHLER,‡ AND PETER SCHIEBERLE*,†,‡

Lehrstuhl für Lebensmittelchemie, Technische Universität München, Lichtenbergstrasse 4, D-85748 Garching, Germany, and Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

3-Aminopropionamide (3-APA) has recently been suggested as a transient intermediate in acrylamide (AA) formation during thermal degradation of asparagine initiated by reducing carbohydrates or aldehydes, respectively. 3-APA may also be formed in foods by an enzymatic decarboxylation of asparagine. Using a newly developed method to quantify 3-APA based on liquid chromatography/tandem mass spectrometry, it could be shown that the biogenic amine was present in several potato cultivars in different amounts. Further experiments indicated that 3-APA is formed during storage of intact potatoes (20 or 35 °C) or after crushing of the cells. The heating of 3-APA under aqueous or low water conditions at temperatures between 100 and 180 °C in model systems always generated more AA than in the same reaction of asparagine, thereby pointing to 3-APA as a very effective precursor of AA. While the highest yields measured were about 28 mol % in the presence of carbohydrates (170 °C; aqueous buffer), in the absence of carbohydrates, 3-APA was even converted by about 63 mol % into AA upon heating at 170 °C under aqueous conditions. Propanoic acid amides bearing an amino or hydroxy group in the α-position, such as 2-hydroxypropionamide and L-alaninamide, were ineffective in AA generation indicating that elimination occurs only from the β-position.

KEYWORDS: Acrylamide; β-alaninamide; 3-aminopropionamide; dansyl chloride; formation; quantitation; GC/MS; glycinamide; LC/MS/MS; potato

INTRODUCTION

Acrylamide (AA) has recently been reported to occur in a number of thermally processed foods, and in particular, high levels were measured in fried, roasted, or baked potatoes (*1*−*7*). Because of the potential health hazard of the amide, several studies have been undertaken in order to find ways to minimize its levels in processed products (*8*−*11*).

In principle, two ways do exist to address this challenge: (i) to modify the parameters used in the manufacturing process, such as pH, temperature, or time and (ii) to clarify the transient intermediates and formation pathways of AA formation. In the meantime, several studies have shown that high temperatures favor AA formation, and thus, lowering the baking or frying temperatures has been proven to effectively lower the amounts of AA in, for example, baked goods and potatoes (*8*−*11*).

On the basis of model studies, asparagine (Asn) has been identified as the most effective precursor of AA quite shortly after the discovery of AA as a "foodborne" toxicant (*12*−*17*). In addition, acrolein and ammonia have also been identified as further precursors in AA formation (*18*). Mottram et al. (*12*) suggested that AA is formed from the amino acid via its Strecker aldehyde as the key intermediate. However, it remains unclear how the aldehyde oxygen is removed in this reaction. Stadler et al. (*13*) also identified Asn as the most effective precursor of AA among 20 other amino acids reacted in the presence of reducing carbohydrates. These authors showed that at 180 °C the corresponding N-glycosides synthesized from Asn and glucose are important reaction intermediates. In labeling experiments using ubiquitously carbon-13 labeled glucose and also nitrogen-15 labeling in the amide as well as in the α-amino group, these authors (*13*) were also able to prove that (i) the carbon backbone in AA stems from the amino acid and (ii) the amide group of the amino acid is transferred into AA, whereas the nitrogen of the α-amino group is lost.

On the basis of data obtained by pyrolysis GC/MS, Yaylayan et al. (*15*) also suggested that a carbohydrate or carbohydrate degradation products are necessary to form AA from Asn and they also considered its formation via a decarboxylated Amadori product, because when Asn was pyrolyzed in the absence of carbohydrates, maleimide was formed, thereby avoiding AA formation.

* To whom correspondence should be addressed. Tel: +49-89-289 132 65. Fax: +49-89-289 141 83. E-mail: Peter.Schieberle@Lrz.tum.de.
† Technische Universität München.
‡ Deutsche Forschungsanstalt für Lebensmittelchemie.

