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Acrylamide in Gingerbread: Critical Factors for Formation and Possible Ways for Reduction

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The influence of ingredients, additives, and process conditions on acrylamide formation in gingerbread was investigated. The sources for reducing sugars and free asparagine were identified, and the effect of different baking agents on acrylamide formation was evaluated. Ammonium hydrogencarbonate strongly enhanced acrylamide formation, but its N atom was not incorporated into acrylamide, nor did acrylic acid form acrylamide in gingerbread. Acrylamide concentration and browning intensity both increased with baking time and correlated with each other. The use of sodium hydrogencarbonate as baking agent reduced the acrylamide concentration by >60%. Free asparagine was a limiting factor for acrylamide formation, but the acrylamide content could also be lowered by replacing reducing sugars with sucrose or by adding organic acids. It is concluded that a significant reduction of acrylamide in gingerbread can be achieved by using sodium hydrogencarbonate as baking agent, minimizing free asparagine, and avoiding prolonged baking.

KEYWORDS: Acrylamide; gingerbread; free asparagine; reducing sugars; baking agent; ammonium hydrogencarbonate; sodium hydrogencarbonate; citric acid; asparaginase

INTRODUCTION

The detection of acrylamide in heated foodstuffs (*1*) led to a worldwide concern because acrylamide is a known neurotoxin and is classified as "probably carcinogenic to humans" (group 2A) by the IARC (*2*). Particularly high concentrations exceeding 1000 μg/kg were found in heated potato products such as French fries and potato crisps (*1*, *3*). It was shown that acrylamide is formed in the Maillard reaction (*4*, *5*). A decrease of the acrylamide concentration at temperatures >170 °C (*4*) pointed to the concurrent elimination of acrylamide, which was first monitored in potatoes (*6*).

Asparagine is considered to be the main precursor for acrylamide formation in foods as shown with ^{15}N-labeled asparagine (*7*). Zyzak et al. provided evidence that asparagine delivers the backbone of the acrylamide molecule, whereas glucose is not incorporated into acrylamide (*8*). However, a reducing sugar is needed for the formation of the Schiff base of asparagine, which is transformed via an oxazolidin-5-one intermediate to a decarboxylated Amadori product that releases acrylamide (*9*). In analogy to the formation of acrylamide from asparagine, the release of vinylogous compounds from other amino acids was assumed. Stadler et al. demonstrated that acrylic acid is formed in copyrolysates of aspartic acid and sugars (*10*).

Gingerbread may contain up to 1000 μg of acrylamide/kg of fresh weight. In Germany, acrylamide contents in gingerbread ranged from <20 to >8000 μg/kg, with an average value of 481 μg/kg and a median of 231 μg/kg (*11*). Konings et al. (*12*) measured acrylamide in Dutch gingerbread products ranging from 260 to 1410 μg/kg (average = 890 μg/kg; median = 1070 μg/kg). Because gingerbread is consumed frequently and all through the year in The Netherlands, these products were estimated to contribute 16% of the total acrylamide exposure of the Dutch population (*12*). In our laboratory preliminary analyses of typical Swiss gingerbread of the 2003 Christmas season showed acrylamide contents from 100 to 800 μg/kg. These relatively high concentrations are in contrast to the low content of free asparagine in cereal flours: In wheat flour free asparagine was determined in the range of 70−300 mg/kg (*13*, *14*). In comparison, potatoes contain up to 4200 mg of free asparagine/kg (*15*). Therefore, the question of an alternative mechanism of acrylamide formation in gingerbread was raised. It was shown that ammonium hydrogencarbonate (i.e., the typical baking agent for gingerbread in Switzerland) strongly enhanced acrylamide formation in a model system for bakery products (*16*) and that acrylic acid and ammonia form high levels of acrylamide in browning model systems (*17*). Because heating of aspartic acid and glucose can produce acrylic acid (*10*), a mechanism other than the thermal degradation of asparagine might contribute to the high acrylamide levels in gingerbread.

