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Acrylamide formation is prevented by divalent cations during the Maillard reaction

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Abstract

The effects of mono- and divalent cations on the formation of acrylamide were studied in a fructose-asparagine model system at 150 and 180 °C. At amounts equivalent to those of asparagine and fructose, added divalent cations, such as Ca^{2+} , were found to prevent acrylamide formation completely, whereas monovalent cations, such as $Na⁺$, almost halved the acrylamide formed in the model system. It was confirmed by mass spectrometric analyses of pyrolyzates that the formation of the Schiff base of asparagines, which is the key intermediate leading to acrylamide, was prevented by the cations. Meanwhile, the reaction proceeded to form brown coloured products. Dipping potatoes into calcium chloride solution inhibited the formation of acrylamide by up to 95% during frying. The sensory quality of fried potato strips, in terms of golden yellow colour and crispy texture, was not adversely affected by this treatment.

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1. Introduction

Detection of high concentrations of acrylamide in common heated foodstuffs in April 2002 [\(Swedish NFA, 2002](#page-6-0)) caused considerable public concern, since acrylamide was found to be carcinogenic in rodents ([Johnson et al., 1986](#page-6-0)) and is classified as a probable human carcinogen ([IARC,](#page-6-0) [1993\)](#page-6-0). These findings caused the European Community [\(EC, 2002](#page-6-0)), and the WHO [\(FAO/WHO, 2002](#page-6-0)) to initiate projects for the minimization of acrylamide content in commercial as well as in homemade foods.

A number of theoretical mechanisms have been proposed for the formation of acrylamide in heated food. Most probably, acrylamide in food results largely from the Maillard reaction between asparagine and a reactive carbonyl, proceeding through intermediates that include a

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Schiff's base ([Mottram, Wedzicha, & Dodson, 2002; Sta](#page-6-0)[dler et al., 2002; Zyzak et al., 2003\)](#page-6-0). Several factors, such as the initial concentration of reactants and their ratio, temperature and time of processing, pH and water activity, have been shown to influence the formation levels of acrylamide in heat-processed foods ([Friedman, 2003\)](#page-6-0). The influence of temperature on the formation of acrylamide has been repeatedly demonstrated ([Becalski, Lau, Lewis, &](#page-6-0) [Seaman, 2003; Biedermann & Grob, 2003; Rydberg et al.,](#page-6-0) [2003; Stadler et al., 2002; Tareke, Rydberg, Karlsson, Eri](#page-6-0)ksson, $&$ Törnqvist, 2002).

Recent studies have indicated that polyvalent cations reduce acrylamide formation in thermally processed snack foods and bakery products ([Elder, Fulcher, Leung, &](#page-6-0) [Topor, 2005; Lindsay & Jang, 2005\)](#page-6-0). In this study, model Maillard and real food systems were employed to investigate the potential formation and degradation of acrylamide during heating in the presence of mono- and divalent cations.

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2. Materials and methods

2.1. Chemicals and consumables

Acrylamide (99+%) and ${}^{13}C_3$ -labelled acrylamide (99%) isotopic purity) were obtained from Sigma (Diesenhofen, Germany) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Fructose and asparagine were obtained from Sigma. Acetic acid, formic acid, sodium chloride, potassium chloride, calcium chloride and magnesium chloride were all reagent grade and obtained from Merck (Darmstadt, Germany). The analytical column Zorbax SB-Aq $(250 \times 4.6 \text{ mm}, 5 \mu \text{m})$ was supplied by Agilent Technologies (Wilmington, DE, USA). Micro-spin centrifuge filters $(0.45 \,\mu\text{m})$ were obtained from Alltech Associates (Deerfield, IL, USA).

Working standards of acrylamide were prepared at concentrations of 0.1, 0.2, 0.3, 0.5, 1.0 and $2.0 \mu g/ml$ in 0.01 mM acetic acid. Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 ml of water, respectively.

2.2. Preparation of reaction mixtures for pyrolysis

A model system composed of fructose (or glucose) and asparagine was used for the determination of concurrent formation and degradation of acrylamide in the presence of monovalent (Na^+, K^+) or divalent (Ca^{2+}, Mg^{2+}) cations. Reactants were combined in a 25 ml test tube (Pyrex closed system). The amounts of fructose, asparagine and cation were 5 µmoles in the reaction mixture. Total reaction volume was adjusted to 100 µl with water in each case, to promote the physical interaction of reactants. The pH value of the reaction mixture was 4.81. Adding 5 or 10μ mol of cation into the reaction mixture did not change the pH value.

Carefully closed test tubes containing the reactants were placed in an oil bath. Reactions were performed at 150 and 180 °C. After predefined heating periods of 5, 10, 15, 20, 30, 45 and 60 min, the tubes were immediately placed on ice.

