Development of New Technologies to Minimize Acrylamide in Food

Entwicklung von neuen Prozesstechniken zur Vermeidung des Acrylamid-Gehaltes in Lebensmitteln

BLL

FEI

Research Association of the German Food Industry
Forschungskreis der Ernährungsindustrie e. V.
German Federation of Food Law and Food Science
Bund für Lebensmittelrecht und Lebensmittelkunde e. V.

AiF-FV 108 ZBG

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Introduction

In 2002 food manufacturers and consumers were faced to a new dimension of food safety problems without any preliminary warnings. The industrial chemical 'acrylamide' was found to be formed in heated foods manufactured and prepared properly in industry and household. Acrylamide is a carcinogen in animals and the amounts found gave reason to concerns about the safety of these products. Instantaneously, worldwide activities have been started to find possible formation pathways in food matrices in order to reduce or to avoid acrylamide. While acrylamide was found first in fried and baked products, following investigations demonstrated that this substance can be detected in a wide variety of heated foods. Therefore, a general solution has to be based on validated data about consumer uptake and exposure of acrylamide from our daily food.

Already in 2002 the German food industry and its associations, the German Federation of Food Law and Food Science (BLL) and the Research Association of the German Food Industry (FEI), initiated an extensive research project according to the complex situation considering toxicological aspects to improve the basis for risk assessment, validation of analytics and, of course, improved manufacturing processes with respect to acrylamide formation. The project was founded by the Ministry of Economics and Labour (BMWA) via AiF in 2003 to 2005 (Project No. AiF 108 ZBG). Up to the beginning all participants e.g. raw material suppliers, food manufacturers and consumers have been integrated in the research approach. Also producers of food manufacturing equipment have been involved in the project to secure the rapid transfer of new process and machinery solutions into the industrial scale of food processing.

According to the global target of the project and the complementary competences required for the scientific approach the following institutes were involved in the project:

- Deutsche Forschungsanstalt für Lebensmittelchemie (DFA), Garching
- Institut für Lebensmittel- und Umweltforschung e. V. (ILU), Nuthetal
- Deutsches Institut für Lebensmitteltechnik e. V (DIL), Quakenbrück
- Bundesanstalt für Ernährung und Lebensmittel (BFEL), Detmold
- Technische Universtität Kaiserslautern, Fachbereich Chemie, Fachrichtung Lebensmittelchemie/ Umwelttoxikologie (TU KL).

Aim of the analytical part of the project (DFA) was to generate validated data regarding acrylamide and formation pathways as a tool for process improvements. Toxicological investigations (TU KL) concerned biological activity of acrylamide and its physiological metabolite glycidamide. The technological project part was related to the relevant food groups cereal and bakery products (ILU), potato products (BFEL) and the influence of plant concepts and heating medium (DIL).

It has to be considered that acrylamide formation during heating of foodstuffs is closely connected with the development of quality parameters like flavor, taste, browning, or texture for fried products or baked goods. For this purpose the reaction mechanisms which lead to formation of acrylamide and of main positive quality parameters have to be identified and the manufacturing process has to be adaptated.

This consolidated project report presents the results of the investigations for every institute and, according to the target of the integrated and systematic scientific approach, the opportunities for minimizing acrylamide contents in foodstuffs, especially in cereal and potato products, resulting from these investigations. It has to be stated that the close cooperation of the institutes generated an added scientific value with respect to all topics investigated.

This integrated network approach has to be continued in the future because the problem acrylamide is not solved yet and its relevance for human health could not be neglected. Especially food preparation in households can cause acrylamide exposures with very limited control opportunities.

Einleitung

Hersteller und Verbraucher von Lebensmitteln sind im Jahre 2002 ohne jede Vorwarnung mit einer neuen Problemdimension konfrontiert worden: Das bisher als Industriechemikalie bekannte Acrylamid wird in hoch erhitzten Lebensmitteln auch bei sachgemäßer Herstellung und Zubereitung gebildet, wobei möglicherweise toxikologisch nicht unbedenkliche Konzentrationen entstehen können. Daraufhin begannen sofort weltweite Aktivitäten zur Ermittlung von möglichen Bildungswegen des Acrylamids in Lebensmitteln, um Acrylamid zu reduzieren bzw. möglichst zu vermeiden. Während Acrylamid zunächst vor allem in frittierten und gebackenen Erzeugnissen gefunden wurde, zeigten nachfolgende Untersuchungen, dass sich diese Substanz in einer Vielzahl von erhitzten Lebensmitteln nachweisen lässt. Daher muss eine generelle Lösung des Problems auf validierten Daten zur Acrylamidaufnahme aus dem gesamten Lebensmittelbereich basieren.

Bereits im Jahre 2002 initiierte die deutsche Lebensmittelindustrie über den Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL) und den Forschungskreis der Ernährungsindustrie e.V. (FEI) ein entsprechendes Kooperationsforschungsprojekt. Bei diesem Projekt wurden, der komplexen Situation angemessen, toxikologische Aspekte einschließlich Risikobewertung, validierte Analytik und natürlich verbesserte Herstellungsprozesse unter dem Aspekt der Acrylamidminimierung berücksichtigt. Dieses Projekt wurde im Rahmen der industriellen Gemeinschaftsforschung durch das Bundesministerium für Wirtschaft und Arbeit (BMWA) und die Arbeitsgemeinschaft industrieller Forschungsvereinigungen (AiF) im Zeitraum von 2003 bis 2005 gefördert (Projekt-Nr. AiF 108 ZBG). Seitens der Wirtschaft wurde dieses Projekt von der gesamten Lebensmittelindustrie sowie dem Maschinen- und Anlagenbau unterstützt und intensiv begleitet.

Entsprechend den globalen Zielen des Projektes und der komplementären Kompetenz, die für den vorgesehenen integrierten wissenschaftlichen Ansatz notwendig ist, waren folgende Forschungsstellen an dem Projekt beteiligt:

- Deutsche Forschungsanstalt für Lebensmittelchemie (DFA), Garching
- Institut für Lebensmittel- und Umweltforschung e. V. (ILU), Nuthetal
- Deutsches Institut für Lebensmitteltechnik e. V (DIL), Quakenbrück
- Bundesanstalt für Ernährung und Lebensmittel (BFEL), Detmold
- Technische Universtität Kaiserslautern, Fachbereich Chemie, Fachrichtung Lebensmittelchemie/ Umwelttoxikologie (TU KL).

Ziel des analytischen Teilprojektes (DFA) war die Erarbeitung von validierten Daten zu Acrylamidgehalten und möglichen Bildungswegen als Grundlage für die Verbesserungen von Prozessen. Die toxikologischen Untersuchungen (TU KL) betrafen sowohl Acrylamid als auch den physiologisch gebildeten Metaboliten Glycidamid. Der technologische Teil war entsprechend der Acrylamidbelastung auf die Gruppen Getreideprodukte und Backwaren (ILU), Kartoffelprodukte (BFEL) sowie auf den Einfluss von Anlage und Erhitzungsmedium (DIL) fokussiert.

Auf der anderen Seite ist aber auch zu berücksichtigen, dass die Acrylamidbildung beim Erhitzen von Lebensmitteln sehr eng mit der Entwicklung wichtiger Qualitätsparameter wie z. B. Geruch, Geschmack, Bräunung oder Textur in frittierten Produkten oder Backwaren verbunden ist. Dafür müssen die entsprechenden Reaktionsmechanismen, die zur Bildung von Acrylamid und wichtiger positiver Qualitätsparameter führen, identifiziert und im Rahmen des Herstellungsprozesses getrennt erfasst werden.

Dieser Projektbericht enthält die Ergebnisse der Untersuchungen aller Institute sowie, dem Ziel des systematischen und integrierten wissenschaftlichen Ansatzes entsprechend, die erarbeiteten Ansatzpunkte für die Minimierung der Acrylamidgehalte in Lebensmitteln, speziell in Getreide- und

Kartoffelprodukten. Die enge Kooperation der Institute ergab einen zusätzlichen wissenschaftlichen Output im Hinblick auf alle bearbeiteten Gebiete. Diese integrierte und vernetzte Herangehensweise muss auch in Zukunft weitergeführt werden, da das Problem Acrylamid auch nach diesem erfolgreichen Projekt noch nicht gänzlich gelöst ist und auch die gesundheitliche Relevanz des Acrylamids noch nicht vollständig geklärt ist. Speziell die Lebensmittelzubereitung im Haushalt kann immer noch im beträchtlichen Maße zur Acrylamidexposition der Verbraucher beitragen, ohne dass bisher entsprechende Eingriffsmöglichkeiten vorliegen.