The aim of the present investigation was to test the hypothesis if ammonia, originating from the baking agent, is incorporated into acrylamide, presumably via reaction with acrylic acid, and

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to find ways to reduce the acrylamide content in gingerbread. The influence of the type and the amount of baking agent and other additives on acrylamide formation was investigated. The paper reports results from more than 180 experiments with different gingerbreads produced in our pilot plant. The critical factors for the acrylamide formation in gingerbread are identified and discussed. Several possible ways to achieve a significant reduction of the acrylamide content in gingerbread are suggested.

MATERIALS AND METHODS

Preparation of Gingerbreads. All ingredients were obtained from JOWA AG (Volketswil, Switzerland), a Swiss producer of gingerbread. The gingerbread dough was prepared with flour (a 70:30 mixture of spelt and wheat, corresponding to flour type 720), inverted sugar syrup, powdered sugar (sucrose), honey (American origin), water, spices, caramel coloring, whole egg, ammonium hydrogencarbonate (baking agent), and lezirol (100 g/L in water, containing milk protein, an acid regulator, lecithin, vegetable oil, and β-carotene). For the experiments with ^{15}N-labeled baking agent a mixture of [^{15}N]ammonium sulfate (CIL, Andover, MA) and sodium hydrogencarbonate (Fluka, Buchs, Switzerland) was used. This mixture contained the same amounts of ammonium and hydrogencarbonate ions as the normal baking agent. Several other ingredients were used in addition to the original recipe: L-asparagine, L-lysine, glycine, L-cysteine, l-aspartic acid, citric acid, tartaric acid, acrylic acid, and sodium hydrogencarbonate, all from Fluka. Asparaginase (from *Escherichia coli*) was purchased from Fluka and Sigma-Aldrich (Steinheim, Germany), diluted with distilled water to a volume of 1.5 mL, and directly added in small portions to the dough during kneading (4 units/kg of dough). Preparation of gingerbreads was performed as closely to the industrial process as possible. The ingredients were mixed according to the prescription obtained from JOWA AG in a Z-kneader (Farinograph; Brabender, Duisburg, Germany, batch size = 300 g of dough). The dough was left at room temperature for 24 h. After a short reworking, the dough was sheeted to 7 mm thickness, cut into pieces of ~50 g (5 cm × 10 cm), glazed (mixture of cracked egg, sucrose, and salt), and then baked in a programmed oven (Schaubackofen Thermody B 160c; Pitec, Oberriet, Switzerland) in a two-step program at 180 °C for 3 min and at 190 °C for 7 min (standard conditions). These conditions gave a product very similar in terms of color, taste, volume, dry matter, and acrylamide to the product prepared by the industry. Immediately after baking, the gingerbreads were glazed with lezirol solution. Product temperature during baking was measured by inserting a temperature sensor (T51; Rotronic, Bassersdorf, Switzerland) in the center of the gingerbread and was recorded every 5 s with a computer.

Measurement of Color, Dry Matter, and pH. Color was measured at three different positions on top of the gingerbread using an optical L^*,a^*,b^* analyzer (Chroma-Meter Cr-300; Konica Minolta Photo Imaging, Dietikon, Switzerland). For determination of the dry matter gingerbreads were cut and homogenized with a household cutter (Moulinette; Moulinex, Paris, France) and dried in an oven at 104 °C for 5 h (in triplicate); dry matter was determined gravimetrically. The pH was measured according to the method of the gingerbread producer: 5.00 g of homogenized gingerbread was mixed with 45.00 g of bidistilled water, and the pH was determined in the slurry under stirring.

Determination of Sugars. Glucose, fructose, and sucrose were determined enzymatically using the test kit from Scil Diagnostics (Martinsried, Germany). An appropriate amount of ingredient was weighed into a 100 mL flask, and ~60 mL of bidistilled water was added. After homogenization (Polytron; Kinematica, Lucerne, Switzerland), 5 mL of Carrez 1 solution [150 g of potassium hexacyanoferrate(II) trihydrate/L; Fluka] and 5 mL of Carrez 2 solution (300 g of zinc sulfate heptahydrate/L; Fluka) were added. The mixture was thoroughly shaken, the foam broken with a few drops of 1-octanol (Fluka), and the volume adjusted to 100 mL with bidistilled water. Filtered samples (Schleicher & Schuell, Dassel, Germany) were subjected to enzymatic analysis using the hexokinase/glucose-6-phosphate-dehydrogenase assay as described by the producer.

