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Addition of Glycine Reduces the Content of Acrylamide in Cereal and Potato Products

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The effects of adding amino acids on the content of acrylamide in potato crisps, French fries, flat breads, and bread crusts were investigated. Addition of glycine or glutamine during blanching of crisps reduced the amount of acrylamide by ∼30% compared to no addition. No effect was found in French fries. Addition of glycine during doughmaking significantly reduced acrylamide in both flat breads and bread crusts. In bread crusts the reduction of acrylamide ranged from 50 to >90% depending on the baking condition. In flat breads the reduction varied between 60 and >95%.

KEYWORDS: Acrylamide; cereal products; French fries; chips; potato crisps; glutamine; glycine; heating time; temperature; blanching

INTRODUCTION

The discovery by Swedish scientists that the probable carcinogen acrylamide (*1*) is formed during heat treatment of starchy foods (*2*) caused much concern for authorities, the food industry, and the public. Since then much effort has been put into understanding its formation and how to reduce the amount in foodstuffs.

The main precursors for this undesirable substance are reducing sugars and the amino acid asparagine (*3*−*9*). However, despite the high content of free asparagine in potatoes (*10*), the limiting factor for the creation of acrylamide in potato products is the content of reducing sugars (*7*, *11*). It is well-known that the storage temperature influences the amount of reducing sugars in potato tubers, but this does not appear to be the case for asparagine (*10*). A quite opposite situation exists in cereal products, for which the crucial factor is the content of asparagine (*12*, *13*).

Decreasing the content of precursors reduces the amount of acrylamide in the products. In potato processing, soaking or blanching can extract these components (*14*−*17*). That kind of treatment is quite sufficient for potato products such as French fries or potato crisps. However, this technique cannot be applied to cereal products such as bread.

One of the main foci for controlling the formation of acrylamide has been heating conditions. Already in the first report on acrylamide in food (*2*) it was found that increased heating temperature and time increased the amount of acrylamide. Similar results have been found in various systems (*6*, *8*, *12*, *15*−*19*). However, in some systems the amount is reduced if high temperatures or long heating times are applied (*4*, *6*, *8*, *9*, *18*−*20*). This seems to be caused by elimination of acrylamide during heating (*13*, *21*). Thus, a low temperature and short heating time will reduce the acrylamide content, and it has been shown that by strict attention to precursor content and processing condition the content of acrylamide in French fries can be reduced to <100 μg/kg (*15*). However, this may be difficult to achieve in an industrial setting or during home preparation and will also be difficult to achieve in dry products such as potato crisps. However, it has been shown that low frying temperature and short frying times followed by postdrying at lower temperatures to acceptable moisture contents substantially reduces acrylamide formation (*14*).

Some results clearly indicate that different additives, such as acids, amino acids, or proteins, reduce the content of acrylamide (*6*, *22*). Reduction of pH seems to reduce the acrylamide content (*6*, *14*, *17*, *23*). It is, however, unclear if these pretreatments impart unwanted flavor to the finished product. Rydberg et al. (*6*) found that the addition of several amino acids or a protein-rich component to potato slurries decreased acrylamide content by >80%. Amrein et al. (*13*) found that the addition of 10000 mg of glycine/kg of dough reduced the content of acrylamide in gingerbread by one-third. Similar results was also found using legume proteins as a coating in fried potato crisps (*22*). Furthermore, Becalski et al. (*8*) found that the addition of rosemary to the frying oil decreased the acrylamide content in fried potato slices if olive oil was used. However, if corn oil was used, an increase in acrylamide content was found. On the other hand, the addition of antioxidants seems to increase the acrylamide content (*20*, *22*). Several patent applications for the

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reduction of acrylamide have been submitted, including some on the addition of amino compounds, including amino acids (*24*−*26*).

Both cereal and potato products prepared at high temperatures usually contain high contents of acrylamide. From the results cited it seemed that the addition of amino acids to the blanching solution during potato processing not only would extract and thereby reduce the amount of acrylamide precursors but also would add a compound that further reduced the content of acrylamide. In the case of baked cereal products, the inclusion of amino acids during processing should have much the same effect and will be easier to handle during production because they can be added directly to the dough. Thus, the aim of this work was to investigate the possibility of reducing the content of acrylamide in cereals and potato-based foods by increasing the content of amino acids before heating.

