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Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms

Frédéric Mestdagh [a,b], Jo Maertens [c], Tatiana Cucu [a], Karel Delporte [a],
Carlos Van Peteghem [b], Bruno De Meulenaer [a,*]

[a] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[b] Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[c] BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

Abstract

The impact on acrylamide formation of several additives was investigated as well as the mechanisms behind it. In a potato powder model system, sodium acid pyrophosphate, citric, acetic and L-lactic acid significantly reduced the final acrylamide content, merely due to the lowering of the pH. Free glycine, L-lysine and L-cysteine also lowered acrylamide, while keeping the pH at its original level. L-glutamine increased the formation of acrylamide. A synergistic acrylamide lowering effect was observed, adding citric acid and glycine or L-lysine to the model system. Yet, a combination of these amino acids with acetic acid appeared to induce a restricted antagonistic acrylamide lowering effect. Calcium and magnesium ions induced a supplementary acrylamide reduction in addition to a lower pH of the food matrix. No lowering effect was however observed upon NaCl addition to the model system.
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1. Introduction

Evidence of the widespread occurrence of acrylamide in fried and baked carbohydrate-rich foodstuffs has stimulated international research. These investigations have contributed to a better insight into the formation pathways, mainly originating from the Maillard reaction between asparagine and carbonyl compounds, such as reducing sugars (Stadler et al., 2004). A number of raw material pretreatments was investigated which could mitigate acrylamide formation. Lowering the pH of the foodstuff would block the nucleophilic addition of asparagine with a carbonyl compound, preventing the formation of the corresponding Schiff base, a key intermediate in the Maillard

reaction and in the formation of acrylamide (Jung, Choi, & Ju, 2003; Kita, Bråthen, Knutsen, & Wicklund, 2004; Pedreschi, Kaack, Granby, & Troncoso, 2007; Rydberg et al., 2003). Furthermore, mono- and divalent cations such as Na^+ or Ca^{2+} were indicated as efficient acrylamide reducing agents. It was postulated that these ions could interact with asparagine so that the Schiff base formation was again prevented (Gökmen & Senyuva, 2007a, 2007b; Lindsay & Jang, 2005a, 2005b; Park et al., 2005). The addition of proteins or free amino acids other than asparagine such as glutamine, glycine, lysine or cysteine was also studied. These components would reduce acrylamide formation by promoting competitive reactions and/or by covalently binding the formed acrylamide through Michael type addition reactions (Bråthen, Kita, Knutsen, & Wicklund, 2005; Claeys, De Vleeschouwer, & Hendrickx, 2005; Cook & Taylor, 2005; Hanley et al., 2005; Kim, Hwang, & Lee, 2005; Low et al., 2006; Rydberg et al., 2003). Combined

treatments with different amino acids (Hanley et al., 2005) or with an amino acid and organic acid (Low et al., 2006) could have an even greater potential to reduce acrylamide formation.

It is however not always clear what is the main mechanism provoking a reduction in acrylamide formation. For instance, addition of Ca^{2+} ions to potato cuts is known to provoke a pH drop, since hydrogen chloride is produced when calcium crosslinks the pectin (Andersson, Gekas, Lind, Oliveira, & Öste, 1994). It can thus be questioned whether the acrylamide mitigating effect in potatoes is not merely due to a decrease in pH. Besides, De Vleeschouwer, Van der Plancken, Van Loey, and Hendrickx (2006) found that an increasing citrate and phosphate buffer concentration seemed to reduce the final acrylamide content, at constant pH. This effect was ascribed to the sterical hindrance caused by the high amount of buffer ions which hinder an effective reaction between the acrylamide precursors. The acrylamide lowering effect of citric acid could thus possibly be attributed to both a pH drop and a spatial hindrance.

Therefore, the main purpose of this investigation was to determine the acrylamide lowering impact of several organic acids, free amino acids and salts, and to find out whether this mitigation is merely due to pH related effects or to other factors. Since most of these additives are already commonly used in the food industry, these components can be more easily applied on industrial scale to mitigate the formation of acrylamide in fried potato products.