2.3. Preparation of potato strips for frying

Potato was cut into strips $(8.5 \text{ mm} \times 8.5 \text{ mm})$ using a French fry cutter and the strip length was adjusted to 70 mm. The strips were divided into three lots. One lot was kept as control without any pretreatment prior to frying. The strips of the other two lots were soaked in a solution of calcium chloride or sodium chloride at a concentration of 0.1 M. After dipping for 15, 30 and 60 min at room temperature, potato strips were withdrawn from the solution and drained for 2 min prior to frying. Two potato strips were dipped into 11 of salt solution in each case. Frying of potato strips was performed using sunflower oil in a 51 oil bath at 170° C for 5 min.

2.4. Analysis of acrylamide

2.4.1. Extraction of acrylamide from the pyrolysates

The pyrolysates were suspended in 1.0 ml of water and the aqueous extract was obtained by vortexing for 1 min. The mixture was transferred into a micro-spin centrifuge filter (0.45 µm) and centrifuged for 5 min at 5000 rpm . Twenty millilitres of clear extract was analysed by liquid chromatography-mass spectrometry (LC-MS) to quantify acrylamide. Each pyrolysate was supplemented with ${}^{13}C_3$ acrylamide (100 ng) as internal standard.

2.4.2. Extraction of acrylamide from the fried potato strips

A procedure described by us elsewhere was used with minor modification (Şenyuva & Gökmen, 2006) Finely ground sample (1 g) was weighed into a 10 ml glass tube with cap. Carrez $1 (500 \,\mu\text{I})$ and Carrez $2 (500 \,\mu\text{I})$ solution were added and the volume was adjusted to 10 ml with 0.2 mM acetic acid. After mixing in a vortex mixer for 2 min, 1 ml of raw extract was transferred into a micro-spin centrifuge filter $(0.45 \,\mu\text{m})$ and centrifuged for 10 min at 10000 rpm at 0 °C. The clear extract (20 μ I) was analysed by LC-MS to quantify acrylamide. Each sample was supplemented with ${}^{13}C_3$ -acrylamide (100 ng).

2.5. LC-MS analysis of acrylamide

The quantification of acrylamide was performed by an Agilent 1100 HPLC system (Waldbronn, Germany), consisting of a binary pump, autosampler and temperaturecontrolled column oven, coupled to an Agilent 1100 MS detector, equipped with atmospheric pressure chemical ionisation (APCI) interface. The chromatographic separations were performed on a Zorbax SB-Aq column, using an isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid, at a flow rate of 0.6 ml/min at 40 °C. Data acquisition was performed either in scan mode or in selected ion monitoring (SIM) mode using the interface parameters: drying gas $(N_2, 100 \text{ psig})$ flow of 4 l/ min, nebulizer pressure of 60 psig, drying gas temperatures 325 °C, vaporiser temperature of 425 °C, capillary voltage of 4 kV , corona current of $4 \mu\text{A}$, fragmentor voltage of 55 eV. Ions monitored were m/z 72 and 55 for acrylamide, m/z 75 and 58 for ¹³C₃-labelled acrylamide, m/z 133 for asparagine and m/z 181 for glucose or fructose.

3. Results and discussion

3.1. The effects of cations on acrylamide formation in asparagine–fructose reaction system

It has been shown that Maillard-driven generation of flavour and colour in heated foods can be linked to the formation of acrylamide. Free asparagine in combination with reducing sugars generates significant amounts of acrylamide when pyrolysed at temperatures greater than 120° C ([Mottram et al., 2002; Stadler et al., 2002\)](#page-6-0).

Fig. 1. Time and temperature-dependent formation of acrylamide (µmol) from asparagine (5 µmol) and fructose (5 µmol) in 100 µl water. Error bars represent standard deviations $(n = 3)$.

We heated equimolar amounts of asparagine and fructose at 150 and 180 °C in sealed glass tubes. The formation of acrylamide followed typical kinetic patterns (Fig. 1). The amount of acrylamide generated in the reaction mixture reached an apparent maximum after 10 min of heating at 180 °C, then, decreased slowly afterwards. Obviously, the content of acrylamide detected in the reaction mixture at 180 \degree C was the net result of the formation and degradation of this compound. Similar results have been reported for a model mixture composed of glucose and asparagine at various molar ratios and in food systems ([Ehling & Shibam](#page-6-0)[oto, 2005; Elmore, Koutsidis, Dodson, Mottram, &](#page-6-0) [Wedzicha, 2005\)](#page-6-0). The decline of the curves is most likely due to polymerisation, as recently reported ([Stadler et al.,](#page-7-0) [2004\)](#page-7-0).