Methods for Acrylamide Quantification and Mechanisms of Acrylamide Formation

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Michael Granvogl, Peter Köhler, Peter Schieberle

1. Introduction

1.1 Analytical Methods for Acrylamide Quantification

Because of its high solubility in water, its high reactivity [1], and the lack of a chromophor, acrylamide is not easy to analyse. To increase the selectivity, and also, the sensitivity in GC analysis, bromination of the double bond in combination with GC/electron capture detection has, therefore, been applied previously [2], mostly by using either methacrylamide or N,N-dimethylacrylamide as the internal standards [3-7]. In addition, methods to quantify acrylamide by direct GC/MS measurement without bromination [8, 9] have been described.

Stable isotope dilution assays, in which an isotopomer of the analyte is used as the internal standard, are among the most reliable methods for quantification of compounds, which are labile and/or reactive. Since acrylamide has been reported as a foodborne toxicant, several methods using 2,3,3-[$^{2}H_3$]-acrylamide as an appropriate internal standard have been reported [10-15]. Because the deuterium labelling is positioned at the double bond in [$^{2}H_3$]-acrylamide, thereby making a deuterium/protium exchange possible during workup, [$^{13}C_1$]-acrylamide [16] and, in particular, [$^{13}C_3$]-acrylamide are now preferably used [5, 14].

In stable isotope dilution assays (SIDA), the differentiation between the analyte and the internal standard is done by recording the respective molecular masses and/or mass fragments. Due to the low molecular weight of acrylamide (71 g/mol), and therefore, also due to its resulting low mass fragment ions, background interference may impede the analysis in the single stage mode. Better sensitivity and selectivity can, thus, be achieved with a three-stage mass spectrometer by monitoring one or more characteristic mass transitions [5, 7, 14, 15].

Although reliable data have been published, up to now, only stable isotope dilution assays using direct analysis of acrylamide have been applied. So, except the bromination procedure, which is quite time-consuming, no method is available that involves the derivatisation of acrylamide prior to MS analysis, thereby increasing the selectivity, in particular, when single-stage MS detection is used. Such methods could be useful, e.g. as reference methods to approve data obtained by multi-stage LC/MS.

1.2 Mechanisms of Acrylamide Formation

Shortly after the characterisation of acrylamide as a constituent of processed foods [5], the thermal degradation of free asparagine in the presence of carbohydrates following Maillard-type reactions has been proposed as the major route in acrylamide formation [17-19]. In addition, it has been shown by labelling experiments that the carbon skeleton in acrylamide as well as the nitrogen stem from asparagine, whereas the nitrogen originates from the amide group [18]. Thus, to generate acrylamide from asparagine from a stoichiometric point of view a loss of CO_2 and NH_3 must occur.

Mottram et al. [17] have suggested the Strecker degradation of asparagine induced by α-dicarbonyl compounds as an important pathway in acrylamide formation, because significant amounts were formed when 2,3-butandione and asparagine were reacted in aqueous solution at high temperatures. From a Strecker reaction of asparagine, after decarboxylation and transamination, the aldehyde 3-oxopropionamide will be generated. However, to form acrylamide from this intermediate, a reduction into 3-hydroxypropionamide followed by a β-elimination of water has to be assumed. However, very recently, Stadler et al. [20] showed that the latter intermediate was not very effective as acrylamide precursor, thereby suggesting only a minor importance of the Strecker reaction in acrylamide formation.

Instead, Stadler et al. [18] had previously proposed the N-glucoside of asparagine as a direct precursor of acrylamide. As established by this group [20], the potassium salt of N-(D-glucos-1-yl)-L-asparaginate was by a factor of twenty-three more effective in acrylamide formation than the respective Amadori product of asparagine, N-(1-deoxy-D-fructos-1-yl)-L-asparagine. In agreement with mechanisms proposed earlier by Yaylayan et al. [21] and Becalski et al. [14], the authors [20] suggested a thermally induced decarboxylation of the Schiff base formed from asparagine and several aldehydes as the key step in acrylamide formation. From the azomethine ylide formed as transient intermediate, the generation of acrylamide is suggested to occur via a β-elimination. This reaction was found to be significantly favoured when α-hydroxy carbonyls, such as 1-hydroxypropan-2-one (acetol) were reacted with asparagine, whereas α-oxo carbonyls were shown to be much less effective [20]. In addition, because the Amadori product was a less effective precursor as compared to the respective N-glucosides, the authors also ruled out the importance of the Amadori product as key intermediate in acrylamide generation. Overall these studies indicated that obviously the degradation of asparagine, or the respective Schiff base, into acrylamide occurs without running through any measurable transient intermediate [22, 23].

In a recent publication, Zyzak et al. [24] were able to identify 3-aminopropionamide as a transient intermediate formed during acrylamide generation from asparagine. However, because only a slight increase in the yields of acrylamide was observed as compared to asparagine itself, the role of 3-aminopropionamide in acrylamide formation was not considered important and, therefore, no quantitative experiments were performed.

1.3 Aims

The purpose of this project was to study possibilities for acrylamide derivatisation to obtain derivatives, which can sensitively be determined by single-stage LC/MS, and to develop a stable isotope dilution assay (SIDA) based on the derivatisation procedure. Because, in particular, smaller companies do not have mass spectrometric equipment, but are obliged to control their products with respect to the concentrations of acrylamide present, there is also a demand for easier and cheaper methods in acrylamide quantification. Thus, another aim of this study was to develop a method for acrylamide quantification by HPLC/fluorescence detection.

Because of discrepancies on the role of the Strecker degradation in acrylamide formation, another aim of the present study was to get further insight into the formation mechanism of acrylamide in foods using different model systems and compounds. Thereby, the importance of 3-aminopropionamide for acrylamide formation needed to be clarified. After emphasising the relevance of 3-aminopropionamide, a method for its quantification in foods should be developed.

The final aim of the project was to identify reactions between acrylamide and food constituents to get information on the behaviour of the amide in processed foods.

2. Results and Discussion

2.1 Methods for Acrylamide Quantification

On the basis of the derivatisation of acrylamide with 2-mercaptobenzoic acid, a selective and sensitive stable isotope dilution assay (SIDA) for acrylamide in foods using [$^{13}C_3$]-acrylamide as internal standard and LC/MS detection was developed (Figure 1). The new method was successfully applied for acrylamide quantification in different foods, e.g. potato chips, crisp bread, rusk, and butter cookies. Optimisation of the sample workup by including an additional step with activated carbon as an adsorptive material and detection by LC/MS-MS in the single reaction monitoring (SRM) mode further increased the selectivity and sensitivity of the method. Thus, complex foods, like coffee and cocoa could also be analysed reliably. Furthermore, a GC/MS based method for acrylamide determination was developed. The most important modification compared to a recently published GC/MS method [25] was the use of solid-phase extraction on Extrelut® columns (silica gel) during sample workup.

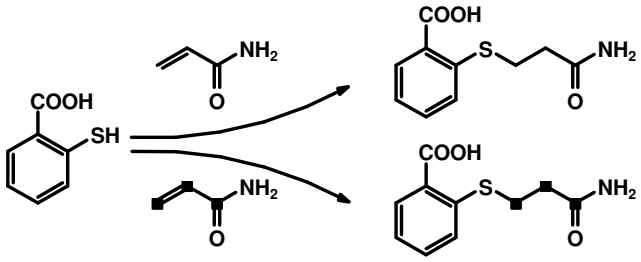

Figure 1: Derivatisation procedure applied for conversion of acrylamide and [$^{13}C_3$]-acrylamide into stable thioethers prior to the stable isotope dilution assay in combination with LC/MS. ■ [^{13}C]-label.

Samples from the same batch were analysed for acrylamide by the new LC/MS and GC/MS methods as well as by the published GC/MS method [25]. The results are summarised in Table 1. All data were in good accordance. The limit of detection was in the range of 5 µg/kg, so that reliable quantitative data could be obtained for acrylamide concentrations above 15 µg/kg. The new methods were published [26] and successfully applied in three proficiency tests of the European Commission [27]. In the case of cocoa, the new LC/MS method with optimised workup and LC/MS-MS detection was the only method, which provided reliable data in this food matrix.

Table 1: Quantitative determination of acrylamide in potato chips and butter cookies from the same batch by using three different methods

	Potato chips		Butter cookies		LOD	LOQ
Method	Conc. [µg/kg]	CV	Conc. [µg/kg]	CV	[µg/kg]	[µg/kg]
LC/MS	517.4	± 0.5	157.4	± 0.4	4.1	12.1
GC/MS	510.8	± 0.4	159.6	± 1.5	4.0	11.8
GC/MS [25]	468.1	± 3.5	158.0	± 0.8	5.6	16.7

CV Coefficient of variation. LOD: Limit of detection. LOQ: Limit of quantification.