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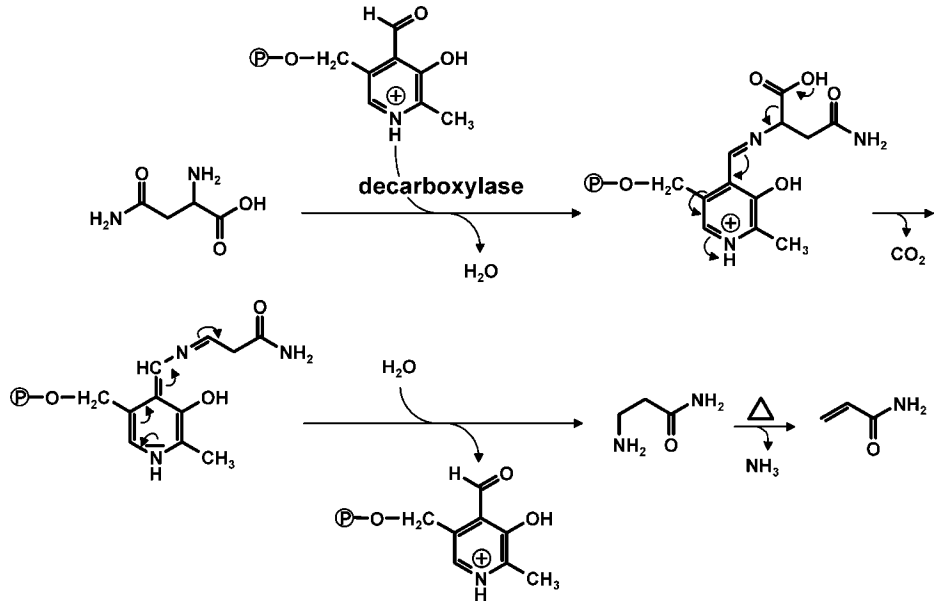

Figure 1. Biochemical pathway of the decarboxylation of Asn into 3-APA with pyridoxal phosphate as cofactor (according to ref *19*).

Zyzak et al. (*16*) suggested that any carbonyl compound may activate Asn by forming a Schiff base, which then decarboxylates upon heating. The decarboxylated Schiff base was suggested to either form AA directly upon further heating or to degrade into 3-APA, which in turn forms AA by elimination of ammonia. As compared to Asn, the amounts generated from 3-APA in a model experiment were reported to be five times higher (*16*).

From a stoichiometric point of view, NH_3 and CO_2 simply have to be eliminated to generate AA from Asn. Besides the Maillard type reactions suggested up to now as the key steps in the formation of AA, it might be assumed that decarboxylases present in the raw materials might generate the biogenic amine 3-aminopropionamide (3-APA) from Asn as indicated in **Figure 1**, which is then thermally deaminated into AA. This process would run without involving reducing carbohydrates. The formation of biogenic amines from amino acids via pyridoxal phosphate as cofactor is a reaction depending on the enzyme present (*19*). Because, to the best of our knowledge, there are no reports on the occurrence of 3-APA in foods, the purpose of the following study was (i) to develop a new method for the quantitation of 3-APA and (ii) to study its effectiveness as a precursor in AA formation in model systems.

MATERIALS AND METHODS

Food Samples. Potatoes of different cultivars (Likaria, Quarta, Selma, Nicola, and Solara) were purchased in a local supermarket.

Chemicals. 3-APA (*β*-alaninamide) hydrochloride was obtained from Chemos (Regenstauf, Germany); [$^2H_3$]-AA (98%) was from CIL (Andover, MA); AA (99.9%), glucose, fructose, and Asn monohydrate were from VWR International (formerly Merck, Darmstadt, Germany); and 5-(dimethylamino)-1-naphthalene sulfonyl chloride (dansyl chloride), glycinamide hydrochloride, L-alaninamide hydrochloride, 2-hydroxypropionamide, pyridoxal 5′-phosphate monohydrate, L-histidine decarboxylase (E.C. 4.1.1.22), L-lysine decarboxylase (E.C. 4.1.1.18), L-glutamic acid decarboxylase type II and type III (both E.C. 4.1.1.15), and pyruvate decarboxylase (2-oxo-acid carboxy-lyase from bakers yeast) (E.C. 4.1.1.1) were from Aldrich (Sigma-Aldrich, Steinheim, Germany). L-Phenylalanine decarboxylase (E.C. 4.1.1.53) was from Fluka (Sigma-Aldrich Chemie, Taufkirchen, Germany). Caution: AA as well as [$^2H_3$]-AA are hazardous and must be handled carefully.

Development of a Method for 3-APA Quantitation. *Preparation of the Reference Derivatives.* 3-APA hydrochloride or glycinamide hydrochloride (150 nmol), respectively, was singly dissolved in aqueous sodium hydrogencarbonate (20 mL; 0.25 mol/L), and the pH was adjusted to 10 ± 0.2 with sodium hydroxide (1 mol/L). Dansyl chloride (10 μmol dissolved in 5 mL of acetone) was added, and the mixture was purged with argon for 30 s and stirred at room temperature for 1 h in the dark. The solution was extracted with dichloromethane (three times; total volume 45 mL), and the organic phases were combined, dried over anhydrous sodium sulfate and, finally evaporated to dryness at about 20 kPa and 35 °C. The residue was taken up in acetonitrile (2.0 mL) and filtered (0.45 μm; Spartan13/0.45RC, Schleicher & Schuell, Dassel, Germany), and an aliquot (200 μL) was made up to 1.0 mL with formic acid (0.1%, v/v in water) for MS analysis. To obtain a calibration curve, defined mixtures of 3-APA and glycinamide were worked-up as described above.