2. Materials and methods

2.1. Reagents and chemicals

NaOH (>99% w/w) and HNO_3 (65% w/w) were used to adjust the pH in the potato powder model system. Furthermore NaCl, $MgCl_2 \cdot 6H_2O$ and K_2HPO_4 were applied, all with a purity >99% (w/w) and delivered by Chem-Lab (Belgium). $CaCl_2$ (36% w/v) and citric acid (>99.8% w/w) were supplied by Brenntag (Belgium), while L-lactic acid (50% w/v) and calcium-L-lactate $\cdot$ $5H_2O$ (>99.9% w/w) were provided by Purac Biochem (The Netherlands). Acetic acid was bought in retail as vinegar (7% v/v). Glycine, L-glutamine, L-lysine and L-cysteine (>99.8% w/w) were supplied by Sigma Aldrich (Belgium). Sodium acid pyrophosphate ($Na_2H_2P_2O_7 > 95\%$ w/w) was delivered by Sibeco (Belgium). All reagents and chemicals used for the acrylamide analysis were described in Mestdagh et al. (2005).

2.2. Preparation of potato powder mixtures in tubular reactor

The artificial mixtures were prepared as described earlier (Mestdagh, De Meulenaer, & Van Peteghem, 2007) using a dried and sieved potato powder (Unilever, Belgium), containing 0.03 g fructose/100 g powder, 0.03 g glucose/100 g powder and 0.89 g asparagine/100 g powder. In short, the potato powder was mixed with water, containing different dissolved compounds, and oil in order to obtain a homogeneous mixture with a final composition of 41% potato powder, 38% water and 21% oil. Prior to heating, the pH was measured by placing the pH electrode (Schott, Germany) directly into the homogenized mixture. 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 deepfryer (Fritel 2505, Belgium), equipped with a thermocouple (Testo 925, Belgium) and with a stirring mechanism to ensure a homogeneous temperature in the oil bath. Heating experiments were performed for 6 min at 170 °C (± 1 °C). With these heating conditions, acrylamide could be quantified over the whole range of pH values investigated. After heating, a quick cooling was established, 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.

2.3. Acrylamide analysis

Acrylamide was determined by LC-MS/MS as described earlier (Mestdagh et al., 2005). 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.

2.4. Statistical analysis

The statistical package R was used to perform the regression and to calculate the 95% confidence intervals, shown in Fig. 1 (Gentleman & Ihaka, 1997). In order to properly assess the 95% confidence intervals on the acrylamide reduction factors in the potato model system, a bootstrap method was applied.
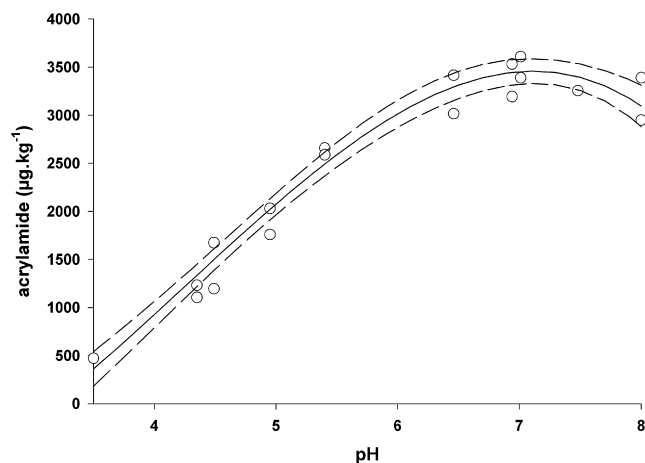

Fig. 1. Acrylamide formation as a function of pH in the potato model system, heated at 170 °C for 6 min. ○: measurements; —: fitted equation; ---: 95% confidence interval on the equation.

3. Results and discussion

3.1. Influence of pH on acrylamide formation in potato model system

The formation of acrylamide was investigated as a function of pH, heating homogeneous potato powder mixtures with different initial pH in a closed tubular reactor. The model system approached the chemical composition of French fries. Moreover, this closed heating methodology eliminated some variable physical and chemical factors normally occurring during the frying process, such as surface deformation, heat flux, water evaporation and oil ingress into the food, as discussed in previous studies (Mestdagh et al., 2005, 2007). The pH of the potato model system was adjusted by addition of NaOH or HNO_3 in a pH range between 3.5 and 8.