In addition to the methods using mass spectrometric detection, a method for acrylamide determination using HPLC separation and fluorescence detection was developed [28]. The basic principle of this method was the nucleophilic addition of a fluorescent thiol compound to the double bond of acrylamide. In model experiments and simple matrices the method showed excellent performance. The sensitivity of the method is yet too low to be recommended for acrylamide analysis in all types of food. There are two main reason for this. First, obviously too many compounds present in food extracts do react with the fluorescent thiol, thereby causing a very high baseline of the fluorescence detector and, secondly, the derivatives seem to form complexes with the food matrix and, thus, the yields of the extraction were very low. Although the application of LC/MS significantly increased the selectivity and sensitivity of the method, our purpose still is the development of a method sensitive and selective enough for acrylamide analysis without MS detection. Further studies are underway to solve this challenge. Nevertheless, the method can be proposed for the sensitive and selective quantitation of acrylamide in aqueous matrices, such as drinking water.

2.2 Model Studies on Acrylamide Formation

For studies on mechanisms of acrylamide formation, two model systems were used. Heating under low moisture conditions was performed in a silica gel matrix containing 10 % of water in closed glass tubes. The second model system simulated heat processing under aqueous conditions. For these experiments, the compounds were dissolved in phosphate buffer at pH 5 or pH 7, respectively, and heated in closed glass tubes.

Initial studies showed that asparagine only formed very minor amounts of acrylamide when heated singly. Addition of glucose or fructose to aspargine and subsequent heating resulted in very high amounts of acrylamide (approx. 10 mmol/mol asparagine), whereas other carbohydrates like lactose or sucrose were less effective (6 to 8 mmol/mol), and polysaccharides were nearly ineffective. Heating methionine, threonine or cysteine instead of asparagine in the presence of glucose also resulted in acrylamide concentrations, but two orders of magnitude below those of the asparagine/glucose system. The amount of water present in the matrix had a very significant effect on the yields of acrylamide: When the reaction of asparagine and glucose or fructose, respectively, was performed in a completely dry system, the yields dropped to about one third of those formed in a matrix with 10 % of water. Interestingly, the yields of acrylamide were further increased, when the reaction was performed at a water content of 25 %. This showed that at least a certain amount of water is required to generate distinct transient intermediates able to release acrylamide upon a thermal treatment.

In addition, asparagine and glucose were reacted in an aqueous buffer at increasing temperatures. These results showed that at temperatures below 160 °C, heating of the aqueous solution at pH 7.0 for 20 min only generated low amounts of acrylamide. However, at 160 °C or 180 °C, respectively, much higher amounts of acrylamide were generated. But, as compared to the same model mixture reacted in a low water system (25 %), the yields in the aqueous solution were clearly lower. Because of the lowered nucleophilic power of the α-amino group of aspargine at pH 5.0, the yields of acrylamide were lower than at pH 7.0, thereby establishing a significant effect of the pH on acrylamide formation.

In further model studies, the two α-dicarbonyl compounds ethanedial (glyoxal) and 2-oxopropionaldehyde (methylglyoxal), known as carbohydrate degradation products, as well as 1-hydroxypropan-2-one (hydroxyacetone) were reacted with asparagine. The results are summarised in Table 2. All intermediates were able to generate acrylamide in significant yields, however, both α-dicarbonyl compounds were less effective than hydroxyacetone or glucose or fructose, respectively. These results suggested that the Strecker reaction should not be the most predominant pathway in acrylamide formation, because, e.g. hydroxyacetone, the effective "catalyst" of asparagine degradation is not likely to initiate a Strecker reaction. Therefore, acrylamide formation via the Amadori compound could be suggested as an important pathway.

Table 2: Influence of different oxo-compounds on the formation of acrylamide from asparagine[a]

Oxo-compound	Acrylamide [mmol/mol asparagine]
2-Oxopropanal (methylglyoxal)	2.1
Ethanedial (glyoxal)	2.2
1-Hydroxypropan-2-one (hydroxyacetone)	6.4
2-Oxopropionic acid (pyruvic acid)	9.9

[a] Asparagine (0.1 mmol) and the respective oxo-compound (0.1 mmol) were mixed with silica gel (3 g, 10 % water content) and heated for 30 min at 170 °C in a closed glass vial.

2.3 Studies on the Significance of 3-Aminopropionamide in Acrylamide Formation

Model studies with 3-aminopropionamide, which was heated with and without glucose showed the very high potential of this compound for acrylamide formation (Table 3). Under certain conditions, the amounts of acrylamide formed from 3-aminopropionamide were by a factor of 750 higher as compared to a model system in which 3-aminopropionamide had been replaced by asparagine. At 180 °C and at pH 7, the yields of acrylamide amounted to nearly 61 mol-%. When carbohydrates were present, the yields dropped to about one half as compared to the experiment in the absence of glucose. These yields obtained from 3-aminopropionamide were by far the highest ever reported for a precursor of acrylamide in the literature.

Table 3: Influence of the temperature on the formation of acrylamide in heated aqueous solutions of 3-aminopropionamide or asparagine, respectively, in the presence of glucose[a]

Temperature [°C]	Acrylamide [mmol/mol] formed from		
	Asparagine	3-Aminopropionamide	3-Aminopropionamide[b]
100	0.01	1.4	1.3
120	0.01	5.5	7.5
140	0.07	55.8	41.1
160	0.7	164.8	147.4
180	1.1	290.6	608.3

[a] 3-Aminopropionamide (1 µmol) or asparagine (250 µmol), respectively, were dissolved in phosphate buffer (10 mL; pH 7.0) and heated for 20 min in the presence of an equimolar amount of glucose. [b] Without glucose

These results suggested that the elimination of ammonia from carbon-3 in 3-aminopropionamide occurs easily and nearly quantitatively under aqueous conditions at high temperatures by a β-elimination as indicated in Figure 2. To prove the importance of this structural feature in acrylamide formation, L-alaninamide and 2-hydroxypropionamide were reacted under the same conditions and the amounts of acrylamide formed were compared to the yields obtained from 3-aminopropionamide (Table 4). The results clearly showed that neither a hydroxyl nor an amino group in the α-position of a propionic acid amide leads to acrylamide formation. Replacement of the amino group of 3-aminopropionamide by a hydroxy group (3-hydroxypropionamide) also caused heat-induced acrylamide formation. However, the acrylamide concentration was substantially lower as compared to 3-aminopropionamide due to the lower potential of water elimination as compared to ammonia elimination. These findings can easily be explained by the different stabilities of the possible tautomers shown in Figure 2.

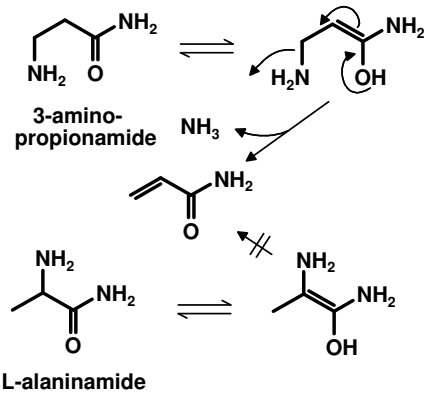

Figure 2: Proposed formation pathway of acrylamide from 3-aminopropionamide. L-Alaninamide for comparison.

Table 4: Heat-induced formation of acrylamide from L-alaninamide, 2- and 3-hydroxypropionamide in comparison to 3-aminopropionamide[a]

Precursor[a]	Acrylamide [mmol/mol precursor]	
	with glucose	without glucose
L-Alaninamide	0.004	0.002
2-Hydroxypropionamide	< 0.0005	0.001
3-Aminopropionamide	109.3	292.3
3-Hydroxypropionamide	n.d.[b]	26.0

[a] Precursor was heated for 30 min at 170 °C in a closed glas vial. [b] not determined.

After the significance of 3-aminopropionamide in acrylamide formation had been established, possible formation pathways were investigated. As it is the biogenic amine of asparagine, a decarboxylase catalysed, biochemical pathway of 3-aminopropionamide formation according to Figure 3 was established. To demonstrate its biochemical formation in foods, a method for the quantitative determination of 3-aminopropionamide using derivatisation with dansyl chloride and LC/MS-MS detection with glycinamide as internal standard was developed [29]. For the first time, 3-amiopropionamide was identified in potatoes and even in foods, respectively. A time-dependent increase of the concentration during storage of mashed potatoes confirmed the biochemical pathway of 3-aminopropionamide formation (Table 5).