High-Performance Liquid Chromatography (HPLC)/MS/MS Analysis. Mass spectra were recorded by means of a triple quadrupole tandem mass spectrometer (TSQ Quantum Discovery, Thermo Electron, Dreieich, Germany) coupled to a Surveyor HPLC system (Thermo Finnigan, Dreieich, Germany) equipped with a thermostated (15 °C) autosampler and a Synergi max RP 80 Å HPLC column (150 mm × 2.0 mm i.d., 4 μm, Phenomenex, Aschaffenburg, Germany; kept at 30 °C) and a max RP precolumn (4 mm × 2.0 mm i.d., Phenomenex). The sample (10 μL) was separated at a flow rate of 0.2 mL/min. The solvent system was composed of (A) formic acid in water (0.1%, w/v) and (B) formic acid in acetonitrile (0.1%, w/v). A linear gradient was applied by increasing the concentration of B from 20 to 50% within 15 min. The effluent between 1.2 and 3.2 mL was directed into the electrospray interface. The mass spectrometer was operated in the positive electrospray ionization mode (ESI^+) with a spray needle voltage of 3.5 kV and a spray current of 5 μA. The temperature of the capillary was 300 °C, and the capillary voltage was 35 V. The sheath and auxiliary gas (nitrogen) was adjusted to 30 and 10 arbitrary units, respectively. The collision cell was operated at a collision gas (argon) pressure of 6.7×10^{-5} kPa.

The derivatives of 3-APA and glycinamide were first characterized by means of their molecular masses obtained in the full scan mode. These were then subjected to MS/MS to determine first the most intense transition of the precursor ions (fragmentation energy 35%) and second to optimize the yields of the product ions by performing a series of runs with different collision energies and flow rates of the sheath and auxiliary gas. These resulted in transitions of *m*/*z* 322 to *m*/*z* 170 (collision energy 29%) and to *m*/*z* 156 (55%) for 3-APA as well as of *m*/*z* 308 to *m*/*z* 170 (25%) and to *m*/*z* 156 (46%) for glycinamide. On both mass filter quadrupoles, the resolution settings were 0.7 (fwhm), the scan time for each transition and single reaction monitoring was 0.20 s, and the scan width was 0.5 amu.

For calibration, single reaction monitoring (SRM) using the ion transitions *m*/*z* 322 to 156 (analyte) and *m*/*z* 308 to 156 (standard) was applied on seven mixtures of 3-APA and glycinamide in defined concentrations (molar ratios 10 + 1 to 1 + 10) and the response factor was calculated as described recently (*20*). Different derivatization times (5, 15, 30, 60, 120, and 180 min) were studied to consider the possibility of different affinities of internal standard and analyte vs the derivatization reagent.

Quantitation of 3-APA in Potatoes. After the addition of tap water (150 mL) to peeled, thick-featured potatoes (150 g; at least from three tubers) and a short homogenization step (30 s) using a blender (MX32, Braun, Kronberg, Germany), the internal standard glycinamide hydrochloride (55.3 μg) was added. For equilibration, the sample was stirred for 60 s and then homogenized using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik, Staufen, Germany) for 60 s. After precipitation of proteins using 5 mL of an aqueous solution of 150 g of $K_4[Fe(CN)_6] \times 3H_2O$/L (Carrez I) and 5 mL of an aqueous solution of 230 g of $Zn(CH_3COO)_2 \times 2H_2O$/L (Carrez II), the suspension was centrifuged twice (first run: 4000 rpm, 10 min at 10 °C; centrifuge GR 412, Jouan, Unterhaching, Germany; second run: 10000 rpm, 10 min at 10 °C; Beckmann J2-HS, München, Germany). To an aliquot (100 mL), sodium hydrogencarbonate (0.25 mol/L, 100 mL) was added and the pH was adjusted to 10 ± 0.2 using sodium hydroxide (2.5 mol/L). After the addition of dansyl chloride in acetone (54 mg in 50 mL), the reaction mixture was stirred at room temperature for 1 h in the dark. The solution was then extracted with dichloromethane (four times; total volume 200 mL), and the organic phases were combined, centrifuged (4000 rpm, 5 min at 10 °C) to separate 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/0.1% formic acid (1.5 mL; 1/5, v/v). After filtration (0.45 μm; Spartan13/0.45RC, Schleicher & Schuell), the sample was analyzed by LC/MS/MS as described above. To determine the recovery, different amounts of the analyte (200, 400, and 600 μg/kg) were added to a potato sample with a known 3-APA concentration. A control run without the addition of glycinamide was performed in each potato cultivar to ensure the absence of the internal standard.

Formation of 3-APA during Storage of a Raw Potato Mash. Potatoes (1.5 kg) were peeled and thick-featured. Aliquots of the material (150 g) were homogenized (30 s) with a blender (MX32, Braun), and glycinamide hydrochloride (55.3 μg) was added. For equilibration, the sample was first stirred for 60 s in the blender and then homogenized for another 60 s using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik). To check the influence of storage time, the samples were magnetically stirred for 30, 60, 180, 300, and 450 min. The 3-APA formed was quantified as described above.