When equimolar amounts of asparagine and fructose $(5 \mu \text{mol each})$ were pyrolysed without cations, a maximum of 0.045 μ mol and 0.085 μ mol of acrylamide was formed at 150 and 180 \degree C, respectively.

[Yasuhara, Tanaka, Hengel, and Shibamoto \(2003\)](#page-7-0) have discussed the oxidation and/or thermal degradation of lipids in fried foods as a possible mechanistic route contributing to the formation of acrylamide via an acrylic acid intermediate. [Lindsay and Jang \(2005\)](#page-6-0) tested this hypothesis by accelerating the oxidation during frying, through the introduction of ferric chloride to the surface of sliced

Fig. 2. Amounts of acrylamide formed (umol) from asparagine (5 µmol) and fructose (5 µmol) in 100 µl water heated in a sealed glass tube, as influenced by the presence of Ca^{2+} and Na^{+} in the reaction mixture.

potatoes before frying. They found that the treatment of potato slices with 1000 ppm $Fe³⁺$ reduced acrylamide formation by 77% compared to the untreated control. They noted that the introduction of Ca^{2+} as a non-transition state cation also reduced acrylamide formation by 59%.

Investigating the role of different mono- and divalent cations in the formation of acrylamide, we found that pyrolysing the equimolar mixture of asparagine and fructose with equimolar amounts of divalent cations, such as Ca^{2+} , led to a complete inhibition of the formation of acrylamide. Meanwhile the Maillard reaction proceeded to form brown coloured end products. The maximum amounts of acrylamide were measured at 15 and 30 min of heating at 180 and 150 °C, respectively, in the presence of mono- and divalent cations. [Fig. 2](#page-2-0) shows the effect of different amounts of Ca^{2+} and Na^{+} on the maximum amount of acrylamide formed during pyrolysis, in comparison to the acrylamide formed without the presence of any cation in the reaction mixture. The maximum amount of acrylamide formed during pyrolysis decreased as the amount of cation increased in the mixture. Ca^{2+} and Na⁺ differed in their effectiveness for the prevention of acrylamide formation. Although the monovalent cation $Na⁺$ prevented the formation of acrylamide

Fig. 3. Extracted ion chromatograms for pyrolysates after heating a mixture of glucose and asparagine (each 10 µmol initially) at 150 °C for 20 min. (a) no cation and (b) 10 μ mol Ca²⁺.

Fig. 3 (continued)

to a certain extent, it was less efficient than the divalent cation Ca^{2+} .

Other cations, namely Mg^{2+} and K^+ ions, exhibited similar behaviour on the inhibition of acrylamide formation to those of Ca^{2+} and Na⁺, respectively (data not shown). So, it is clear that the effect of mono- and divalent cations toward the prevention of acrylamide formation during the Maillard reaction occurs in a systematic manner.

[Lindsay and Jang \(2005\)](#page-6-0) have hypothesised that ionic associations involving the ions and charged groups on asparagine and related intermediates were likely to be involved. However, no concrete proof of this hypothesis has been provided yet, and information about how the

presence of cations would affect the formation of typical Maillard reaction products is still lacking. In order to investigate any possible interaction between the charged groups of asparagine and the divalent cation, a reaction mixture composed of glucose, asparagine and Ca^{2+} was heated at $150 \degree C$ for 15 min. Mass spectrometric analyses of pyrolysates did not confirm any ionic associations between the charged groups of asparagine and Ca^{2+} . [Zhou](#page-7-0) [et al. \(2003\)](#page-7-0) have reported that Ca^{2+} may form a complex with poly(acrylamide), but such a complex was also not confirmed between Ca^{2+} and acrylamide in this study.

The presence of cations in the reaction mixture influenced the rate of decomposition of acrylamide precursors significantly. Adding cations into the reaction mixture increased the rate of glucose decomposition while most asparagine remained unreacted.

Mechanistic studies have proposed that N-glycosides and related compounds formed in the early phase of Maillard reaction are key intermediates leading to acrylamide ([Stadler et al., 2002, 2004; Yaylayan, Wnorowski, &](#page-7-0) [Perez-Locas, 2003; Zyzak et al., 2003](#page-7-0)). The first step in acrylamide production is the formation of a Schiff base between the carbonyl and α -amino group of asparagine by means of the dehydration of the N-glycosyl compound. Our results showed a clear impact of Ca^{2+} on acrylamide formation. When Ca^{2+} was lacking in the reaction mixture, both the Schiff base of asparagine (MW 294) and acrylamide (MW 71) formed during heating at 150° C for 20 min, as clearly seen in the corresponding extracted ion chro-matograms [\(Fig. 3](#page-3-0)a). However, the presence of Ca^{2+} prevented the formation of the Schiff base, and thus, of acrylamide during heating ([Fig. 3](#page-3-0)b).