In addition to the biochemical pathway, also the heat-induced formation of 3-aminopropionamide was established. Upon heating of the asparagine/glucose system, 3-aminopropionamide was identified as a reaction product. To prove that 3-aminopropionamide was formed in the asparagine/2-oxopropionic acid model, its concentrations were monitored parallel with the acrylamide formation. In the asparagine/2-oxopropionic acid model, 3-aminopropionamide was present in quite high amounts already in the first minutes of the reaction (Table 6). At this early stage, however, only low amounts of acrylamide were present. The fact that 3-aminopropionamide was formed prior to acrylamide clearly corroborates that 3-aminopropionamide is an important transient intermediate in the formation of acrylamide during thermal degradation of asparagine.

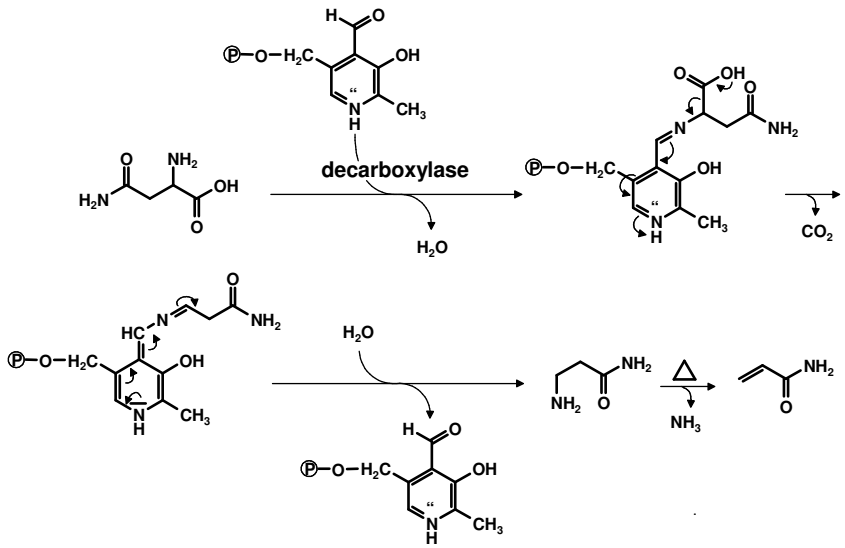

Figure 3: Biochemical pathway of the decarboxylation of asparagine into 3-aminopropionamide with pyridoxal phosphate as cofactor.

Table 5: Time course of the formation of 3-aminopropionamide in a raw potato mash stored at 20 °C for 480 min

	Acrylamide [µg/kg fresh weight] formed after					
Cultivar	0 min	30 min	60 min	180 min	300 min	450 min
Quarta	226.5	247.7	283.8	324.4	372.4	410.5
Selma	330.2	360.1	410.4	457.9	475.9	750.2

Table 6: Formation of 3-aminopropionamide and acrylamide from asparagine and 2-oxopropionic acid[a]

Reaction time [min]	3-Aminopropionamide		Acrylamide	
	[µg]	[mmol/mol asparagine]	[µg]	[mmol/mol asparagine]
5	26.3	2.99	0.6	0.08
10	18.7	2.13	2.1	0.30
20	24.7	2.80	11.3	1.60
30	38.0	4.32	13.3	1.87

[a] Asparagine (0.1 mmol) and 2-oxopropionic acid (0.1 mmol) were mixed with silica gel (3 g; 10 % water content) and heated for 30 min at 170 °C in a closed glass vial.

3-Aminopropionamide might also be formed as an intermediate in the Strecker reaction of asparagine and an α-dicarbonyl compound as suggested in Figure 4. Decarboxylation of the Schiff base may lead to the formation of three different tautomers (I-III in Figure 4). Hydrolysis of tautomer II would lead to 3-oxopropionamide, the typical Strecker aldehyde, which would only be able to generate acrylamide after reduction to 3-hydroxypropionamide (Figure 4, Table 4). Contrary, if tautomer III is hydrolysed, 3-aminopropionamide will be formed, which in turn will yield acrylamide after thermal treatment. Based on these suggestions it is obvious why the Strecker reaction is not as effective in acrylamide formation, because only tautomer III will yield a transient intermediate able to generate acrylamide. However, it still needs to be clarified, which tautomer is mainly formed in the Strecker reaction.

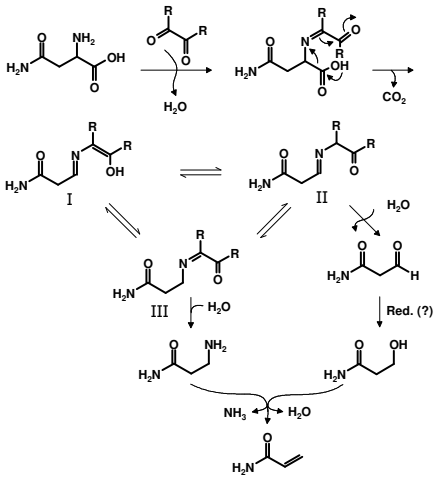

Figure 4: Hypothetical reaction pathway suggesting the formation of 3-aminopropionamide and acrylamide via a Strecker-type degradation of asparagine initiated by an α-dicarbonyl compound.

3-Aminopropionamide can also be formed as a transient intermediate in a system containing asparagine and an α-hydroxycarbonyl compound, e.g. a carbohydrate or a degradation product such as hydroxyacetone according to Figure 5. In this Amadori-type rearrangement of asparagine the tautomerisation of the Schiff´ base yields enaminol II. Such compounds have recently been shown to be very susceptible to oxidation [30]. The vinylogous ß-keto acid formed by an oxidation step will easily decarboxylate yielding an α-oxo-imine, which may then be hydrolysed into 3-aminopropionamide and an osone (Figure 5). 3-Aminopropionamide may then thermally be converted into acrylamide according to Figure 3.

2.4 Studies on Binding of Acrylamide to Glutathione

Acrylamide was reacted with glutathione in aqueous solution at room temperature for 1 hour and the mixture was analysed by LC/MS. Two newly formed compounds were identified. Their mass spectra suggested structures formed by addition of the thiol and/or the amino group of glutathione to the double bond of acrylamide. However, further studies are necessary to find out, whether acrylamide is bound to food constituents temporarily or permanently and whether acrylamide can be released from these products by heating.

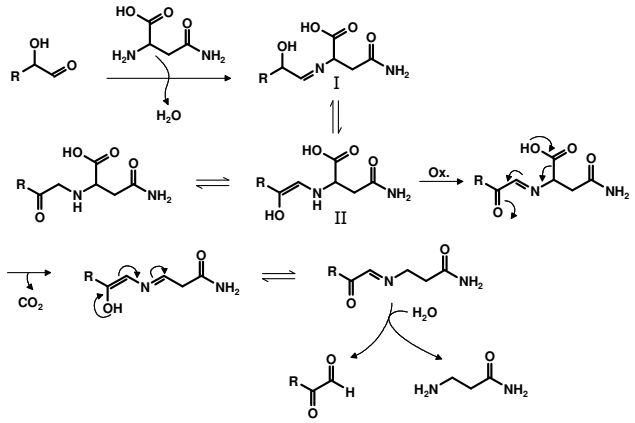

Figure 5: Hypothetical formation pathway leading to 3-aminopropionamide after oxidation of an enaminol formed in the Amadori-type rearrangement of asparagine and its subsequent decarboxylation and hydrolysis.

2.5 Carry-over Studies with Acrylamide in a Feeding Trial

A feeding trial with Chinese quails was carried out to demonstrate a possible carry-over of acrylamide from the feed into the meat, the liver, the eggs and the serum of the animals [31]. Three groups of 10 quails were fed for 24 days with feed containing 17, 663, and 2473 µg acrylamide/kg. The respective amounts of acrylamide were not added, but generated by heating the feed. The excreta, the eggs, the liver, the brest muscle and the serum were analysed for acrylamide after certain periods of time. The results have shown that acrylamide, in particular, when present in the feed at concentrations above 1000 µg/kg, was detectable in the eggs, the serum, the meat and was also excreted in a significant degree. Acrylamide could not be detected in all liver samples and in the meat of the animals fed with lower levels of acrylamide in the diet. The free acrylamide content in eggs and edible tissue, however, were lower than in most thermally processed foods, and should, therefore, not contribute significantly to the average daily intake of acrylamide in humans.

3. Conclusions

From the analytical point of view the project provided new methods for the quantitative analysis of acrylamide, especially, in complex food samples. In particular the method using LC/MS-MS in combination with an optimised sample workup can be recommended for difficult food matrices like coffee or cocoa.