Enzymatic Formation of 3-APA from Asn. Asn monohydrate (75 mg) and the cosubstrate pyridoxal 5′-phosphate monohydrate (5 mg) were added to phosphate buffer (75 mL; 0.01 mol/L). Each model was incubated in the presence of several decarboxylases for 16 h at 37 °C (25 °C for pyruvate decarboxylase) and at the pH optimum of each decarboxylase (4.5−6.0) with stirring. After the addition of glycinamide hydrochloride (110.6 μg) to an aliquot of the solution (10 mL), the pH was adjusted to 10 ± 0.2 using sodium hydrogencarbonate and sodium carbonate. The quantitation of the 3-APA formed was done as described above for the potato samples.

Model Studies on AA Formation. *Model I.* Equimolar amounts of Asn and several carbohydrates or carbonyl compounds, respectively (0.1 mmol each), were singly homogenized with silica gel (3 g, KG 60, 0.063−0.200 mm; VWR International, formerly Merck; containing 10% of water) and heated at 170 °C for 30 min in closed glass vessels. 3-APA hydrochloride (0.001 mmol), L-alaninamide hydrochloride (2.5 mmol), and 2-hydroxypropanoic acid amide (2.5 mmol) were treated in the same way but also without carbohydrates added.

Models II. Equimolar amounts (0.25 mmol each) of Asn and the respective carbohydrate were dissolved in phosphate buffer (0.1 mol/L; pH 5.0 = model II 5 or pH 7.0 = model II 7; 10 mL) and were heated at different temperatures (100−180 °C) for 20 min in closed glass vessels. 3-APA hydrochloride (0.001 mmol) and L-alaninamide hydrochloride (2.5 mmol) were treated in the same way but also without carbohydrates added. The quantitation of the AA formed was carried out as described recently (*21*).

Quantitation of Asn. A buffer solution (20 mL; see below) containing the internal standard norleucine (15 mg) and *n*-octanol (20 μL for foam suppression) was added to peeled and thick-featured potatoes (5 g) and homogenized with an Ultraturrax (Jahnke & Kunkel, Oberstaufen, Germany) for 5 min. After centrifugation (10000 rpm, 30 min at 20 °C; Beckmann J2-HS, München, Germany), the supernatant was filtered (0.45 μm; Spartan13/0.45RC, Schleicher & Schuell) and diluted 1 + 9 with a buffer solution prepared as follows: Lithium acetate dihydrate (16.3 g), formic acid (7.5 mL), thiodiglycol (20 mL; 25%, w/w in water), and octanoic acid (0.1 mL) were dissolved in water (900 mL), and the pH was adjusted to 2.20 with trifluoroacetic acid and made up to 1000 mL with water. The Asn concentration was analyzed by means of an amino acid analyzer LC 3000 (Onken, Gründau, Germany). For calibration, defined mixtures of Asn and norleucine were analyzed.

Table 1. Influence of the Temperature on the Formation of AA in Thermally Processed Aqueous Solutions of 3-APA or Asn, Respectively, in the Presence of Glucose[a]

	AA formed (mmol/mol)[b] from		
temperature (°C)	Asn	3-APA	3-APA[c]
100	0.01	1.4	1.3
120	0.01	5.5	7.5
140	0.07	55.8	41.1
160	0.7	164.8	147.4
160[d]	0.9	129.5	234.4
180	1.1	290.6	608.3

[a] 3-APA (1 μmol) or Asn (250 μmol), respectively, were dissolved in phosphate buffer (10 mL; pH 7.0) and, after addition of an equimolar amount of glucose, heated for 20 min (model II 7). [b] Number of replicates, 3–5; coefficient of variation, ±5% or below. [c] Glucose was omitted. [d] The buffer was substituted by tap water.

RESULTS AND DISCUSSION

Formation of AA from 3-APA. A recent publication (*16*) had pointed to 3-APA as a transient intermediate in AA formation during the thermal degradation of Asn. As compared to an Asn/glucose mixture, the amounts of AA formed from 3-APA were reported to be five times higher (*16*). However, neither a method for 3-APA quantitation nor the exact quantitative data on the yields obtained from 3-APA were reported. Thus, its efficacy as a precursor of AA remained unclear.

To study the effectiveness of 3-APA in AA generation, the amine was first reacted at elevated temperatures in phosphate buffer at pH 7.0 in the presence or absence of glucose and the AA formation was monitored. The results showed that about 5.6 mol % of 3-APA was converted into AA already after a thermal treatment for 20 min at 140 °C (**Table 1**). Under these conditions, the amounts from 3-APA were by a factor of about 800 higher as compared to a model system in which 3-APA had been replaced by Asn. Increasing the temperature up to 180 °C drastically increased the yields of AA from 3-APA up to 29 mol %, whereas Asn again was less effective (**Table 1**). Interestingly, when glucose was omitted from the model system, at high temperatures the amounts of AA formed from 3-APA were even higher than in the presence of glucose (**Table 1**). In addition, the formation of AA from 3-APA was measurable already at 100 °C (0.1 mol %) and did also occur in the absence of phosphate ions, e.g., in tap water (**Table 1**). In general, the yields obtained from 3-APA were by far the highest ever reported for a precursor of AA in the literature.