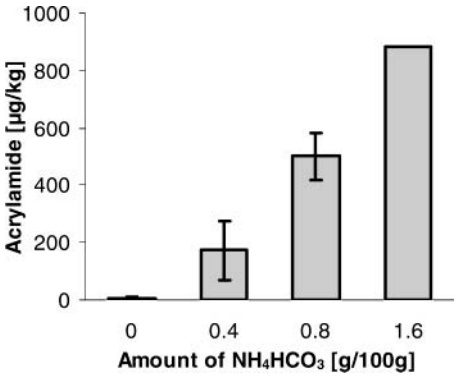

Figure 1. Acrylamide content in gingerbreads produced with different amounts of ammonium hydrogencarbonate as baking agent (error bars are ± standard deviation, $n = 2$, except for 1.6 g/100 g).

Measurement of Free Asparagine. An appropriate amount of the sample (generally 10 g) was weighed into a 100 mL flask and mixed (Polytron) with ~60 mL of aqueous solution of trifluoroacetic acid (1 g/L; Fluka). Both Carrez 1 and 2 solutions (5 mL each) were added, and the mixture was intensively shaken. Foam was broken by a few drops of 1-octanol and the volume adjusted to 100 mL with trifluoroacetic acid solution. After filtration (Schleicher & Schuell), samples were diluted 1+1 with 0.16 M lithium citrate buffer (pH 2.2, PVP physiological; Laborservice Onken, Gründau, Germany) and thoroughly mixed. Diluted samples were filtered through a 0.45 μm HPLC membrane filter (Titan; Infochroma, Zug, Switzerland) and subjected to analysis by cation-exchange chromatography followed by postcolumn derivatization with ninhydrin (Biochrom 30, Biochrom, Cambridge, U.K.) as described by Lebet et al. (*18*). Injection volume was 50 μL, and quantification was done by comparison with an external standard.

Analysis of Acrylamide. Two whole gingerbreads, baked under the same conditions, were cut into small pieces and homogenized in a household cutter (Moulinex). Acrylamide was analyzed as described by Biedermann et al. (*19*): To 10 g of sample and 30 g of tap water were added the internal standards ([$^{13}C_3$]acrylamide in acetonitrile, CIL; methacrylamide, Fluka) in a concentration of 500 μg of standard/kg of sample. The mixture was intensively homogenized (Polytron). After swelling in a water bath at 70 °C for 30 min, 10 g of sample was extracted with 40 mL of 1-propanol (Fluka). After settling of the solids, 10 mL of the clear supernatant was subjected to azeotropic evaporation. Acrylamide was extracted from the residue with 3 mL of acetonitrile (Fluka) and twice defatted with hexane (Fluka). To determine the overall yield of sample preparation, 5 μL of butyramide solution (25 μg/mL in acetonitrile; Fluka) was added to 1.5 mL of defatted acetonitrile extract, which was then analyzed by GC-MS. GC-MS involved an 8000 series gas chromatograph with on-column injector (Fisons Instruments, Milan, Italy) coupled with an SSQ 710 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA). The precolumn (TSP deactivated, i.d. = 0.53 mm) and the separation column (BGB-Wax, 12 m, i.d. = 0.25 mm) were both from BGB Analytik (Böckten, Switzerland). GC conditions were as described in ref *19*. Mass spectrometry with positive chemical ionization monitored the ions m/z 72 (acrylamide), m/z 75 ([$^{13}C_3$]acrylamide), m/z 86 (methacrylamide), and m/z 88 (butyramide). Results were calculated as acrylamide concentration in fresh gingerbread without corrections relative to dry matter. If the overall yield was <40%, the analysis was repeated from the evaporation step.