From the postulated pathways of acrylamide formation (Figures 4 and 5) it can be concluded that the Strecker-type degradation of asparagine might be subject of special interest. If it would be possible to direct the reaction sequence of the Strecker-degradation to a preferential formation of 3-oxopropionamide, the Strecker-aldehyde of asparagine, at the expense of the formation of 3-aminopropionamide, the formation of acrylamide should be clearly decreased, because from 3-oxopropionamide no direct route leads to acrylamide. Formation of acrylamide from this compound would only be possible after reduction to 3-hydroxypropionamide, which may be converted to acrylamide by the elimination of water. However, there is no evidence that the required reduction occurs during heat processing. Furthermore, the acrylamide formation potential of 3-hydroxypropionamide is substantially lower as compared to 3-aminopropionamide showing by far the highest potential.

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Tests to minimize the acrylamide content of carbohydrate-rich cereal based food

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1. Introduction

The project was aimed at investigating the complex factors influencing the formation of acrylamide in cereal based food, with special regard to the composition of the raw materials, the effect of ingredients, as well as technological and technical parameters. The dependence of acrylamide formation on composition of raw materials was characterized in order to derive starting points for minimization strategies. The determination of the effect of technological parameters was aimed at the modification of existing plants and technologies as well as the design of unconventional processes for the production of baked goods. Food models as well as real food systems were used for the definition of the variables. The results achieved for raw materials, the baking process, extrusion and frying technologies will be discussed in the following.

2. Material and methods

Determination of the content of acrylamide

The analysis of acrylamide was performed according to LC-MS-MS. Acrylamide-2,3,3-d_3 (Fa. Cambridge Isotope Laboratories, Inc., USA) was used as recovery standard. The 1-propanol extract was transferred to acetonitrile and separated on a reversed-phase C18-column (Fa. Phenomenex). A water-methanol mixture (90/10) was used as elution agent. The detection was performed in the coupled mass spectrometer by Quadrupol (AP/365, Fa. Applied Biosystems, USA). The determination limit is fixed at least at 30 µg/kg and at 10 µg/kg for unproblematic matrices, respectively.

Determination of the content of free asparagine

The determination of the content of free asparagine is based on the method according to Algermissen et al. [1].

Determination of the content of free sugars

The determination of pre-existing glucose, fructose, sucrose, and maltose is performed enzymatically according to the hexokinase method by test combinations of Roche Diagnostics.

Raw materials

Commercial grain, flours and milling products (wheat flour type 550, rye wholemeal, maize grits, oatmeal) were used.

Model system for baked goods

For model baking tests a food model consisting of rye wholemeal and water was used which facilitated the individual variation of influences and interactions of raw materials, ingredients and baking conditions. Sensory influences remained unnoticed. The mass of a density of app. 0.54 g/ml is produced from 36.5 % rye wholemeal and 63.5 % water in a Hobart mixer. The temperature of the mass is kept lower 13 °C in order to achieve a high viscosity. For the subsequent thermal treatment a temperature-controlled waffle iron at a temperature of 330 °C and a baking time of 5 min is used. In order to avoid uneven browning of the product the mass is fed into the iron in equal amounts of 110 g. Product moisture amounts to 25-30 % after thermal treatment. Afterwards the product is subjected to drying at 45 °C for 8-10 h. The moisture

content of the dried product amounts to 4-6 %. The dried products were ground in an impact mill before acrylamide analytics.

Model brown gingerbread

Formula: 46 % invert sugar, 1.3 % ammonium bicarbonate raising agent, 0.4 % potash, related to flour; baking time: 24 min.

3. Influence of raw materials and ingredients on the acrylamide development

3.1 Influence of cereal components on the acrylamide development

a) Influence of the content of free asparagine in raw materials on the acrylamide content of the products

Free asparagine is regarded to be an important reactant of reducing sugars for the development of acrylamide [2-6].

At first, it was tested whether there is a correlation between the content of free asparagine in the raw materials and the content of acrylamide in the product. Then, possibilities of reducing the content of free asparagine in flour were investigated. The following approaches were tested:

— Reduction of the acrylamide content by the use of flours of low extraction degree

— Reduction of the acrylamide content by specific selection of varieties.

For the examination of the correlation between the content of free asparagine in the raw material rye flour and the acrylamide content of the baked product the rye model system for baked goods described under 2 was used.

Passage flours of rye with asparagine contents of 125 to 1261 mg/kg d.m. were used as raw material.

In the model a significant correlation between the extraction degree, the content of free asparagine and the acrylamide content after baking was observed (Figure 1). The increased content of free asparagine in wholemeal, as against flours of low extraction degree, also leads to higher acrylamide contents of the baked product. Thus, the reduction of the asparagine content of the raw material flour is a suitable way to minimize the acrylamide content of the baked goods.

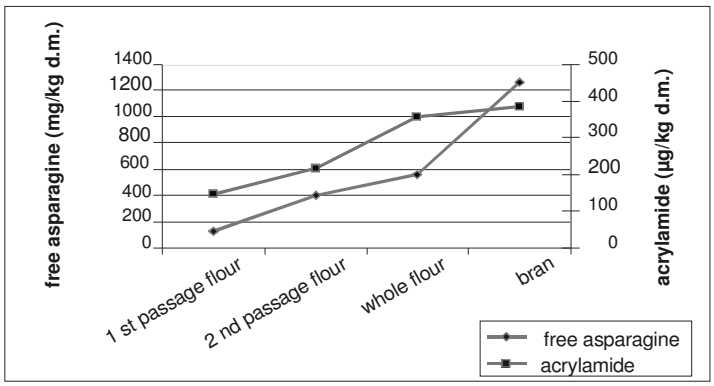


Figure 1: Correlation between free asparagine in flour and acrylamide in model baked goods.

For the reduction of the asparagine content of flour a special selection of varieties is possible as well as the use of flours of low extraction degree.

Investigations on the reduction of the acrylamide content by special selection of varieties

Different rye varieties of one or more sites were tested. Differences in the content of free asparagine from 319 mg/kg (Born var.) to 880 mg/kg (Amilo var.) were determined for the different rye varieties. Also,

significant differences between samples of varying sites or cultural methods were determined. For the Avanti var. of conventional cultivation site specific differences in the content of free asparagine from 472 to 716 mg/kg were determined. For the varieties Nikita and Born samples of conventional and ecological cultivation were tested. Again, differences were determined, the lower asparagine contents not necessarily were assigned to ecological but also to conventional cultivation. The content of free asparagine within one variety of different sites can vary by up to more than 100 %. The site factors therefore can exceed the varietal differences. Due to the overlap of varietal and site specific differences of the contents of free asparagine, the reduction of the contents of free asparagine by selection of low-asparagine varieties is not practicable.

b) Reduction of the acrylamide content by using flours of low extraction degree

In analyses of passage flours the distribution of the content of free asparagine in the rye kernel was determined. Contrary to the variety analyses in which the complete wholemeal is analysed, the analyses of passage flours show a positive correlation between the protein content, the content of free asparagine and minerals (Figure 2).

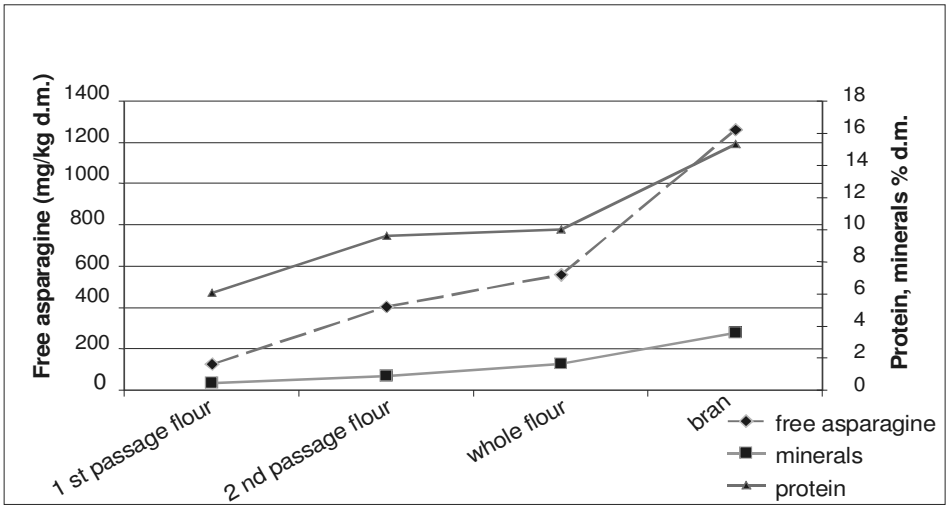


Figure 2: Contents of free asparagine, minerals and proteins in flour passages.

The distribution gradient of free asparagine is significant. From the peripheral layers of the kernel of 1164 mg/kg over the content in the wholemeal and the 2nd to 1st passage, it drops to approx. one tenth. The endosperm which is contained in the 1st passage, has the lowest content of free asparagine. The content increases with increasing ratio of peripheral layers, bran having the highest values.