Further experiments were performed to study the influence of the pH and low water conditions on AA formation from 3-APA (**Table 2**). The data again proved 3-APA to be a very potent precursor: when 3-APA was reacted in aqueous solution

Table 2. Influence of the Processing Conditions on the Formation of AA from 3-APA

carbohydrate added	AA (mmol/mol 3-APA)		
	model I[a]	model II 5[b]	model II 7[c]
no	292.3	194.9	627.7
glucose	109.3	129.3	277.0
glucose[d]	52.6	ND	ND
glucose[e]	36.4	ND	ND
fructose	111.3	101.2	243.4

[a] The reaction was performed for 30 min at 170 °C under low water conditions. [b] The reaction was performed under aqueous conditions for 20 min at 180 °C and pH 5.0. [c] The reaction was performed under aqueous conditions for 20 min at 180 °C and pH 7.0. [d] The time was reduced to 5 min. [e] The temperature was reduced to 120 °C. Number of replicates, 5–10; coefficient of variation, ±5% or below.

Table 3. Formation of AA from L-Alaninamide and 2-Hydroxypropionamide As Compared to 3-APA[a]

precursor[a]	AA (mmol/mol)[b]	
	with	without glucose
L-alaninamide	0.004	0.002
2-hydroxypropionamide	<0.0005	0.001
3-APA	109.3	292.3

[a] The precursor was reacted at 170 °C (model I). [b] Number of replicates, 3–5; coefficient of variation, ±5% or below.

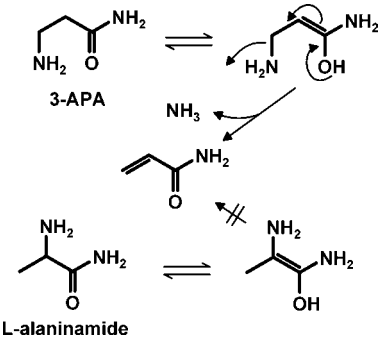

Figure 2. Proposed formation pathway of AA from 3-APA. L-Alaninamide for comparison.

at 180 °C and at pH 7.0 and in the absence of carbohydrates, the yields of AA amounted to nearly 63 mol %. Lowering of the temperature to 120 °C and also shortening of the heating time to 5 min (model I) reduced the amount of AA formed but still led to a significant generation. In all experiments, the yields were significantly higher as compared to the same experiments with Asn (data not shown).

When carbohydrates were present, in all model systems, the yields were mostly lower as compared to the reaction in the absence of carbohydrates (**Table 2**), and also, lowering the pH from 7.0 to 5.0 in model II clearly lowered the amounts of AA.

The results corroborate the easy elimination of ammonia from carbon-3 in 3-APA as suggested by Zyzak et al. (*16*). To prove the importance of this β-amino group in AA formation, L-alaninamide and 2-hydroxypropionamide were reacted under the same conditions and the amounts of AA formed were compared to the yields obtained from 3-APA (**Table 3**). The results clearly showed that neither a hydroxyl nor an amino group in the α-position of propanoic acid amide led to AA formation. This might be explained by the different stabilities of the possible tautomers shown for 3-APA and 2-hydroxypropionamide in **Figure 2**. The slight acidity of H-atoms α to an amide (peptide bond) in a protein is well-known, and thus, the elimination from the tautomers shown in **Figure 2** seems probable.

Method for Quantitation of 3-APA. Up to now, 3-APA has not been reported as a food constituent. It might be formed enzymatically from Asn by decarboxylases as indicated in **Figure 1**. Because no exact method for the quantitation of 3-APA in foods was available, a method using glycinamide as the internal standard followed by a derivatization with dansyl chloride (**Figure 3**) and, finally, LC/MS/MS measurement of the derivatives was developed. In a first experiment, both derivatives were synthesized separately and their ESI mass spectra were recorded. The data showed clear base peaks at *m*/*z* 322 for the analyte and at *m*/*z* 308 for the internal standard (**Figure 4**).

