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Importance of Oil Degradation Components in the Formation of Acrylamide in Fried Foodstuffs

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This study investigates the importance of selected oil degradation components and some analogues in the formation of acrylamide. For this, a model system containing silica gel, PBS buffer, and oil was heated in a closed tubular reactor, under practically relevant heating conditions. Several probable acrylamide precursors were mixed together with free asparagine in the model system, such as partial glycerides, glycerol, acrolein, acrylic acid, and several aldehydes. Only the heated model system containing acrolein and asparagine showed a significantly higher acrylamide content compared to the control to which only asparagine was added. It was postulated that a nucleophilic 1,2-addition of the α-amino group of free asparagine to the carbonyl function of acrolein would lead to the formation of acrylamide. This hypothesis could partially be confirmed, replacing acrolein with other α,β-unsaturated aldehydes. However, the contribution of acrolein to the overall formation of acrylamide appeared to be negligible in the presence of a reducing sugar, indicating that in foodstuffs the importance of acrolein and other oil degradation products is probably small.

KEYWORDS: Acrylamide formation; food; modeling; oil degradation; LC-MS/MS

INTRODUCTION

To reduce acrylamide in foodstuffs, the formation mechanisms should be clarified, as well as the factors influencing it. Acrylamide formation is closely linked to the Maillard reaction. The free amino acid asparagine and reducing sugars have been until now considered to be the main precursors of acrylamide (*1*). Fried foodstuffs, such as potatoes, are susceptible to acrylamide formation because they contain these compounds in relatively high amounts.

Although it was previously demonstrated that the fatty acid composition of the deep-frying oils did not significantly influence the final acrylamide content (*2*), additional formation pathways, starting from lipids, may exist (*3*–*8*). It was suggested that triacylglycerols partially hydrolyze during frying, followed by dehydration of glycerol to acrolein. This three-carbon compound may oxidize to acrylic acid, which can finally react with ammonia to form acrylamide, as shown in **Figure 1**. Acrolein could also be formed upon pyrolysis of triacylglycerols, without glycerol as an intermediary product. Moreover, monoacylglycerols decompose above 150 °C in an elimination reaction to acrolein and a free fatty acid (*6, 9*). Consequently, oil hydrolysis products may act as acrylamide precursors. However, the importance of these pathways within the framework of acrylamide formation in foodstuffs is still not completely clear. From previous studies on potato products (*10*), it could be concluded that the investigated oil degradation precursors did not significantly contribute to the formation of acrylamide. However, these potato products also contained reducing sugars, which participate in the conversion of free asparagine to acrylamide (*11*). In this study, it was therefore questioned whether oil degradation products could contribute more significantly to the formation of acrylamide in the absence of reducing sugars, under practically relevant heating conditions. In addition, the contribution of oil degradation products to the overall formation of acrylamide was investigated in the presence of different reducing sugar contents.

MATERIALS AND METHODS

Reagents and Chemicals. Phosphate-buffered saline (PBS) (pH 7.4) consisted of 0.135 M NaCl, 1.5 mM KH_2PO_4, 8 mM $NaH_2PO_4 \cdot 12H_2O$, and 2.7 mM KCl. These reagents as well as sulfuric acid were supplied by Chem-Laboratory, Zedelgem, Belgium. Acrylic acid, acrolein, crotonaldehyde, methacrolein, pentanal, hexanal, octanal, decanal, *trans*-cinnamaldehyde, glucose, and asparagine were from Acros Organics, Geel, Belgium. Glycerol was obtained from Sigma-Aldrich, Bornem, Belgium, and ammonia 25% (w/v) was from Chem-Laboratory. Grace Davison (Worms, Germany) delivered the silica gel (Davisil, 0.06−0.20 mm,

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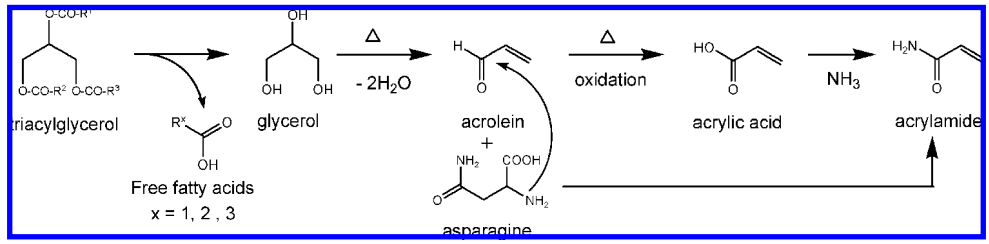

Figure 1. Formation of acrylamide after hydrolysis of triacylglycerol (*5, 6, 8*).