Thus, it was demonstrated that for a reproducible reduction of the asparagine content flours of low extraction degrees can be used.

3.2 Evaluation of different cereal varieties as raw material for extruded products

The cereal varieties maize, rice, wheat, rye and oats as important raw materials for extruded products and breakfast cereals were tested to their potential to develop acrylamide during extrusion.

For the characterization of the raw materials the contents of asparagine and free sugars as important reactands were determined. The values are summarized in Table 1. The low contents of asparagine in different rice samples but also in grits and wheat flour are obvious. The asparagine contents of rice bran, especially of rye wholemeal and oat flour are higher by the 5 to 25fold. The reducing sugars and sucrose are contained in highly differing amounts.

Tests with maize and rice were performed in the laboratory single-screw extruder, tests with wheat, rye and oats in the twin-screw extruder at varying temperature and moisture. For all the tests the parameters were

selected such as to obtain well expanded, sensorily appealing products. For the characterization of the extrudates, firmness, bulk density and expansion index were determined as well as the sensory evaluation performed.

Table 1: Contents of asparagine and free sugars of selected cereal varieties

Cereal sample	asparagine	glucose	fructose	sucrose	maltose
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
round grain rice	13.0	1000	100	1000	
long grain rice	12.7	500	<100	700	
Thai long grain rice	18.4	900	100	700	
US-parboiled rice	19.9	1100	300	5600	
rice bran	177.9	600	300	30700	
maize grits	95.2	310	80	240	<50
wheat flour (type 550)	118.1	700	400	3000	700
oat flour	503.7	<100	<100	11780	<100
rye wholemeal 1	458.8	2400	1500	9900	3100
rye wholemeal 2	684.8	2500	1300	11000	4200
rye wholemeal 3	666.5	1700	640	12930	7250

The tests were performed on three levels of extrusion temperature and moisture contents of raw material.

Due to the low contents of reducing sugars and asparagine, maize and rice had low acrylamide contents of the extrudate, the values partly being at the detection limit. The acrylamide contents of maize extrudates amounted to 40 to 124 µg/kg (180 °C, 15 % moisture). Rice extrudates had an even lower acrylamide formation below 50 µg/kg.

The acrylamide contents of wheat extrudates amounted up to 114 µg/kg.

The values clearly show that analogeously to rice and maize flour, wheat too has a low potential to form acrylamide which is due to the low asparagine content.

Oat flour was treated at extrusion moisture contents of 16 to 26 % as well as without wetting (11.5 %). The results of acrylamide determination were very low despite of the high asparagine content (>500 mg/kg). The acrylamide content amounted to <30 µg/kg at all the temperature and moisture values. Only the unwetted sample which was processed with a moisture content of 11.5 % had an acrylamide content of 58 µg/kg.

In rye extrusion tests acrylamide contents of 200 to 1200 µg/kg were induced, depending on the extrusion conditions (Table 2 and Figure 8). The high acrylamide contents mainly are caused by the high contents of asparagine and reducing sugars, compared with the other cereal varieties (Table 1). Therefore, rye has a high potential to form acrylamide. Rye as raw material for extrusion is of importance especially for the production of flat bread. According to literature and internet searches (www.lua.rlp.de; www.vzhh.de), these products have acrylamide contents of <60 to 590 µg/kg.

Table 2: Acrylamide contents in rye wholemeal extrudates

mass temperature	moisture extrusion	of specific mechanical energy E_{sm}	dry matter	acrylamide	acrylamide
°C	%	Wh/kg	%	µg/kg	µg/kg d.m.
150	19	123	91.3	259	284
150	17	127	93.0	687	738
150	16	190	92.8	916	987
160	19	130	91.9	463	504
160	17	155	92.8	781	842
160	16	190	92.1	1226	1331
170	19	131	92.1	781	848
170	17	134	92.5	1188	1284
170	16	175	91.8	1242	1353

3.3 Influence of formula components on the development of acrylamide at the example of extrusion and baked goods

The following components could have a potential effect on the development of acrylamide: mono-, di- and oligosaccharides, pH-influencing components, aw-influencing components.

a) Analysis of sugar-containing ingredients and sweeteners

For the production of extrudates (flat bread, breakfast cereals) several ingredients are used (skim milk powder, flavour malt flour, salt, cocoa, rework). These ingredients were tested as to their influence on the formation of acrylamide. Most of the ingredients for extrudates contain mono-, di- and oligosaccharides (e.g. milk powder, malt flour, sugar). For characterizing their effect extrusion tests were performed with rye wholemeal and several ingredients. The dosage was determined by practice relevant formulas under sensory aspects.

All the practice relevant ingredients of wholemeal extrudates which increase the content of reducing sugars in the mixture significantly increase the acrylamide content, compared with extrudates without additives. Comparable results were achieved also with the use of various ingredients in roasting processes.

For a clear presentation the values in Figure 3 are related to the standard (100 %). Roasting was based on a temperature of 250 °C and a time of 45 s. The increased content of mono-, di- and oligosaccharides in the formula supports the development of acrylamide. The results have the same trend as those for rye extrudates. The development of acrylamide was enhanced in the following grading: glucose>fructose>sucrose. Inuline and malt syrup also increased the acrylamide content. The addition of salt decreased significantly the acrylamide content. The effect was observed also in the mixture of salt, sugar and malt syrup; despite the sugar containing ingredients the mixture ranged slightly below the standard.

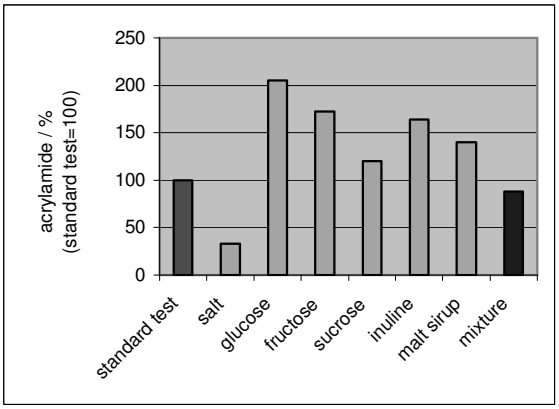


Figure 3: Influence of components on development of acrylamide in cornflakes (250 °C, 45 s). Dosage: salt, malt syrup (1 %), glucose, fructose, sucrose, inuline (5 %); mixture: 1.5 % salt, 4 % sucrose, 1 % malt syrup.

Especially fructose caused an extreme increase of acrylamide development and thus is the main cause of the partly very high acrylamide contents of diabetic products. Therefore, the substitution of fructose by other less reactive sweeteners is an important aspect for the development of formulas. Here, sugar alcohols (Isomalt, sorbite) are suitable which are reducing the acrylamide content compared to the standard sample.

Isomalt is a sugar substitute which chemically belongs to the group of disaccharid alcohols and is a mixture of glucosyl sorbite and glucosyl mannite. Compared with sucrose the sweetening power amounts to app. 0.5-0.6. The test results are demonstrated in Figure 4. Low additions of Isomalt do not have any influence on the acrylamide development. A clear decrease of the acrylamide content of the product was observed at additions of 5 and 10 %. The influence of sorbite on the development of acrylamide can be compared with that of Isomalt. The substitution of fructose by sugar alcohols is one approach to reduce the acrylamide content of diabetic products.

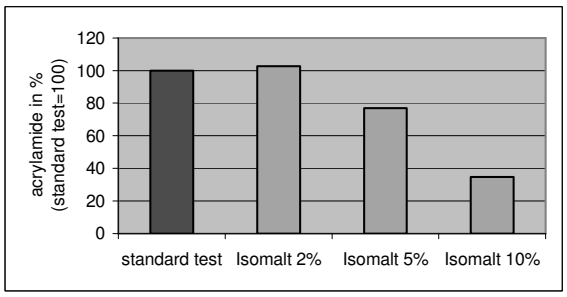


Figure 4: Influence of isomalt on the relative acrylamide content of rye extrudates.

b) Components influencing the pH-value and water activity

The dependence of the development of acrylamide on the pH-value [7] can be a suitable approach to reduce acrylamide contents. Ingredients which frequently are used for baked goods and extrusion products are suitable: citric acid, ascorbic acid, salt (NaCl, KCl), or glycerol.

Influence of citric acid and ascorbic acid

The results in Figure 5 imply that especially acids have a siginificant influence on the acrylamide content. The results were confirmed by the production of brown gingerbread for which the dough was adjusted to pH 4.5 by citric acid.

The tests have shown that the minimization of acrylamide is possible by the addition of citric acid in a sensorily justifiable range.