MATERIALS AND METHODS

Chemicals. Glycine and glutamine were obtained from Sigma-Aldrich (Deisenhoffen, Germany). All other solvents and chemicals used were of analytical grade.

Raw Material. For crisps production the potato variety Asterix was used. After harvest, the tubers were stored for 1−2 months at 8 °C and before the experiment at 4 °C for 1 month. Storage was conducted in a storage room with monitored temperature and moisture. For French fries production the variety Beate was used. All potatoes came from specialized farms in Norway. All flours were from Cerealia Mills AS (Oslo, Norway).

Potato Crisps. Potato tubers was washed, peeled, and sliced (1.8 mm). After washing in water, 100 g of potato slices were soaked (60 min/20 °C) or blanched (2 min/80 °C) in water, 0.01, 0.02, and 0.05 M glycine solution, and 0.01, 0.02, and 0.05 M glutamine solution (for all experiments, solution/potato = 2:1 w/w). After blanching, the slices were dried on absorbent paper, and 100 g samples were fried in palm oil for 4 min at 170 °C in a 10 L electric frying pan (Masterline, Italy); acrylamide content was measured. All experiments were run in duplicates.

French Fries. Potato strips were obtained from a commercial plant (GRO-Industry, Tønsberg, Norway). Samples, 1000 g, were blanched in water or 0.05 M glycine and glutamine solutions at 70, 80, and 90 °C for 10 min. One set of samples was also blanched for 15 min in water, and another set in two steps: first, 5 min in water followed by 5 min in amino acid solutions. In all of the experiment 8 L of solution was used. After blanching, potato samples were dried at 20 °C for 5 min in an air oven and fried in palm oil for 1 min at 180 °C in a 10 L electric frying pan (Masterline). The French fries were frozen and transported to the production plant, where they were fried in palm oil for 3 min at 180 °C. The samples were kept frozen until analysis for acrylamide content. All experiments were run in duplicates.

Flat Breads. Doughs contained various amounts of glycine, 53% whole meal rye flour, 46% water, and 0.9% salt. Baking time, temperature, and added glycine were varied according to a central composite design. The variations were between 5 and 30 min, between 120 and 260 °C, and between 0 and 400 mmol of glycine/kg of flour, respectively. The doughs were rolled to an even thickness of 0.6 mm using a commercial rolling machine and baked in an electrically heated stone baking oven (Sveba, AB Svenska Bakungsfabriken, Fristad, Sweden). After baking, the samples were kept frozen until they were analyzed for acrylamide.

Breads. Breads with added glycine were made from 31.6% white wheat flour, 31.6% whole meal rye flour, 34.2% water, 0.7% salt, 0.3% dry yeast, and 1.5% fat, using commercial baking equipment. Hearth breads were baked in a fan oven (Revent, Revent International AB, Upplands Väsby, Sweden). Baking time, temperature, and added glycine were varied according to a central composite design, the variations being 15−45 min, 180 and 280 °C, and 0 and 400 mmol of glycine/kg of flour, respectively. Slices of ∼150 g were cut from the center of three breads, and the crusts (19.4−27.5% of the slices) were manually separated and kept frozen until they were analyzed for acrylamide.

Table 1. Content of Acrylamide (Micrograms per Kilogram) in Potato Crisps after Blanching[a]

amino acid	concn (M)	blanching (2 min/80 °C)
control[b]		6188 a
water		4127 b
glycine	0.01	2482 c
glycine	0.02	2572 c
glycine	0.05	1842 d
glutamine	0.01	4316 b
glutamine	0.02	3076 c
glutamine	0.05	3917 b

[a] Numbers followed by different letters are significantly different ($p \leq 0.05$). [b] No blanching or soaking used.

Acrylamide Analysis. Acrylamide was analyzed by NILU (NILU, Norway) using LC-HRMS as described elsewhere (*14*, *18*). All values of acrylamide content are expressed as milligrams per kilogram of fresh weight. The lowest level of quantification (LOQ) for acrylamide was 25 μg/kg. In the statistical analysis all values below the LOQ were set at 25 μg/kg in order not to overestimate the reduction in acrylamide content.