3.2. The effects of cations on acrylamide formation in potato strips

Potato products such as French fries and crisps were among the food items containing highest amounts of acrylamide (Swedish NFA, 2002 UK; Şenyuva and Gökmen, [2005; FSA, 2002\)](#page-6-0). Therefore, we decided to confirm the effect of mono- and divalent cations on acrylamide formation in French fries during the frying process. It has been successfully shown that acrylamide formation takes place mainly at the surface and in near-surface regions, because during the process of frying, the conditions in this part of the potato strip becomes favourable for acrylamide formation, as a result of simultaneous drying (Gökmen, Palazoğlu, & Ş[enyuva, 2006; Taubert, Harlfinger, Henkes,](#page-6-0) Berkels, $&$ Schömig, 2004). So, any treatment that inhibits the reaction responsible for the formation of acrylamide on potato surface would be a viable approach to limit the amount of acrylamide formed during frying. Dipping potato strips into the solutions of calcium chloride or sodium chloride was, therefore, applied as a simple pretreatment prior to frying in this study.

The results obtained for potato confirmed that divalent cations are capable of inhibiting the formation of acrylamide. Without any pre-treatment, as much as 711 ± 33 µg/kg of acrylamide formed upon frying potato strips at 170° C for 5 min. The amount of acrylamide could be decreased by dipping potato strips into the solutions of sodium chloride and calcium chloride. The amount of acrylamide formed decreased as the dipping time increased for both sodium chloride and calcium chloride. However, when water was used alone for that kind of pretreatment, the results were inconclusive, having only a limited effect on the amount of acrylamide formed during frying as was also noted previously by other researchers (Grob et al., 2003; Haase, Matthäus, & Vosmann, 2003; Kita, Bråthen, Knutsen, & Wicklund, 2005). So, the inhibition of acrylamide formation during frying was mainly attributed to the presence of mono- or divalent cations in the potato strips after the dipping treatment, rather than the reduction of acrylamide precursors by dipping (Table 1).

As also observed in the reaction mixture composed of asparagine and fructose, the divalent cation, Ca^{2+} was more effective than the monovalent cation $Na⁺$. When compared to the amount of acrylamide formed in potato strips without pre-treatment, percentage inhibition of acrylamide formation increased to ca. 95% by dipping in calcium chloride solution for 60 min at room temperature (Table 1). In addition, the sensory quality of the fried potatoes, in terms of golden yellow colour, crispy texture and salty taste could also be maintained. As shown in [Fig. 4,](#page-6-0) fries prepared by dipping in 0.1 M CaCl₂ solution had similar surface colour to that of the control. This was the evidence that the Maillard reaction had taken place on the surface of the potato strips, leading to the formation of colour without the formation of acrylamide.

Lowering the pH or the concentrations of acrylamide precursors as suggested by other researchers to limit acrylamide

Table 1

Effect of dipping into calcium chloride and sodium chloride solutions on the inhibition of acrylamide formation during frying

Dipping		Loss by dipping treatment, $(\%)^a$			Acrylamide $(\mu/kg)^b$	Inhibition $(\%)$
Solution	Time (min)	Glucose	Fructose	Asparagine		
Water	15		Δ		655 ± 18	
	30				628 ± 59	12
	60		21	19	589 ± 41	17
0.1 M NaCl	15	6			430 ± 58	40
	30	11		12	328 ± 39	54
	60	17	18	20	302 ± 41	58
0.1 M CaCl ²	15		6	6	151 ± 14	79
	30			11	60 ± 8	92
	60	19	17	21	40 ± 3	95

^a Glucose, fructose and asparagine concentrations of potato strips were 0.45, 1.82 and 1.86 g/kg (fresh weight).
^b The concentration of acrylamide formed upon frying at 170 °C for 5 min in potato strip without pretrea

Fig. 4. Images of potato strips after deep frying at 170 °C for 5 min, which indicate dipping treatment (0.1 M CaCl² or NaCl for 60 min) has no adverse effect on the formation of colour.

formation is difficult to put into practice since the final product has a noticeable sour taste or unsatisfactory surface colour (Jung, Choi, & Ju, 2003; Kita et al., 2005).

Based on the results obtained for fried potatoes here, dipping of potato strips into a solution of calcium chloride at moderate concentrations may be a viable approach for the minimisation of acrylamide content, in commercial as well as in homemade foods. Calcium chloride, as a food additive (E 509), is widely used as a firming agent during processing. It could now be used by the food industry to control the formation of acrylamide.

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