RESULTS AND DISCUSSION

Influence of the Baking Agent. To investigate the influence of ammonium hydrogencarbonate on acrylamide formation in gingerbread, this baking agent was added in different amounts (normal amount is 0.8 g/100 g of dough) to the dough, and samples were baked under standard conditions. Acrylamide concentrations are shown in **Figure 1**. Gingerbread prepared according to the recipe contained 501 μg of acrylamide/kg with a relative standard deviation of 16% ($n = 9$). This is well within

the range of reported acrylamide levels in gingerbread products (*11*, *12*). The amount of ammonium hydrogencarbonate had a very strong influence on the acrylamide formation: In its absence almost no acrylamide was formed (~10 μg/kg), but the product was unsatisfactory because it lacked browning and leavening. When 0.4 g of NH_4HCO_3/100 g was added, the acrylamide content decreased to one-third (170 μg/kg) and the color was too bright, whereas 1.6 g/100 g led to a strong increase in the acrylamide content (880 μg/kg) and enhanced browning. Apparently, ammonium hydrogencarbonate strongly promotes the formation of acrylamide in gingerbread which was also shown in different model systems for bakery products (*16*, *20*). The amount of added ammonium hydrogencarbonate correlated with pH and color (*L* value): The more baking agent added, the higher was the pH and the darker was the product. Ammonia boosts the browning from the Maillard reaction, which was also shown by Izzo and Ho (*21*).

To check if the promotion of acrylamide formation was due to the incorporation of ammonia into acrylamide and therefore due to an alternative mechanism, gingerbread with ^{15}N-labeled baking agent [$(^{15}NH_4)_2SO_4$ + $NaHCO_3$] was produced. In a control experiment with unlabeled ammonium sulfate and sodium hydrogencarbonate, a normal product with a slightly increased acrylamide content was obtained. Gingerbreads with the labeled baking agent were baked under standard (3 min at 180 °C and 7 min at 190 °C) and more drastic conditions (12 min at 200 °C) to enhance temperature-dependent effects. No [^{15}N]acrylamide was detected in any of the products. Addition of L-aspartic acid (1000 mg/kg), which is a precursor of acrylic acid (*10*), or acrylic acid (1000 mg/kg) resulted in no detectable [^{15}N]acrylamide in gingerbread prepared with labeled baking agent. In standard samples and samples with added L-aspartic acid, only trace amounts of acrylic acid were detected. Addition of L-aspartic acid or acrylic acid to normal dough (each time 1000 mg/kg) did not lead to a significant increase in the acrylamide content: 551 and 573 μg/kg, respectively (the standard product of same batch contained 562 μg/kg). Thus, the N atom from the baking agent is not incorporated into acrylamide and the amidation of acrylic acid by ammonia from the baking agent does not contribute to the high acrylamide content in gingerbread. A mechanism for acrylamide formation other than by thermal degradation of asparagine is unlikely in gingerbread. However, formation of acrylic acid from aspartic acid and reaction of ammonia with acrylic acid leading to acrylamide were shown in model systems (*10*, *17*). Formation of acrylic acid from aspartic acid and glucose or fructose started at 150 °C (*10*), and reaction of ammonia with acrylic acid was performed at 180 °C for 30 min (*17*). For the amidation of aliphatic acids by ammonia, heating at 160 °C for 5 h is needed for a yield of ~80% (*22*). Measurement of the temperature within gingerbreads during the baking process showed that the temperature stayed below 100 °C in the first 6 min due to water evaporation and did not exceed 110 °C until the end of the baking process (**Figure 2**). This demonstrates why the formation of acrylamide via amidation of acrylic acid is unlikely to occur in gingerbread. A possible explanation for the promoting effect of ammonium hydrogencarbonate on the acrylamide formation could be the reaction of asparagine with reactive carbonyls. Glyoxal and methylglyoxal are formed from glucose and fructose in Maillard reaction models (*23*) and have been shown to react more quickly with amino acids than glucose or fructose do (*24*). Besides these compounds, many other α-dicarbonyls and α-hydroxycarbonyls are formed from reducing sugars in the Maillard reaction (*25*), and the sum of these reactive carbonyls might be responsible for the high yield of acrylamide. This hypothesis is supported by the finding that glyoxal and glyceraldehyde formed more acrylamide from asparagine than glucose did (*8*). Thus, the promoting effect of ammonium hydrogencarbonate on the formation of acrylamide might be indirect by providing more reactive carbonyls originating from the reaction of ammonia with reducing sugars. The fact that almost no acrylamide is formed in gingerbread prepared without NH_4HCO_3 corroborates this hypothesis.