To prevent matrix interferences and for increased selectivity, MS/MS was applied for unequivocal quantitation. By fragmentation of the protonated molecule ions, MS/MS spectra of dansyl 3-APA and dansyl glycinamide were obtained (**Figure 5**). From each fragment spectrum, the most abundant ion transitions were selected and used for quantitation by LC/MS/MS in the SRM mode. Ion transitions *m*/*z* 322 to *m*/*z* 170 and *m*/*z* 322 to *m*/*z* 156 (analyte) and *m*/*z* 308 to *m*/*z* 170 and *m*/*z* 308 to *m*/*z* 156 (internal standard), respectively, were recorded. Next, for calibration, seven model mixtures containing defined amounts of 3-APA and glycinamide (10 + 1 to 1 + 10) were measured. From the data obtained, a calibration curve was drawn (**Figure 6**) and the response factor was calculated to be 0.61 according to a procedure described recently (*20*). Additional investigations showed that the response factor did not differ, if the amounts of analyte and internal standard were varied within a wide range of molar ratios showing no different affinities of standard and analyte vs the derivatization reagent. Furthermore, also, no differences in yields were observed when varying the derivatization time between 5 and 180 min.

Identification and Quantitation of 3-APA in Potatoes. In a first series of experiments, five different cultivars of potatoes

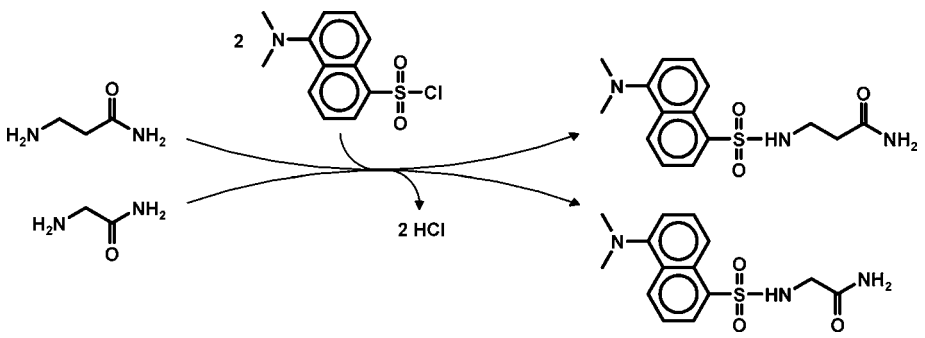

Figure 3. Derivatization procedure applied to convert 3-APA and the internal standard glycinamide into stable dansyl sulfonamides prior to LC/MS/MS analysis.

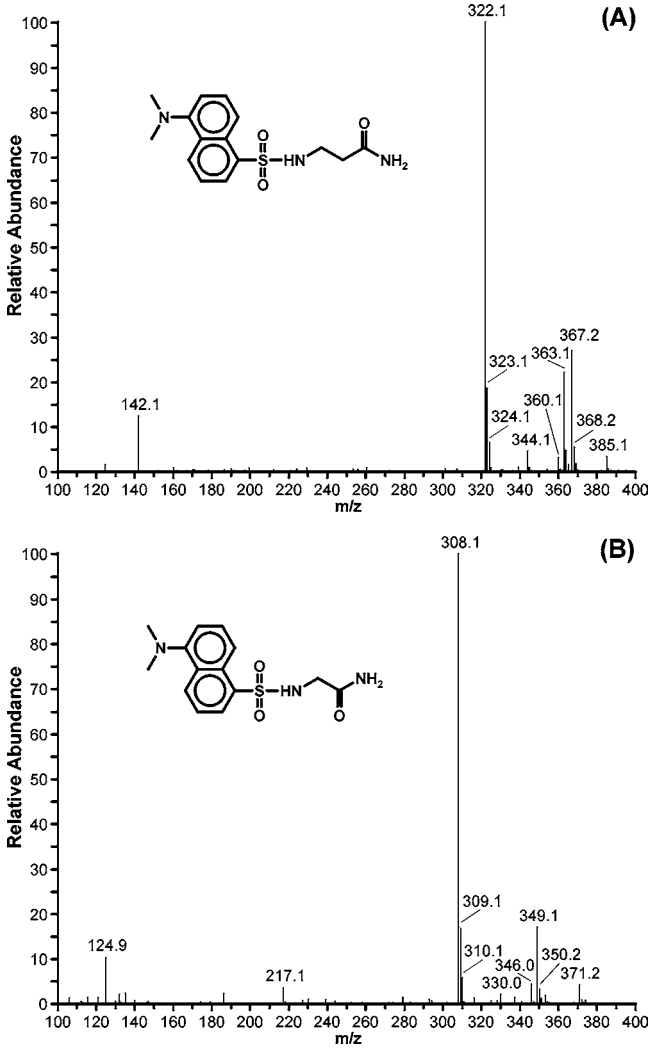

Figure 4. Mass spectra (ESI positive) of the derivatives obtained from 3-APA (**A**) and glycinamide (**B**).