Table 1. Formation of Acrylamide in a Silica Gel Model System Containing Asparagine and Several Probable Acrylamide Precursors, upon Heating at 170 °C for 5 min in the Tubular Reactor

reactant added to asparagine in model system	acrylamide content[a] (μg/kg) ($N \geq 3$)
control (PBS)	360 a
glucose	9925 b
diacylglycerol	386 a
monoacylglycerol	364 a
glycerol	360 a
acrolein	1430 c
acrylic acid	316 a
methacrolein	1745 c
crotonaldehyde	408 a
trans-cinnamaldehyde	623 a
pentanal	319 a
hexanal	370 a
octanal	348 a
decanal	250 a

[a] Different letters indicate significant difference ($P < 0.05$) by Tukey test.

containing 10% water). All reagents and chemicals used for the acrylamide and sugar analysis were of analytical grade (>99%, w/w) as described earlier (*2*).

Preparation and Heating of Homogeneous Mixtures. Artificial mixtures were prepared as described earlier (*2*) using a dried and sieved potato powder (Unilever, Brussels, Belgium), initially containing 0.03 g of fructose/100 g of powder, 0.03 g of glucose/100 g of powder, and 0.89 g of asparagine/100 g of powder. In short, the potato powder was mixed with water and fresh sunflower oil to obtain a homogeneous mixture with a final composition of 41% potato powder, 39% water, and 20% fresh sunflower oil. The oil freshness was assessed by means of the *p*-anisidine value (*12*). In a similar way, artificial mixtures were prepared, in which silica gel was used instead of potato powder. These mixtures consisted of 41% SiO_2, 20% oil, and 39% PBS. Prior to homogenization, several components, mentioned in **Table 1**, were dissolved in the aqueous or oil fraction. Subsequently, 1 g of the mixture was introduced as a cylinder (diameter = 1 cm) in a stainless steel tubular reactor (internal diameter = 1 cm). Then the reactor was sealed and heated in a deep-fryer (Fritel 2505, Hasselt, Belgium), equipped with a thermocouple and a stirring mechanism to ensure a homogeneous temperature in the oil bath. Heating experiments were performed for 6 min at 170 ± 1 °C. After heating, a quick cooling was established by submerging the reactor in an ice bath for 2 min. Finally, the 1 g mixture was analyzed for its acrylamide content. All reported acrylamide levels are the average of at least two heating experiments.

Acrylamide Analysis. Acrylamide was determined by LC-MS/MS as described earlier (*2*). After aqueous extraction, using [2,3,3-D_3]acrylamide as internal standard, the acrylamide extract was further cleaned up by solid-phase extraction. The extract was analyzed using LC-MS/MS with positive electrospray ionization.

Sugar Analysis. Mono- and disaccharides were quantified by GC analysis as described earlier (*13*). Briefly, after aqueous extraction, addition of an internal standard (phenyl β-D-glucopyranoside), and cleanup, the filtrate was derivatized and injected in a GC equipped with a flame ionization detector.

Statistical Analysis. The average acrylamide contents were compared using analysis of variance (one-way ANOVA) and post hoc multiple comparison of means (Tukey).

RESULTS AND DISCUSSION

To investigate the importance of oil degradation products with respect to other acrylamide precursors, it was essential to devise a model system free of sugars. Therefore, the sugars were completely extracted from the potato powder used in preceding studies (*2, 10*). Several cold water extraction steps were performed until no sugars were detected anymore in the lyophilized powder. Similar to previous investigations, a model system was reconstituted, composed of 41% sugar free potato powder, 20% fresh sunflower oil (with a *p*-anisidine value of < 1), and 39% PBS. The PBS was used to have an initial pH of 6.0 of the fresh mixture and to stabilize the pH during heating. It could be assumed that the free asparagine was also largely extracted from the powder. Therefore, it was again added to the model system in a similar amount as in potatoes, being 0.46 mmol/10 g of mixture. Heating experiments in the tubular reactor were performed, adding only asparagine and asparagine in combination with glucose to the model system. Because the glucose content in potatoes is in general about 10 times lower compared to the asparagine content (*14*), it was added to obtain a final content of 0.046 mmol of glucose/10 g of mixture. Surprisingly, no significantly different acrylamide content was found between either heated mixtures, being, respectively, 978 and 1041 μg/kg. Also, after heating, no reducing sugars were detected in either mixture. It was assumed that the starch, present in the sugar-free powder, was hydrolyzed to some extent during heating in the tubular reactor, liberating sugars which could in situ react with the present asparagine to form acrylamide in a similar way as in the model system to which glucose was added. Because the presence or eventual generation of reducing sugars during the heating step was not desirable in the current experimental setup, the sugar-free potato powder appeared to be an unsuitable matrix for heating.