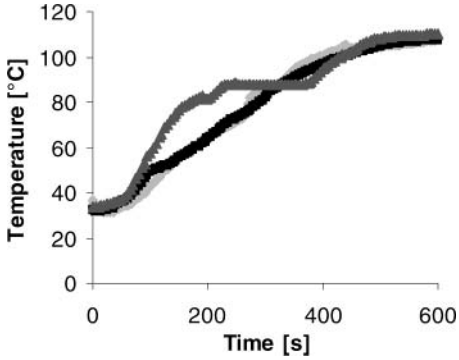

Figure 2. Progression of temperature within a gingerbread during the baking process (3 min at 180 °C, 7 min at 190 °C; three independent determinations).

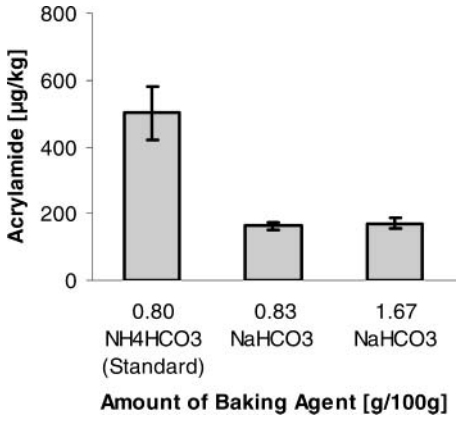

Figure 3. Acrylamide contents in gingerbread with different baking agents (error bars are ± standard deviation; $n = 9$ for NH_4HCO_3; $n = 2$ for $NaHCO_3$).

The influence of sodium hydrogencarbonate, which is an alternative baking agent, was also investigated (**Figure 3**). Its application reduced the acrylamide content to one-third, whereas only 1.67 g of $NaHCO_3$ led to a product with a color comparable to that of the standard product (*L* value = 47.3): *L* values were 45.1 (1.67 g of $NaHCO_3$/100 g) and 51.3 (0.83 g of $NaHCO_3$/100 g). However, the pH was significantly higher in the samples with sodium hydrogencarbonate, 8.2 and 8.8, respectively, when compared to the standard product (pH 6.9), and the product had an alkaline taste. This sensory taint could be compensated for by adding some citric or tartaric acid, which might reduce the acrylamide formation even more as confirmed by preliminary experiments (results not shown). Sodium hydrogencarbonate allows the preparation of gingerbreads with a substantially lower acrylamide concentration and acceptable browning and sensory properties (taste, volume) and is therefore a valuable alternative baking agent. These results also show that a more alkaline pH does not necessarily imply a higher acrylamide content in gingerbread. Other factors such as the presence of ammonia have a stronger impact.

Table 1. Concentrations of Sugars and Free Asparagine in the Ingredients of Gingerbread (Referred to Fresh Weight; $n = 2$)

ingredient	glucose (g/100 g)	fructose (g/100 g)	sucrose (g/100 g)	free asparagine (mg/kg)	contribution to total glucose and fructose (%)	contribution to total free asparagine (%)
flour	0.03	0.03	0.44	139	0.2	87.5
inverted sugar syrup	34.52	31.74	4.54	–[a]	52.3	–
powdered sugar	–	–	96.4	–	–	–
honey	30.69	39.66	3.37	64	38.8	8.5
caramel coloring	31.71	23.77	5.79	–	8.6	–
spices	0.77	0.81	0.57	212	0.1	3.2
whole egg	0.39	–	–	31	0.1	0.7

[a] –, not detected.