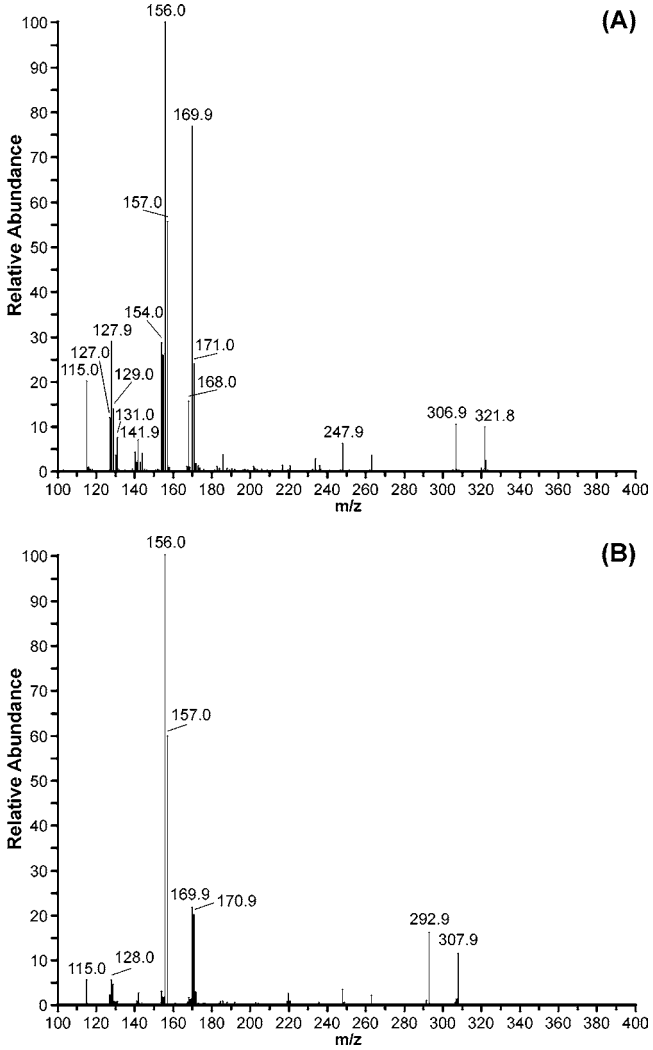

Figure 5. MS/MS spectra (ESI positive) of the protonated dansyl sulfonamides of 3-APA (**A**) (collision energy 55%) and glycinamide (**B**) (collision energy 46%).

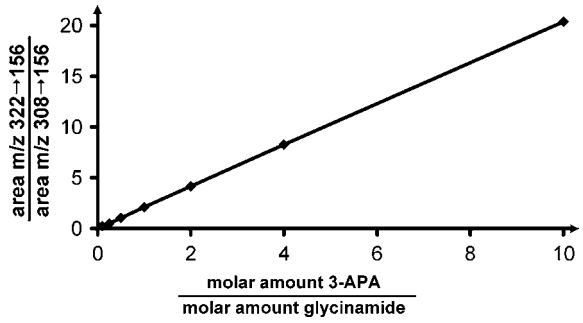

Figure 6. Calibration curve obtained by analysis of seven mixtures of 3-APA and glycinamide in a defined molar ratio using derivatization with dansyl chloride.

were analyzed for their content of free 3-APA. In **Figure 7**, the UV trace obtained for a potato extract is contrasted to the LC/MS/MS traces used for quantitation. The data clearly suggest the method as quite sensitive for 3-APA quantitation in a food matrix.

As indicated in **Table 4**, all cultivars contained 3-APA, which is for the first time reported here as a constituent of potatoes or even foods, respectively. The concentrations varied between the cultivars but did not show clear correlations to the amounts of free Asn. For example, Nicola was highest in Asn but lowest in 3-APA.

It might be assumed that the formation of 3-APA is enhanced during storage of intact potatoes. To prove this assumption, the potatoes of each cultivar were either stored for 5 weeks at 20 °C (cool storage) or 12 days at 35 °C (warm storage). The amounts of 3-APA were measured and contrasted to the amounts measured in the unstored samples (**Table 4**). The data revealed a clear increase in 3-APA after 5 weeks at 20 °C. However, some cultivars, in particular Selma, showed a comparatively higher amount of 3-APA indicating a more effective bioconversion rate.

Storage of the potatoes at 35 °C, but for a shorter period of time, led to a more significant formation of 3-APA. Again, clear differences were observed between the cultivars, with Selma and Likaria followed by Solara showing a much higher formation as compared to, for example, Quarta.

To follow the idea of the presence of an Asn decarboxylase activity, potatoes were crushed and stored for a maximum of 8 h at 20 °C. As shown for two cultivars (**Figure 8**), the "active" Selma and the "less active" Quarta, 3-APA was formed with increasing the storage time, in particular, in the sample prepared from Selma.