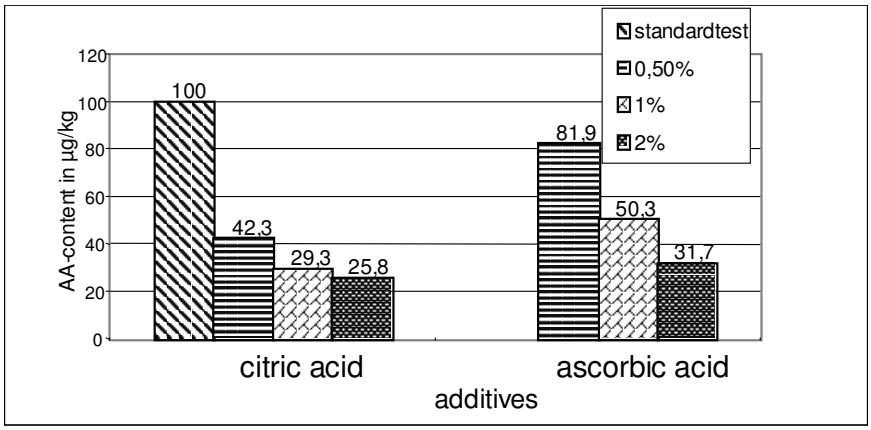


Figure 5: Influence of acids on the development of acrylamide.

Influence of salts

Results of extrusion tests indicate that salts have a decisive influence on the development of acrylamide. Tests with the model system for baked goods have shown that a significant reduction of the acrylamide content is possible by the use of NaCl and KCl in the formula. The acrylamide content was decreased with increasing dosage. The results of KCl are given as an example.

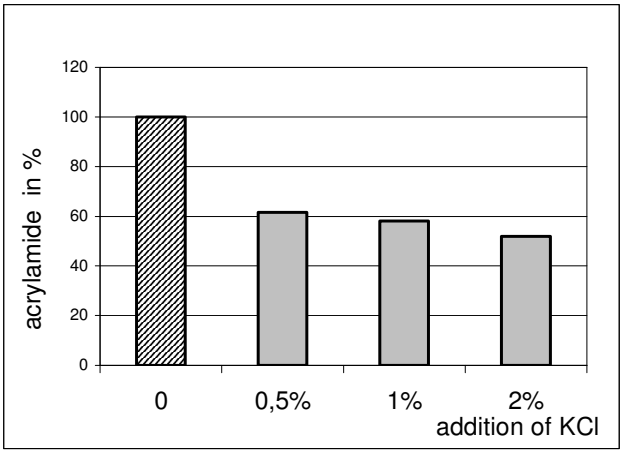


Figure 6: Influence of KCl on the development of acrylamide.

Although the reduction in the model system was significant, the quantity added to the real food is limited by sensory aspects, as also stated in [9].

c) Influence of raising agents and dough conditioners on the development of acrylamide

In the production of baked goods, the effect of dough conditioners and raising agents is of special importance because they influence decisively the pH-value of the dough. The effects could be the basis of minimization strategies. The model system of baked goods was tested at the example of brown gingerbread.

In the test series

- baking powder
- commercial ammonium carbonate (ABC raiser)
- potash (potassium carbonate)
- malt flour

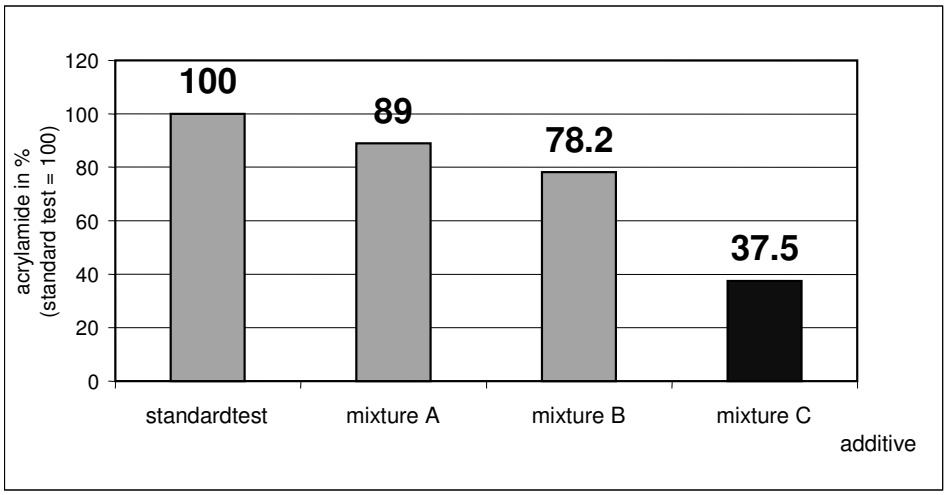
were added in different quantities, related to flour, to the basic formula of the model system. For the sake of clear conclusions, provoking quantities were tested which generally are not applied in practice. The results are summarized in Table 3.

Table 3: Effect of additions to the model system and to brown gingerbread, respectively

raw material	addition	influence on acrylamide content in	
	[%]	model system	brown ginger bread
baking yeast	0.5	not significant	--
raising agent			
baking powder	1 to 10	--	--
ABC raiser	1 to 5	--	--
potash	2	+	+
dough conditioner			
with malt flour	1 to 2	--	not determined
with lecithine	1 to 2	--	not determined
with DAWE	1 to 2	++	not determined
malt flour	1 to 7	--	-- (bread)
salt			
NaCl	0.5 to 2	+	+
KCl	0.5 to 2	+	--
acids (pH-influence)			
ascorbic acid	1 to 2	++	+
citric acid	1 to 2	++	--
pH-regulation 4,5	-	++	+++
a_w-value influences			
glycerine	2 to 5	++	+
potato flakes	1 to 10	++	+
sugars and sweeteners			
isomalt	1 to 10	+	+
glucose	1 to 10	--	--
sorbit	1 to 10	+	

Legend: + reducing (up to 10 %, compared with the initial value); ++ strongly reducing (up to 40 %, compared with the initial value); +++ very strongly reducing (up to 80 %, compared with the initial value); - no effect or increasing the acrylamide content

The use of salts which have a reducing effect in model systems did not have the expected effect in brown gingerbread. The dosage also was limited by sensory aspects which allowed an addition of max. 1 %. The combination of acids and salts (Figure 7) caused reductions up to 37 %. The additions did not have any sensory effect. The gingerbread had an acceptable taste and chewability, the volume was not influenced.



mixture A: 1 % NaCl + 0.5 % citric acid mixture B: 0.5 % KCl + 1 % citric acid mixture C: 0.5 % NaCl + 1 % citric acid

Figure 7: Influence of acids and salts combined, on the development of acrylamide.

4. Influencing the development of acrylamide by technique and technology

4.1 Extrusion

Extrusion tests were performed in two models of extruder:

— Single-screw laboratory extruder, model DN 20 (Brabender), working length 20 D. Dosage by K-TRON Soder twin-screw compact dosator; model KMV KT 20

— Twin-screw co-rotating extruder, model MPF 50 (APV Baker Perkins), working length 15 D.

Beside the thermal treatment, the mechanical energy input during extrusion influences the conversion of the material. The mechanical energy input depends on the rotational speed, the throughput and the viscosity of the material mixture which is reflected in the torque. For the evaluation of the process of extrusion the specific mechanical energy input (SME) was calculated.

The tests were performed in three ranges of temperature and moisture as n^3-plan in order to define limiting parameters in the acrylamide development.

— Extrusion temperatures: 130 °C to 180 °C

— Material moistures: 15 % to 21 %.

The tests of the influence of technological parameters on the development of acrylamide demonstrated the direct dependence of the cereal raw materials used on the extrusion temperature. Temperatures >170 °C caused a significant increase of the acrylamide content of the extrudate. The increase of extrusion moisture by 1-2 % already resulted in a significant reduction of the acrylamide contents.

The dependence of the acrylamide content on the extrusion temperature and moisture for the extrusion of rye is demonstrated in Figure 8. Already a slight increase of the extrusion moisture reduced significantly the acrylamide content of the extrudate.

These trends were observed also in tests with further additives in the extrusion mixture.

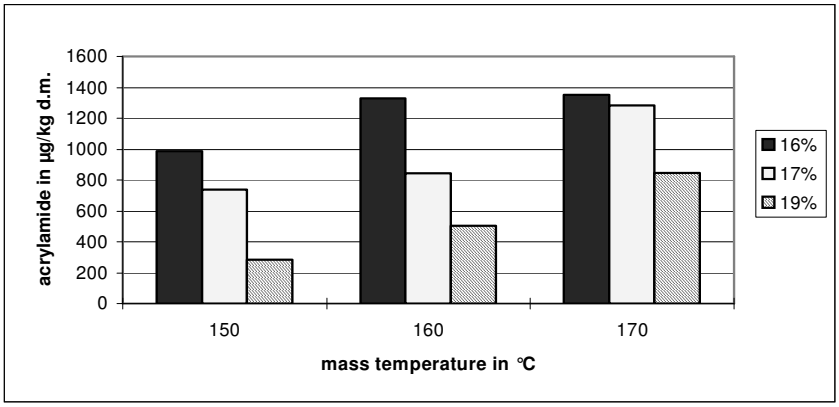


Figure 8: Acrylamide contents in rye extrudates at varied mass temperature (150-170 °C) and moisture levels (16-19 %).