Sources of Sugars and Free Asparagine. The precursors for acrylamide formation in foods were determined in all of the ingredients, and their contribution to the total amount in the gingerbread dough was calculated (**Table 1**). The method for the determination of free asparagine was verified in a small interlaboratory comparison: The flour lot was analyzed by four laboratories with four methods (different extraction, separation, and detection techniques; internal and external standards for quantification). The average concentration of free asparagine was determined to be 132 mg/kg with a relative standard deviation of 9.3% ($n = 4$). Flour was clearly the main source for free asparagine in the gingerbread dough. Honey contributed <10% of the free asparagine. The spice mixture contained almost twice as much free asparagine as the flour, but contributed only a marginal part. In contrast to free asparagine, the contribution to reducing sugars from the flour was negligible. The inverted sugar syrup, honey, and caramel coloring contributed together 99% of the total amount of glucose and fructose and outnumbered the part from the flour by far. Interestingly, honey was a source for all of the compounds that are involved in acrylamide formation. In the literature very similar values are reported: Sporns et al. found about 37 g of fructose, 33 g of glucose, and 0−5.5 g of sucrose per 100 g in various honeys (*26*). Speer and Montag reported concentrations of free asparagine in the range of 3.6−81.8 mg/kg in different honeys (*27*). In hard red spring wheat varieties approximately 0.02 g of fructose, 0.04−0.07 g of glucose, and 0.1−0.3 g of sucrose per 100 g were found (*28*). Concentrations of free asparagine in wheat are reported in the range of 69−297 mg/kg (*13*, *14*). The concentration of free amino acids and sugars in cereal flours increases with a higher degree of extraction during milling (*29*). Our values are all well in the range of those cited in the literature.

In one experiment, honey, inverted sugar syrup, and caramel coloring were replaced by sucrose solutions containing the same amount of sucrose instead of glucose and fructose. This virtual depletion of reducing sugars resulted not only in a reduction of the acrylamide content by a factor of 20 (acrylamide content = 25 μg/kg) but also in insufficiently browned products. The lack of reactive carbonyls obviously led to a strong decrease of the acrylamide formation and the Maillard reaction in general, which is in accordance with the literature (*8*, *9*, *16*, *25*, *30*). "White gingerbread" is a specialty in some regions of Switzerland, prepared with NH_4HCO_3 and sucrose instead of honey and inverted sugar syrup and baked at 230 °C for 15 min. It contains only little acrylamide (<10 μg/kg). Ammonium hydrogencarbonate is thus a critical factor only if reducing sugars are present, its promoting effect is not related to free asparagine, and sucrose does not contribute to the formation of acrylamide (*15*, *16*).

One kilogram of fresh gingerbread dough contains ~80 mg of free asparagine, and after standard baking, 500 μg of acrylamide/kg are found. This corresponds to a yield of 0.6% acrylamide based on free asparagine, which is higher than the usually reported yields of ~0.1% (*4*, *7*, *17*, *31*). Biedermann and Grob found even higher yields (3.5 and 4.8%) in model systems consisting of wheat flour, fructose, and ammonium carbonate (*16*). These high yields are probably due to the high molar ratio of fructose/asparagine of 6, whereas in our study this ratio was almost 100 times larger. Thus, free asparagine is a limiting factor, and its addition to the flour before the dough was prepared led to drastic increases in the acrylamide contents of baked gingerbreads (**Figure 4**).

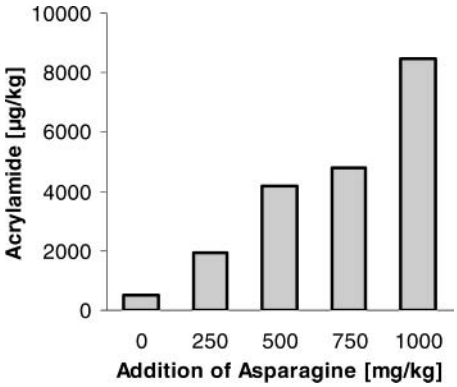

Figure 4. Effect of added free asparagine on acrylamide formation in gingerbread.