Experimental Design and Statistical Analysis. Experimental design and statistical analysis were performed using Minitab, version 14 (Minitab Inc., State College, PA). Tukey's multiple-range tests were performed to determine significant differences ($p \leq 0.05$) among treatment means. Central composite designs were used in two of the experiments to be able to calculate response surface equations of the type

$$\text{response} = c_1A + c_2B + c_3C + ... + c_nA^2 + c_{n+1}B^2 + ... + c_mAB + c_{m+1}AC + ... + c_zBC + ... \quad (1)$$

where A, B, and C are the variables and c_1, c_2, ... are the coefficients.

RESULTS

Potato Crisps. The blanching condition and acrylamide contents in the potato crisps are shown in **Table 1**. Soaking at 20 °C for 1 h in either glycine or glutamine reduced the acrylamide content by 45%, from 6188 to 3394 μg/kg. No difference was found between glycine and glutamine. Blanching for 2 min at 80 °C gave the most effective reduction of the acrylamide. Glycine reduced the acrylamide contents more effectively than glutamine (**Figure 1**), and although blanching in water gave a reduction of ∼30%, an additional reduction to 70% was observed when the blanching solution contained 0.05 M glycine. No substantial difference was found between the two lowest concentrations of glycine. However, an additional reduction was found when the concentration was increased to 0.05 M.

French Fries. The blanching conditions and amounts of acrylamide in the French fries are shown in **Table 2**. Only a few significant ($p \leq 0.05$) differences in acrylamide content were found among the samples. No significant differences in acrylamide formation were detected due to the blanching conditions. However, glycine tended to lower the amount of acrylamide, averaging 237 μg/kg compared to 295 μg/kg for pure water. No effect of glutamine was found (average = 312 μg/kg). Furthermore, the acrylamide content tended to increase with increasing blanching temperature, the averages ranging from 267 μg/kg at 70 °C to 296 μg/kg at 90 °C. No effect on the acrylamide content was found between blanching for 10 min in amino acid solution only and blanching for 5 min in water followed by 5 min in amino acid solution.

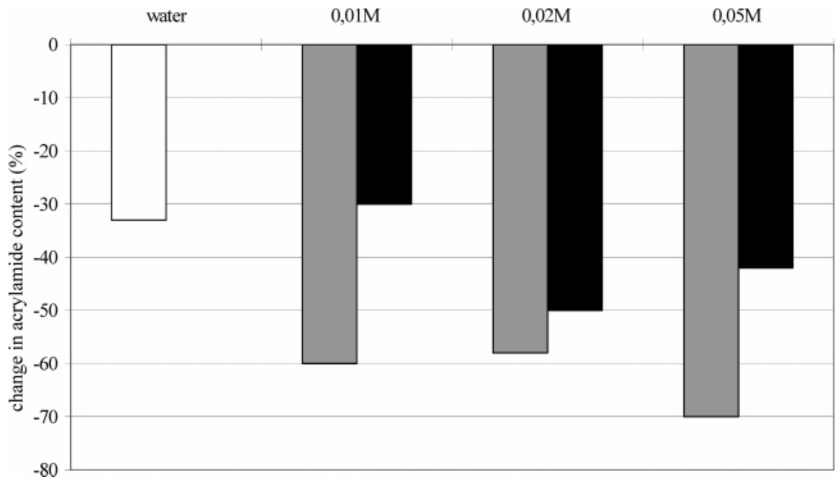

Figure 1. Effect of blanching (2 min/80 °C) in amino acid solution on acrylamide content in potato crisps: (white bar) water; (gray bars) glycine; (black bars) glutamine.

Table 2. Blanching Conditions and Acrylamide Values for French Fries

solution	temp (°C)	time (min)	acrylamide[a,b] (μg/kg)
water	70	10	244 ab
water	70	15	404 a
water	80	10	264 ab
water	80	15	211 ab
water	90	10	311 ab
water	90	15	338 ab
glycine	70	10	302 ab
glycine	70	5 + 5[c]	219 ab
glycine	80	10	235 ab
glycine	80	5 + 5[c]	189 b
glycine	90	10	214 ab
glycine	90	5 + 5[c]	262 ab
glutamine	70	10	183 b
glutamine	70	5 + 5[c]	317 ab
glutamine	80	10	380 a
glutamine	80	5 + 5[c]	323 ab
glutamine	90	10	328 ab
glutamine	90	5 + 5[c]	343 ab

[a] Pooled standard deviation = 57.9. [b] Acrylamide values followed by different letters are significantly different ($p \leq 0.05$). [c] Five minutes in water followed by 5 min in the amino acid solution.