Therefore, the potato powder in the model system was replaced with inert silica gel, similar to previous investigations (*15*) in which silica gel appeared to be inert enough as a matrix. To simulate the real food composition as much as possible, fresh sunflower oil, PBS, and free asparagine were again added in similar amounts as in the above-mentioned experiments. The initial pH of all mixtures was again stabilized at 6 by means of PBS. A silica gel model system with only asparagine being added in the liquid phase was used as a control (**Table 1**). Other probable acrylamide precursors, mentioned in **Table 1**, were added in the same molar amount as glucose in the above-mentioned experiments to have a precursor/asparagine ratio of 0.1.

As shown in **Table 1**, an acrylamide content of 360 μg/kg was measured in the model system containing only asparagine as acrylamide precursor. It was indeed previously demonstrated that free asparagine may release acrylamide by thermally initiated decarboxylation and deamination (*8, 16, 17*). However, the acrylamide content found in these preceding studies was lower, most probably due to the somewhat different experimental setup applied. In contrast to the model system with the

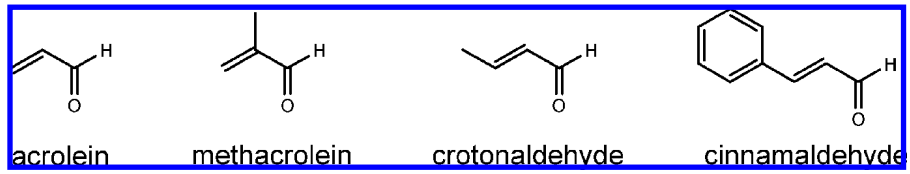

Figure 2. Chemical structures of acrolein, methacrolein, crotonaldehyde, and cinnamaldehyde.

sugar-free powder, addition of glucose dramatically increased acrylamide formation to 9925 μg/kg. This content is about 10 times higher than in the previous experiments with the sugar-free powder. This can be explained by the fact that the silica gel model system did not contain other free amino acids (besides asparagine) or proteins, which are known to compete with asparagine for available sugar when the reducing sugars are limiting (*18*). On the basis of the reaction mechanism proposed in **Figure 1**, several oil hydrolysis compounds were subsequently evaluated, including diacylglycerol, monoacylglycerol, glycerol, acrolein, and acrylic acid.

The addition of diacylglycerol, monoacylglycerol, glycerol, or acrylic acid to the asparagine-containing model system did not lead to a significant increase in acrylamide formation compared to the control, containing only asparagine (**Table 1**). However, according to **Figure 1**, ammonia is required to form acrylamide from acrylic acid. It was not known, though, how much ammonia was liberated from asparagine in the silica gel model system upon heating. Therefore, an additional heating experiment was performed in which acrylic acid and ammonia were heated in the tubular reactor without the presence of asparagine. No acrylamide was, however, formed (results not shown). These results are in contrast to earlier studies (*8, 19*) in which more intense heating treatments were applied (170 or 180 °C for 30 min). Under the current and practically more relevant heating conditions, the postulated formation mechanism of acrylamide via acrylic acid could not be confirmed.

Interestingly, the heated model system containing both acrolein and asparagine showed a significantly higher acrylamide content compared to the control, to which only asparagine was added, but this increase was less pronounced compared to the model system with asparagine and glucose (**Table 1**). Heating of acrolein with ammonia in the absence of asparagine did not lead to acrylamide formation (results not shown). Consequently, it could be postulated that a nucleophilic 1,2-addition of the α-amino group of free asparagine to the carbonyl function of acrolein would lead to the formation of acrylamide, via the Schiff base. After decarboxylation, an imine is formed, which hydrolyzes to 3-aminopropionamide, which is known to efficiently release acrylamide (*17*). However, the α-amino group of asparagine could also react with acrolein via the so-called Michael addition (1,4-addition). After tautomerization, an intermediate with a structure similar to that of the Amadori compound is formed. Due to the absence of the imine in the β-position of the carboxylic acid group, this compound would, however, not easily decarboxylate, as was demonstrated previously (*20, 21*). Consequently, this Michael addition pathway is not very likely to generate acrylamide.