Addition of only 250 mg of asparagine to 1 kg of dough resulted in a 4 times higher acrylamide content. When 1000 mg/kg was added, the acrylamide concentrations rose to >8000 μg/kg, whereas the color of the product was only somewhat darker than that of standard gingerbread. This shows that free asparagine largely determines the acrylamide formation in gingerbread. Consequently, the decomposition of free asparagine prior to baking is supposed to result in a decrease in acrylamide formation. This hypothesis was tested by adding an asparaginase during dough preparation in order to hydrolyze the amide group of asparagine. Gingerbreads from this dough contained 228 μg of acrylamide/kg (average of three independent experiments), which corresponds to a decrease of 55% of the normal acrylamide content. Taste and color were virtually identical to those of the standard product, which is a clear advantage of this approach to reduce acrylamide. Analysis of the fresh dough treated with asparaginase revealed that it still contained 22 mg/kg free asparagine. Therefore, ~75% of the total free asparagine had been degraded, which explains why acrylamide formation was not fully inhibited. The incomplete hydrolysis was probably due to the limited mobility of the enzyme and the substrate within the dough. These results indicate that a significant reduction of the acrylamide content could be achieved by choosing ingredients with a lower content of free asparagine or by applying an asparaginase. It was shown that incubation of a potato matrix with an asparaginase can effectively reduce the

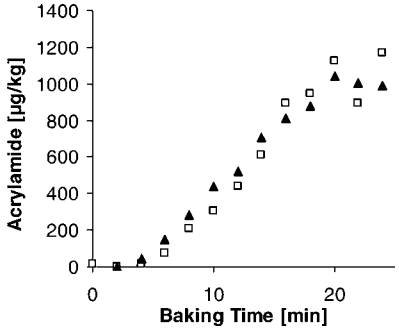

Figure 5. Influence of temperature and time on acrylamide formation during baking: (□) 180 °C; (▲) 200 °C.

acrylamide content (*8*). Thus, the application of this enzyme to different food matrices prior to heating should be further investigated.

Influence of Process Conditions. Gingerbreads were baked under various temperature−time combinations to investigate the influence of temperature and time on acrylamide formation. **Figure 5** shows the acrylamide concentrations measured during baking at 180 and 200 °C. Although the temperature stayed below 100 °C in the first 6 min (see **Figure 2**), some acrylamide was already formed in this period. Even in the raw dough traces of acrylamide were detected. It seems that the presence of ammonia allows the formation of acrylamide at temperatures <100 °C. Acrylamide formation at 60 °C and even at room temperature was reported in various model systems, provided that ammonium carbonate and fructose were present (*16*). The acrylamide concentrations increased steadily in the first 20 min of the baking process. A linear rather than an exponential correlation between acrylamide concentration and time could be assumed. In contrast to French fries, where most of the acrylamide is formed in the last minute of the frying process (*32*), acrylamide was formed almost evenly and over a broader period of the baking process. At 200 °C the acrylamide contents were slightly higher than that at 180 °C during the first 15 min. During baking at 180 °C the *L* value and the acrylamide content were strongly correlated ($R^2 = 0.9474$): The darker the product was, the higher the acrylamide concentrations. The extension of the baking process beyond the necessary time (10 min) resulted in a further increase of the acrylamide content. Thus, prolonged baking or excessive browning should be avoided in order to minimize the acrylamide content.

A lower temperature combined with a prolonged baking time did not result in lower acrylamide contents if the same browning of the product was to be achieved. It was generally observed that a prolonged baking at lower temperatures resulted in an even higher acrylamide content. For instance, gingerbread baked at 160 °C for 20 min exhibited the same color as a sample prepared at 200 °C for 10 min, but the acrylamide contents were 910 and 440 µg/kg, respectively. Thus, a shorter baking at higher temperatures is more suitable to contain the formation of acrylamide in gingerbread. At all temperatures tested (160, 180, and 200 °C) the acrylamide content decreased after a baking time of 20 min, pointing to some elimination of acrylamide. To determine the extent of elimination, [$^{13}C_3$]acrylamide (500 µg/kg) was added to the dough during mixing, and gingerbreads were baked at 180 °C for different lengths of time. Analysis was performed by using only methacrylamide as internal standard. **Figure 6** shows that the elimination of acrylamide took place from the first minutes on, but its extent was limited: Only about one-third of the added [$^{13}C_3$]acrylamide was eliminated after 10 min, and after 28 min ∼50% was still present. The elimination of acrylamide is of no practical importance because baking exceeding 10 min results in unacceptably dark and dry products with high acrylamide contents.