Breads. The processing conditions and acrylamide contents found in bread crust are presented in **Table 3** and the coefficients for the response surface in **Table 5**. The effect of neither time nor temperature was significant ($p \leq 0.05$), but a clear dependence on glycine was found ($p = 0.008$). Also, the quadratic term in glycine and the interaction term glycine × temperature was significant, $p = 0.003$ and 0.05, respectively. Added glycine reduced the acrylamide content up to ∼100−150 mmol/kg of flour (**Figure 2**). The differences between the addition levels were, however, not significant. To further test the effect of the variables glycine, baking time, and baking temperature, the results were investigated for statistical significance by one-way ANOVA. The measurements at 15 and 45 min were not included in the testing of the effect of time because only one measurement was done at each of these times. For the same reason the data points at 180 and 260 °C were omitted from the testing for temperature effects. Because glycine reduced the acrylamide content even at the lowest level of addition (**Table 3**), acrylamide values (200, 140, and 160 μg/kg) for the zero addition level of glycine from a previous experiment using the same experimental setup (*18*) were included in the testing of effects of glycine addition. This confirmed that the addition of glycine reduced the acrylamide content in the bread crusts ($p = 0.000$), but no significant differences were found between the levels of glycine addition. Neither time ($p = 0.12$) nor temperature ($p = 0.7$) significantly reduced the content of acrylamide in bread crusts.

Table 3. Variable Levels and Acrylamide Contents in Bread Crusts

glycine (mmol/kg of flour)	time (min)	temp (°C)	acrylamide (μg/kg)
0	30	230	202
81	21	200	36
81	21	260	58
81	39	200	<25
81	39	260	102
200	15	230	<25
200	30	180	<25
200	30	230	<25
200	30	230	41
200	30	230	<25
200	30	230	<25
200	30	230	<25
200	30	230	<25
200	30	280	<25
200	45	230	<25
319	21	200	103
319	21	260	<25
319	39	200	<25
319	39	260	<25
400	30	230	<25

Table 4. Variable Levels and Acrylamide Contents in Flat Breads

glycine (mmol/kg of flour)	time (min)	temp (°C)	acrylamide (μg/kg)
0	17.5	190	1522
81	10.1	148	<25
81	10.1	232	101
81	24.9	148	<25
81	24.9	232	110
200	5.0	190	<25
200	17.5	120	<25
200	17.5	190	529
200	17.5	190	579
200	17.5	190	365
200	17.5	190	342
200	17.5	190	365
200	17.5	190	381
200	17.5	260	166
200	30.0	190	598
319	10.1	148	<25
319	10.1	232	156
319	24.9	148	<25
319	24.9	232	152
400	17.5	190	309

Table 5. Coefficients and *p* Values for the Response Surface Equations for Bread Crusts and Flat Breads

	bread crusts		flat breads	
term[a]	coefficient	*p* value	coefficient	*p* value
C	56.4	0.04	−4039.1	0.007
T	−10.5	0.68	84.1	0.43
Tp	0.60	0.84	43.2	0.59
Gly	0.80	0.008	−4.3	0.13
T × T	−0.03	0.77	−2.1	0.19
Tp × Tp	−0.003	0.77	−0.11	0.04
Gly × Gly	0.002	0.003	0.007	0.28
T × Tp	0.06	0.13	0.002	1.00
T × Gly	−0.13	0.19	−0.002	0.99
Tp × Gly	−0.006	0.05	0.002	0.92

[a] C = constant, T = time, Tp = temperature, Gly = glycine.