The acrylamide contents of the mixtures, heated at 170 °C for 6 min, are shown in Fig. 1. A third order mathematical model was built which correlates the pH of the mixtures to the final acrylamide content. Also the 95% confidence intervals are plotted in Fig. 1. Acrylamide formation showed an optimum between pH 7 and 7.5, which was slightly lower compared to previous studies where an optimum pH around 8 was obtained (Rydberg et al., 2003). Furthermore, a clear decrease in acrylamide content was noted upon acidification of the potato powder matrix, as observed earlier (Jung et al., 2003; Kita et al., 2004; Rydberg et al., 2003).

3.2. Influence of additives on acrylamide formation in potato model system

The mathematical model in Fig. 1, describing acrylamide formation as a function of pH, was subsequently used to evaluate whether the mitigation of acrylamide formation due to addition of specific additives was merely attributable to a pH reduction or to another mitigating mechanism. Therefore, several components were added to the potato powder at a level of 50, 100 or 200 μmol/g mixture. These molar levels are in the same order of magnitude as the acrylamide precursor asparagine, present in the potato powder. The compounds were added separately or in combination with other additives, as shown in Table 1. Again, the potato model system proved its fit-for-purpose since the added components could be mixed homogeneously throughout the entire matrix, enabling a better evaluation of their chemical impact on acrylamide formation. The pH of the mixtures was determined just before heating. In Table 1, the acrylamide content in each heated mixture was compared with the acrylamide content in the heated potato powder mixture to which no additives were added. This control mixture had a pH of 5.4 before heating and generated upon heating acrylamide up to an average contamination level of 2492 μg kg^{-1}. Subsequently, the acrylamide content in each mixture was compared with the acrylamide content, obtained from the mathematical model, shown in Fig. 1, at the same pH level of the

experimental mixture evaluated. This enabled to make a clear distinction between acrylamide mitigation due to a pH effect and possible other acrylamide mitigating or promoting effects (last column of Table 1).

As shown in Table 1, three organic acids were evaluated, which are already used in the food industry as acidity regulator, flavouring agent or preservative. Addition of these acids significantly reduced the pH, in comparison with the control mixture to which only water was added. At a concentration of 100 μmol/g mixture, citric acid was the most efficient acidifier, followed by L-lactic and acetic acid, as can be explained by the acid dissociation constants. The acrylamide content followed the same trend. Comparing the acrylamide contents with the control mixture at the same pH, no significant additional decrease or increase could be detected. The additional acrylamide lowering effect of citric acid, as suggested earlier (De Vleeschouwer et al., 2006), appeared not to be significant and could thus not be confirmed. Similarly, also the other organic acids mitigated acrylamide formation only due to their impact on the product pH, at the applied concentration levels.

Subsequently, the impact on acrylamide formation of four free amino acids was investigated. L-lysine and glycine belong to the group causing the most intense Maillard browning. L-glutamine, structurally resembling L-asparagine, belongs to the amino acid group producing intermediate browning, while the sulphur-containing L-cysteine is known to generate the lowest browning (Ajandouz & Puigserver, 1999; Ashoor & Zent, 1984). The nucleophilic sulphur atom of L-cysteine and the amino groups of the other amino acids might readily give rise to Michael type addition reactions with acrylamide (Fennell et al., 2005; Stadler et al., 2004). These amino acids might also reduce acrylamide formation by competing with asparagine to react with reducing sugars in the Maillard reaction. As shown in Table 1, these components only marginally changed the pH of the mixtures, compared to the control mixture. There was however a significant impact on the acrylamide formation. L-cysteine appeared to reduce the acrylamide content in the most effective way, with a reduction of about 92%, followed by L-lysine (39%) and glycine (24%). These results confirm previous investigations and above-mentioned hypotheses, although there were some differences in degree of reduction between different studies (Bråthen et al., 2005; Kim et al., 2005; Rydberg et al., 2003). Rydberg et al. (2003) for instance found a more efficient acrylamide reduction of glycine compared to L-lysine in a potato model system. On the other hand, Kim et al. (2005) showed quite similar reductions of L-lysine and glycine in blanched potato crisps. In a dough system, L-cysteine was even observed to be the least efficient acrylamide lowering agent of the three. When this sulphur-containing amino acid was however applied in current experiments, unpleasant off-flavours were detected upon opening the tubular reactor, making its application in real foodstuffs unacceptable to consumers. Interestingly, L-glutamine significantly promoted the formation of