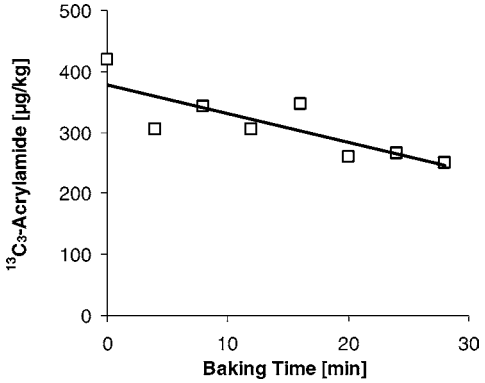

Figure 6. Elimination of [$^{13}C_3$]acrylamide in gingerbread during baking at 180 °C.

Table 2. Effect of the Addition of Organic Acids on the Acrylamide Content, Color, and pH ($n = 1$)

organic acid	amount (mg/kg)	acrylamide (µg/kg)	*L* value	pH
no addition		501[a]	47.3[a]	6.9[b]
citric acid	5000	133[c]	56.0[c]	5.6[c]
	10000	12[c]	55.3[c]	5.0[c]
glycine	2000	430	41.2	7.0
	10000	151	38.4	6.5
L-cysteine	500	368	48.7	6.7
	2000	380	42.7	6.4
L-glutamine	2000	587	41.9	6.8
L-lysine	2000	542	41.7	7.1

[a] $n = 9$. [b] $n = 5$. [c] $n = 2$.

Dry matter content increased steadily during baking and reached ∼85% after the standard baking process. Dry matter and acrylamide content both increased steadily during baking (e.g., at 180 °C) and were strongly correlated with each other ($R^2 = 0.9874$). However, addition of some extra water (5 g/100 g of dough) during preparation of the dough led to a 25% increase in acrylamide. This shows that the observed correlation between dry matter and acrylamide content is rather coincidental than causal.

Organic Acids To Reduce the Acrylamide Content. Various experiments with the addition of different organic acids were performed to check their ability to reduce the acrylamide content in gingerbread (**Table 2**). Addition of 0.5 and 1.0 g of citric acid/100 g of dough resulted in drops of pH to 5.6 and 5.0, respectively, and in a reduction of the acrylamide concentration by factors of 4 and 40, respectively. At the same time, browning was also substantially reduced. Gingerbread with 1 g of citric acid/100 g had a clearly acidic taste and its leavening was not sufficient, probably due to the forced protonation of NH_3 whereby the gas volume was reduced during baking. The reduction of the acrylamide formation by citric acid has already been reported in French fries and various model systems (*33*−*35*). The protonation of the α-amino group of asparagine hinders the formation of the N-substituted glycosylamine, which may explain the reduced acrylamide content and the lesser browning.

A moderate addition of L-glutamine, L-lysine, or glycine (2000 mg/kg of dough) did not lower the acrylamide contents, but enhanced browning. This might be due to the higher number of available α-amino groups that undergo Maillard reaction, resulting in more melanoidins. However, a large addition of

glycine (10000 mg/kg of dough) reduced the acrylamide content to one-third, whereas the browning was even stronger and the pH slightly lower. Glycine is known to strongly enhance browning (*36*) and to react readily with α-dicarbonyls (*24*). Thus, the observed effect could be due to the competition of the amino acids for the reactive carbonyls and/or the elimination of formed acrylamide by a reaction with glycine. L-Cysteine showed a tendency to reduce the acrylamide content and the pH, but these samples had also an unpleasant taste and odor, presumably caused by S-containing decomposition products of cysteine. In a potato model system the acrylamide content was reduced by up to 92% if amino acids had been added (*35*). However, the authors used far larger amounts of amino acids (5000−20000 mg/kg), which probably explains the strong effect. Only a moderate addition of citric acid (≤5000 mg/kg) seems to be suitable to reduce the acrylamide content in gingerbread because it also affects browning, leavening, and taste. Browning (e.g., in samples with an alternative baking agent) could be enhanced by the addition of amino acids, in particular, glycine. Further research is needed to find the optimal combination of process conditions, ingredients and additives to produce gingerbread of good quality and with a low acrylamide content.

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