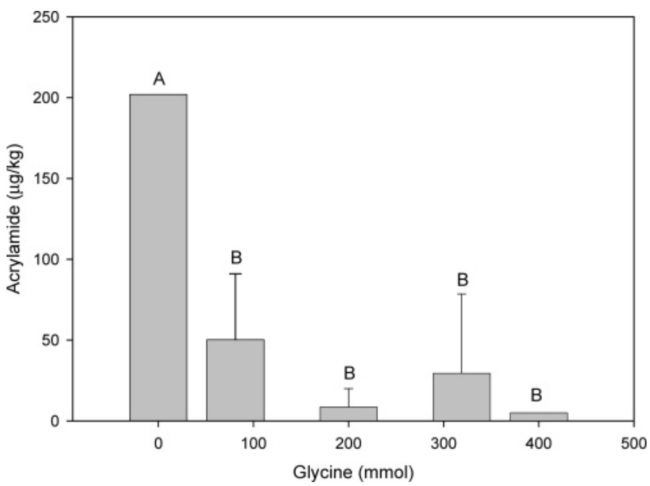

Figure 2. Effect of addition of glycine on the amount of acrylamide in bread crusts. Bars designate standard deviation and different letters significant differences ($p \leq 0.05$). 0 and 400 mmol/kg, $n = 1$; 81 and 319 mmol/kg, $n = 4$; 200 mmol/kg, $n = 10$.

Flat Breads. Variable levels and acrylamide contents for the flat breads are shown in **Table 4**. In this case only the quadratic term in temperature was significant ($p = 0.04$). The effects of time, temperature, and glycine addition were further tested by one-way ANOVA as described for the bread crusts. The values for acrylamide obtained from the previous experiment without glycine additions (*18*) were 1790, 1480, and 2060 µg/kg. This showed that the addition of glycine significantly reduced the content of acrylamide ($p = 0.000$) (**Figure 3**). In this case, an effect of the amount of glycine addition was found ($p = 0.016$ without the zero addition), the 200 mmol addition giving increased acrylamide contents compared to 81 and 319 mmol. Similarly, effects of baking time ($p = 0.046$) and temperature ($p = 0.029$) were found. In both instances, the middle variable levels, 17.5 min and 190 °C, respectively, gave rise to increased acrylamide content.

DISCUSSION

A clear reduction in acrylamide content was found in all systems except for French fries (**Figures 1−3**).

The content of acrylamide found in the potato crisps was high, but still in line with earlier results from the same experimental setup (*14*). The results for the potato crisps indicated that glycine reduced the amount of acrylamide more effectively than glutamine (**Figure 1**). Rydberg et al. (*6*) found similar reductions for both of these amino acids. The apparent discrepancy may

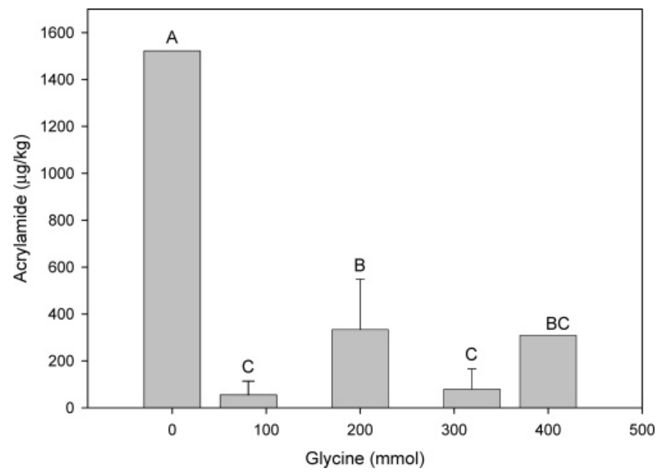

Figure 3. Effect of addition of glycine on the amount of acrylamide in flat breads. Bars designate standard deviation and different letters significant differences ($p \leq 0.05$). 0 and 400 mmol/kg, $n = 1$; 81 and 319 mmol/kg, $n = 4$; 200 mmol/kg, $n = 10$.

be explained by the different systems used. Rydberg et al. used homogenized potato slurries with amino acids added during the homogenization. This ensured that the amino acids were evenly distributed throughout. In the most common industrial procedures potato crisps are made from sliced potatoes and are not reconstituted. In non-reconstituted potato crisps, the amino acid has to be transported into the potato tissue, which can be done by soaking or blanching. The rate of transportation is dependent on both the temperature and the size of the molecule. Thus, in agreement with our results, it would be expected that an elevated temperature would increase the diffusion rate. Furthermore glutamine, being the larger, will be less effectively transported into the tissue. Differences in raw materials may also contribute to the different results. It is well-known that potatoes vary greatly in their ability to form acrylamide due to differences in sugar content (*7*, *10*). Furthermore, glycine is known to be more reactive in the Maillard reaction than glutamine (*27*), which may be of importance if the effect of the addition of glycine is caused by competition in the Maillard reaction. However, as discussed later, a reaction between glycine and acrylamide seems to be the more likely cause of the decrease in measured acrylamide content when glycine is added.