Table 1

Impact of addition of several components at different concentrations on the pH and acrylamide content in the potato model system, heated at 170 °C for 6 min

Added component	Concentration µmol (g mixture)$^{-1}$	pH mixture	Acrylamide content (µg kg^{-1})	% Change in acrylamide content	
				Compared to control[a]	Compared to control at same pH[b]
Citric acid	100	3.7	553	−78[*]	6
	50	4.0	754	−70[*]	−19
Acetic acid	100	4.5	1341	−46[*]	−10
	50	4.7	1613	−35[*]	−9
L-lactic acid	100	4.2	937	−62[*]	−16
L-cysteine	50	5.5	208	−92[*]	−92[*]
L-glutamine	50	5.3	3745	50[*]	57[*]
Glycine	50	5.4	1891	−24[*]	−25[*]
L-lysine	50	5.4	1522	−39[*]	−39[*]
Citric acid + glycine	50 + 50	4.1	697	−72[*]	−31[*]
Citric acid + L-lysine	50 + 50	4.1	566	−77[*]	−42[*]
Acetic acid + glycine	50 + 50	4.8	1429	−43[*]	−20[*]
Acetic acid + L-lysine	50 + 50	4.7	1285	−48[*]	−27[*]
NaCl	200	5.3	2359	−5	1
	50	5.3	2250	−10	−7
$Na_2H_2P_2O_7$	100	5.0	1869	−25[*]	−10
K_2HPO_4	100	6.8	3487	40[*]	2
$MgCl_2$	100	4.6	1058	−58[*]	−34[*]
$CaCl_2$	100	4.5	459	−82[*]	−70[*]
	50	5.0	1353	−46[*]	−34[*]
	100[c]	5.7[c]	1165	−53[*]	−58[*]
Calcium-L-lactate	100	5.0	1282	−49[*]	−38[*]
	50	5.0	1313	−47[*]	−38[*]

[a] Control = potato powder mixture to which only water was added (pH 5.4).
[b] At the pH of the experimental mixture evaluated.
[c] pH of unheated mixture adjusted with NaOH.
[*] Significant change ($P < 0.05$).

acrylamide with 50% (Table 1). In literature, conflicting results were found for this amino acid. Several studies showed a significant decrease upon addition of free L-glutamine (Bråthen et al., 2005; Hanley et al., 2005; Rydberg et al., 2003). Similar to our results, Claeys et al. (2005) observed an increase, although this was not attributed to a direct acrylamide formation from the amino acid. These results indicate that the efficiency of free amino acids to lower the acrylamide content may depend on the experimental set-up, such as the heating conditions or the way of applying these components to the foodstuff (e.g. by soaking or blanching or by homogeneous mixing).

Furthermore, a combination of organic acids (citric and acetic acid) with glycine and L-lysine was tested to check possible synergistic effects on acrylamide mitigation between organic acids and amino acids (Low et al., 2006). Again, the mixtures containing citric acid had a lower pH and lower acrylamide content compared to the mixtures with acetic acid and compared to the control mixtures with water. L-lysine showed once more an additional acrylamide mitigating effect in comparison with glycine. Interestingly, in the combination of amino acids with organic acids, acetic acid seemed to have an antagonistic effect, while the effect of citric acid was merely synergistic. Hence, a less pronounced reduction in acrylamide forma-

tion was obtained within the combined acetic acid/L-lysine addition (27%) compared to the addition of L-lysine (39%), if evaluated against the control at the same pH.