To further investigate this hypothesis, several α,β-unsaturated aldehydes with different substituents on the α- (R^1) and β-carbons (R^2) were heated with asparagine in the model system (**Figure 2**). The heated mixtures containing asparagine and methacrolein showed an increase in acrylamide formation similar to those of the mixtures with asparagine and acrolein (**Table 1**). Also, crotonaldehyde and cinnamaldehyde increased, in combination with asparagine, the formation of acrylamide, compared to the control containing only asparagine, although

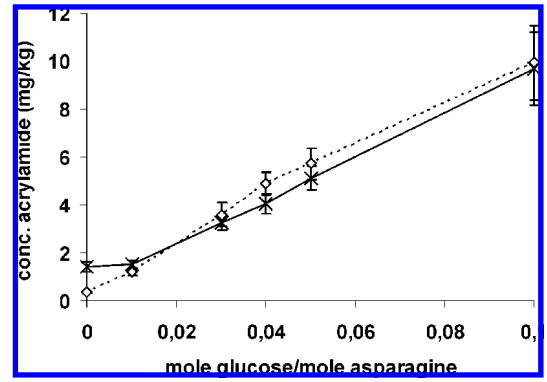

Figure 3. Acrylamide formation as a function of several molar glucose/asparagine ratios of a silica gel model system containing 0.1 mol of acrolein/mol of asparagine (×) and a model system containing initially only asparagine (◇), heated in the tubular reactor at 170 °C for 5 min.

not significantly. For cinnamaldehyde, this could be explained by the fact that the water solubility of this reactant is lower compared to acrolein. The reaction with asparagine, present in the aqueous fraction of the model system, would thus be less probable. On the other hand, it was expected that the β-methyl group of crotonaldehyde would hinder the 1,4-addition, which could possibly promote the 1,2-addition (*22*) and thus acrylamide formation. On the contrary, the mixture containing crotonaldehyde had a lower acrylamide content compared to the mixture to which methacrolein was added, suggesting that the 1,2-addition was not stimulated. Of course other reactions between the several intermediary components may also occur and complicate the unraveling of the overall reaction mechanism.

Furthermore, several known oil oxidation products, such as pentanal, hexanal, octanal, and decanal (*23*), were evaluated in the asparagine-containing silica gel system, but these compounds did not increase the formation of acrylamide compared to the control (**Table 1**). Similar to cinnamaldehyde, the poor water solubility of these aldehydes might explain this outcome. Previously, it was, however, postulated that octanal and decanal were able to react with asparagine to form acrylamide (*4, 17*). Our results indicate that the formation of acrylamide in the presence of these aldehydes and asparagine could merely be attributed to decarboxylation and deamination of asparagine, at the applied practically relevant heating conditions.

In the presence of asparagine, it was thus observed that acrolein significantly enhanced acrylamide formation, compared to the control containing only asparagine. In contrast to a common deep-frying process, these experiments were performed in a closed reactor. Because frying temperatures are far above the boiling point of acrolein (51 °C), this compound may readily evaporate from the foodstuff being fried (*3, 24*). The importance of this chemical pathway in real foodstuffs such as French fries remains thus questionable. In this respect, the current experimental design in a closed reactor can thus be seen as a worst-case event. In addition, the significance of this pathway should be evaluated in the presence of other acrylamide precursors, such as glucose. Therefore, in a final series of experiments the

glucose concentration was gradually increased in the applied model system, from 0 to 0.1 mol of glucose/mol of asparagine, in a silica gel model system containing 0.046 mmol of acrolein and 0.46 mmol of asparagine/10 g of mixture. Similar experiments were performed with a model system containing only 0.46 mmol of asparagine/10 g of mixture. The results (experiments performed in quadruplicate) are presented in **Figure 3** and show that acrylamide gradually increased as the glucose content was enhanced. The difference between the model system with and without acrolein, however, disappeared when 0.01 mol of glucose/mol of asparagine was added, indicating that the contribution of acrolein to the overall formation of acrylamide was negligible in the presence of reducing sugars in the model system matrix. From the above-mentioned results, the importance of the suspected acrylamide precursors acrolein and other oil degradation products in general appeared thus to be negligible compared to that of reducing sugars, as evaluated by the silica gel model system. Because the experiments in this study were performed in a closed reaction system, it can be expected that volatile oil degradation components such as acrolein probably contribute even less to the formation of acrylamide in an open environment, during food preparation.

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