In French fries acrylamide is assumed to be formed primarily on the surface (*15*) due to water evaporation keeping the interior at a lower temperature. In biscuits it has been shown that the temperature in the center is lower than at the surface and that more acrylamide is formed in the latter part (*20*). Thus, even though the blanching conditions were different with longer blanching times, it was expected that blanching would have the same effect as for the crisps. The lack of any significant effect of the addition of amino acids to the blanching solution in this case may be due to a less effective leaching out of the precursors. At the temperature used cells may rupture and provide more asparagine and reducing sugars, which can be expected to diffuse to the surface. Thus, ever-new supplies of the precursors for acrylamide formation will be available even though they are depleted due to Maillard reactions and a simultaneous leaching into the surrounding solution.

The results for breads and flat breads clearly demonstrate that the addition of glycine to the doughs effectively reduces the content of acrylamide in the finished products. Even at the lowest addition, 81 mmol of glycine/kg of flour, reductions of 73 and 96% were obtained for bread crusts and flat breads, respectively (**Tables 3** and **4**). This is a much larger decrease than that found by Amrein et al. (*13*) for the addition of glycine to gingerbreads, even though the level of addition seems to be similar (133 mmol/kg of dough). However, they did not give the exact recipe for the gingerbreads.

The apparent increase in acrylamide content when 200 mmolof glycine/kg of flour was added is probably caused by the fact that most of these samples were baked at 190 °C. This is approximately the temperature found to give the higest content of acrylamide in this system (*18*). Thus, increasing the amount of glycine added gave no further decrease in either case. As for the potato products, the acrylamide content in the flat breads and bread crusts without glycine was the same as previously found in the same systems (*18*). It has been shown that in breads the amount of acrylamide increases with time and temperature, whereas for flat breads the level of acrylamide decreased at long baking times and went through a maximum at ∼200 °C (*18*), as can be seen from the positive linear and negative quadratic response surface coefficients in time and temperature (**Table 5**). The ANOVA analysis showed that the highest acrylamide content was at a baking temperature of 190 °C. This was not caused by the fact that the flat breads with no glycine addition were baked at this temperature. When the zero addition samples were eliminated from the calculation, the p value was reduced from 0.029 to 0.001.

A similar maximum was found for the baking time at 17.5 min. As for the temperature, this was not caused by the zero addition samples because the p value was reduced from 0.046 to 0.004 when they were kept out of the calculation. Also, in the previous experiment with the same experimental setup a decrease in acrylamide content was found with increasing baking time (*18*). In that case, no initial increase with increasing time was found. The different time dependence indicated in the present experiment can be explained by differences in baking temperature between the samples. Most of the samples baked for 17.5 min were baked at 190 °C, which is expected to give high acryalmide contents. On the other hand, temperatures of 148 and 232 °C were used for the samples baked for 10.1 min. Thus, the time and temperature dependence of acrylamide content in flat breads is independent of whether glycine is added or not.

For bread crusts, the response surface indicated that the time and temperature dependence differed from what was previously found (*18*) (negative first-order and second-order coefficients for time, positive first-order and negative second-order coefficients in temperature). However, no effects of time, temperature, or level of glycine addition were found by ANOVA analysis. This, together with the low content of acrylamide found in this case, indicates that the apparent difference probably is of no importance.

Thus, the addition of glycine reduces the amount of acrylamide in bread and flat bread. In potato crisps, both glycine and, to a lesser extent, glutamine have a reducing effect on the content of acrylamide. It is uncertain whether the same is the case for French fries. This may provide a method for reduction of acrylamide. The reason for the effect is not clear, but at least two explanations seem to be reasonable. Glycine may compete effectively with asparagine in the Maillard reaction, or glycine may react with acrylamide. However, in a model system consisting of starch, asparagine, and glucose the acrylamide content was reduced after prolonged heat treatment (*18*), indicating that acrylamide reacts with amino acids. Furthermore, acrylamide is known to react with the NH_2 group in glycine (*28*).

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