Besides, the effect of several salts was evaluated in the potato model system (Table 1). NaCl did not appear to significantly reduce the acrylamide content, even at a concentration of 200 µmol/g mixture, which corresponds to about 1% (w/w). This is in contrast to previous investigations, where significant acrylamide reductions were obtained at this concentration level. These earlier reported experiments were however performed in an aqueous asparagine/glucose model system (Kolek, Simko, & Simon, 2006). Yet, in real foodstuffs, NaCl appeared to have more acrylamide lowering properties compared to model systems (Franke, Sell, & Reimerdes, 2005; Gökmen & Senyuva, 2007a), as investigated in a subsequent study (Mestdagh, De Wilde, Delporte, Van Peteghem, & De Meulenaer, 2007). In addition, the effect of sodium acid pyrophosphate ($Na_2H_2P_2O_7$) was evaluated. Pyrophosphate is already used in the potato processing industry to reduce darkening of the blanched potato cuts, caused by a reaction between Fe^{3+} and chlorogenic acid present in the potato cuts (Mazza & Qi, 1991). This additive lowered the pH of the mixtures and accordingly the acrylamide content with 25% (Table 1), but no significant additional reduction, apart

from the lower pH, was assessed. In a previous study (Pedreschi et al., 2007), no acrylamide lowering effect at all was found in French fries, blanched in water containing sodium pyrophosphate. Yet, the pH was not monitored. Also for K_2HPO_4, no supplementary acrylamide inhibiting effect was found at the investigated concentration level (Table 1). In contrast, the acrylamide content increased with 40%, compared to the control mixture to which no additives were added. This was due to the increased pH, since no significant difference was assessed with the control mixture at the same pH. In a previous study, it was suggested that phosphate ions exerted an inhibiting effect on acrylamide formation, apart from the pH effect (De Vleeschouwer et al., 2006). This could however not be confirmed in the current model system at the applied concentration level.

Finally, $CaCl_2$ and $MgCl_2$ were investigated. $MgCl_2$ is already used as a coagulant and $CaCl_2$ as a firming agent during fruit and vegetable processing (Andersson et al., 1994; Carbonell, Oliveira, & Kelly, 2006). These compounds did significantly lower the final acrylamide content in the model system, although this effect was more distinct for $CaCl_2$ (Table 1). In addition to the expected acrylamide reduction due to the pH drop as a result of the interaction of Ca^{2+} with pectin (Andersson et al., 1994), an additional interaction was however observed leading to an acrylamide mitigation of 70% compared to the control at the same pH. For Mg^{2+}, this interaction seemed less pronounced (34%), at 100 µmol/g mixture. In order to further clarify this, the pH drop in the potato powder was counteracted by addition of NaOH to the $CaCl_2$ containing mixture prior to heating. Still a significant difference (58%) with the control mixture at the same pH was observed, confirming the efficient acrylamide reducing effect of calcium, next to its pH lowering effect, as it was previously postulated (Gökmen & Senyuva, 2007a; Lindsay & Jang, 2005a, 2005b; Park et al., 2005). Recently, evidence was found that cations such as Ca^{2+} or Mg^{2+} would change the reaction path from the Maillard reaction toward dehydration of glucose (Gökmen & Senyuva, 2007b). Since calcium appeared to efficiently lower acrylamide formation, calcium-L-lactate was also investigated (Table 1). Similar reductions were obtained, compared to $CaCl_2$, for the 50 µmol/g mixture. In contrast to $CaCl_2$, the acrylamide content and pH did however not further drop at a calcium-L-lactate concentration of 100 µmol/g mixture.

4. Conclusion

From the experiments with the potato powder model system, it could be concluded that the addition of free glycine and L-lysine gave significant acrylamide reductions, while keeping the pH of the mixture at its original level. Addition of organic acids and sodium acid pyrophosphate also significantly mitigated the final acrylamide content, but merely due to the lower pH. Ca^{2+} and Mg^{2+} ions combined a reduction in pH with an additional acrylamide lowering effect, previously ascribed to the binding of divalent ions to asparagine in order to prevent the Schiff base formation, which is a key step in acrylamide formation (Gökmen & Senyuva, 2007a; Lindsay & Jang, 2005a, 2005b). In the subsequent study (Mestdagh et al., 2007), these mitigation strategies will be evaluated in a real food system, being potato crisps. In addition, the final product quality of these treated foodstuffs will be assessed by means of sensory analysis of potato crisps.

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