Enzymatic Decarboxylation of Asn in Model Systems. Several decarboxylases such a glycine decarboxylase, glutamate decarboxylase, tryptophan decarboxylase, arginine decarboxylase, or ornithine decarboxylase have been reported to occur

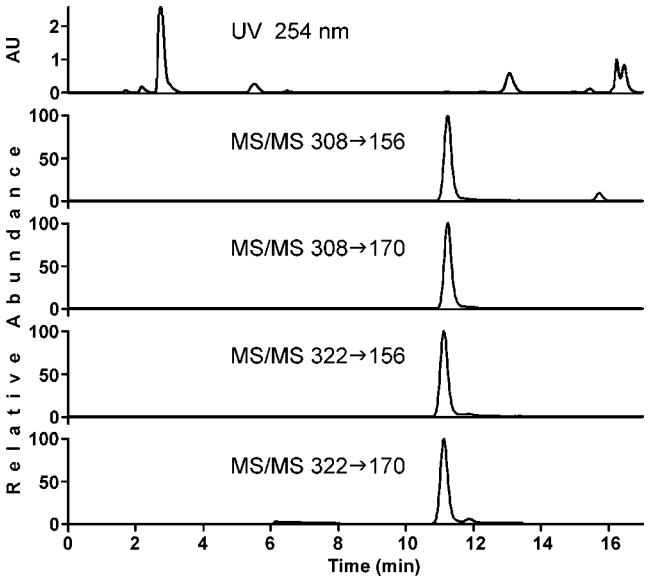

Figure 7. LC/MS/MS chromatograms obtained in the analysis of 3-APA in a potato sample (cultivar Quarta) containing about 220 μg of 3-APA/kg.

Table 4. Influence of Storage on the Generation of 3-APA in Potatoes[a]

	3-APA (μg/kg fresh weight)[b]			
cultivar	fresh	cool stored[c]	warm stored potatoes[d]	Asn (g/kg)
Likaria	265.1	360.6	1045.0	5.03
Nicola	135.5	188.3	336.0	6.30
Quarta	223.0	291.5	323.0	4.48
Selma	293.8	747.5	1753.5	5.05
Solara	159.5	238.2	676.3	4.58

[a] Concentration of free asparagine (Asn) in fresh potatoes for comparison. [b] Analyses were performed in triplicates. Coefficient of variation: ±6% or below. [c] 5 weeks at 20 °C (cool stored). [d] 12 days at 35 °C (warm stored).

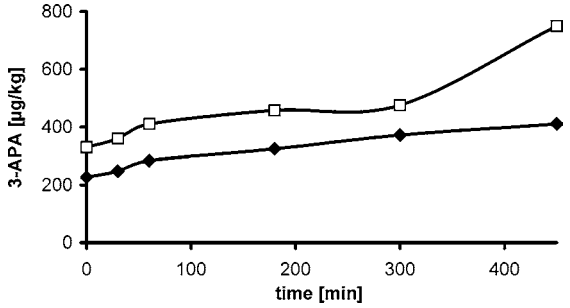

Figure 8. Time course of the formation of 3-APA in a raw potato mash stored at 20 °C for 480 min; Selma (–□–); Quarta (–◆–).

in potatoes as well as many other foods (*22*−*26*). However, no data are available on Asn decarboxylase in potatoes or foods in general, and consequently, no Asn decarboxylase is commercially available. Thus, we first studied the ability of a variety of commercially available decarboxylases to liberate 3-APA from Asn as a side activity. The results showed that among the "nonspecific" decarboxylases selected, only histidine decarboxylase was able to form 3-APA (**Table 5**). However, although the amounts were by a factor of about 140 higher as compared to the control experiment, only about 0.1% of Asn was converted into 3-APA.

In summary, the results suggest an alternative reaction pathway in foods leading to AA formation by a combination

Table 5. Amounts of 3-APA Generated from Asn by Different Decarboxylases[a]

decarboxylase	3-APA (μg)[b]
control[c]	0.05
histidine	7.10
glutamic acid type III	0.61
glutamic acid type II	0.52
phenylalanine	0.15
lysine	0.05
pyruvate	0.05

[a] A 13.2 mg amount of Asn were incubated in phosphate buffer (75 mL) in the presence of the respective decarboxylase for 16 h at 37 °C (25 °C for pyruvate decarboxylase) at the respective pH optimum (4.5–6.0). [b] Number of experiments, 3–6; coefficient of variation, ±1% or below. [c] Incubation without decarboxylase.

of a biochemical reaction generating 3-APA, which will then generate AA in high yields upon heating. Although 3-APA is undoubtedly present in many foods in lower concentrations as compared to free Asn, the much higher effectiveness of 3-APA in terms of yields as compared to Asn suggest it as an additional precursor in AA formation.

Although in potatoes, 3-APA will not be a more effective precursor of AA as compared to the Asn/carbohydrate reaction, it should be stressed that once 3-APA is formed, AA can be effectively generated also under aqueous conditions without going through the Maillard reaction. Thus, this reaction might explain AA formation from raw materials low in Asn.

ABBREVIATIONS USED

AA, acrylamide; 3-APA, 3-aminopropionamide; Asn, asparagine; GC/MS, gas chromatography/mass spectrometry; LC/MS/MS, liquid chromatography/tandem mass spectrometry.

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