4.2 Roasting processes

For testing the roasting processes for cornflakes production, maize gelatinates were prepared by extrusion and flaked. Roasting was performed in the laboratory fluid-bed roaster (NOVOPACK). The parameters sample quantity and air throughput were optimised in preliminary tests and constantly maintained in the subsequent tests. The parameters roasting temperature and time were varied. The parameters were selected to obtain sensorily appealing cornflakes of pleasant roasting result.

The technology of cornflakes production includes two thermal treatments. At first, cooked maize grits and gelatinates are produced which are roasted after flaking. The production of these intermediate products is of no critical relevance for the development of acrylamide because the temperatures applied are below 100 °C. The tests were performed on the basis of cooked maize grits and grit gelatinates which were produced in the extruder. The acrylamide contents measured after this processing step were below 30 µg/kg. Corresponding results are described also in literature [8].

The tests for the production of cornflakes were based on ingredients which were prepared from grits by cooking. Fluid-bed roasting was performed at different roasting temperatures and times.

The influence of roasting temperature and time on the development of acrylamide in cornflakes is shown in Figure 9. The increase of temperature from 230 to 250 °C at constant roasting time of 40 s resulted in an increase of the acrylamide content. The reduction of time to 25 s at further increase of temperature to 270 °C reversed the trend. The results imply that the acrylamide content of cornflakes can be maintained with a balanced roasting regime with regard to temperature and time. Short roasting times and high temperatures are an approach to minimize the development of acrylamide. Corresponding test parameters have to be determined with regard to the production plant and adapted to the sensory requirements.

The acrylamide content in dependence on the moisture of the final product is shown in Figure 10. The drier the product after roasting the higher the acrylamide content. Therefore, the final moisture is of decisive importance. For sensory and storage-technical considerations, cornflakes should have maximum moisture for the reduction of the acrylamide content.

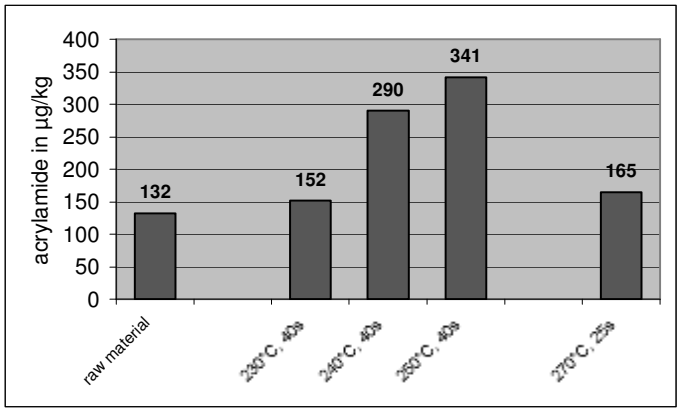


Figure 9: Influence of roasting parameters on the development of acrylamide in cornflakes.

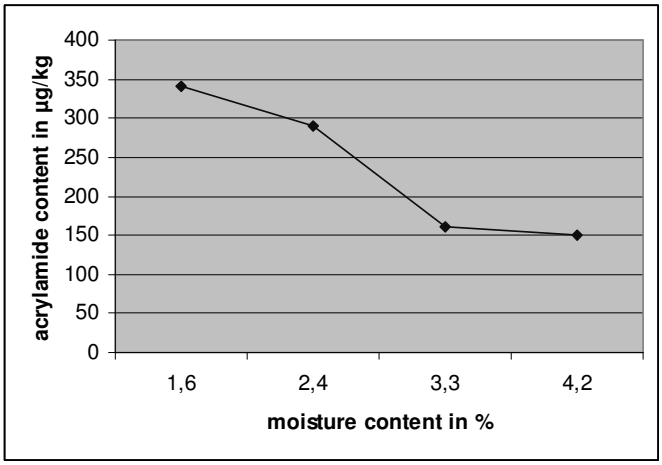


Figure 10: Correlation of acrylamide content and moisture after roasting.

4.3 Influence of technological parameters on the development of acrylamide during baking

a) Influence of dough making on the acrylamide content

The process parameters to which the products are subjected during the production process play an important role for the development of acrylamide. Baking temperature, duration of influence, kind of heat transfer, and product moisture are the decisive technological parameters [10].

The parameters are acting in a complex system and with alternating effects. Therefore, the single steps of dough making and baking process were tested.

For the determination of influence of fermentation conditions on development of acrylamide test series were performed with variation of the humidity in the proving room during production of mixed rye bread (60 % rye flour, 40 % wheat flour). Proof of the dough pieces generally was performed at a humidity of 80 % and a temperature of 32 °C. The temperature was kept at a constant level during the tests. An increase of the humidity by 5 % lead to a significant reduction of the acrylamide content by 22 %.

Further, the shape and surface treatment of the bread loaf was evaluated. For this purpose rye bread (oven bottom loaf, tinned loaf, wetted surface, mealy surface) was prepared and baked. The difference of the acrylamide content between oven bottom loaves and tinned loaves amounted to 43 %. The lower contents of the tinned loaves are explained by the lower portion of crust. Differences in connection with the surface treatment were not observed.

b) Dependence of the development of acrylamide on the temperature-time profiles

According to Granvogel et al. [11] an increased development of acrylamide was observed at temperatures of 140 °C and more. In the classic baking process at 230 °C, at least after 70 % of baking time the critical temperature range for the development of acrylamide was reached in the crust. With increasing baking time principally the crust temperature also increases [12].

The reduction of the acrylamide content is possible by changing the baking conditions, especially by reducing the baking temperature. The increase of product moisture and the reduction of baking time are suitable steps. For the technical implementation, the measurement of baking climate is recommended in order to control and regulate damping. In practice the results also were used for the production of crisp bread. The acrylamide content could be reduced by changing the baking conditions.

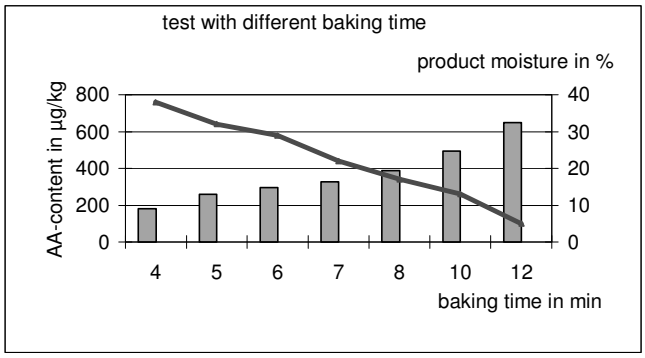


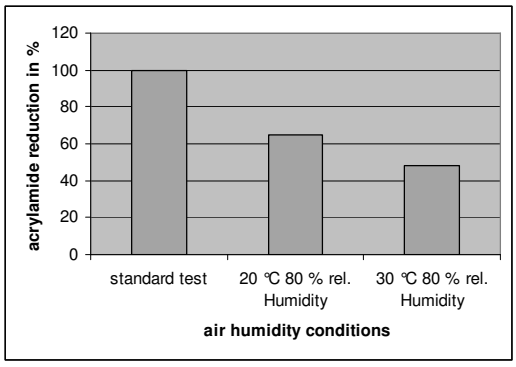
Figure 11: Influence of baking time and product moisture on the acrylamide content.

c) Influence of treatment conditions after baking

The treatment of the products after baking with moisture (spraying of a defined water quantity on the surface of the baked goods) resulted in a reductions of the acrylamide content by 18, 50 and 10 %, respectively, for crisp bread, mixed rye bread, and gingerbread, respectively. These results can be used in the gingerbread production.

A frequently applied technology in gingerbread production is baking up to a product moisture of app. 10 % for a better storage ability and subsequent conditioning of the products. Depending on the technological parameters the process time is variable. This post-treatment is aimed at a product moisture of 17 % (eatable, soft crumb). This process can take up to 48 h. In industrial production, the baked warm gingerbread frequently is sprayed after baking.

Storage tests of gingerbread at defined post-treatment conditions showed that the acrylamide content was reduced probably because of the volatility in steam [16].



Storage time: 5 days; Product moisture: before conditioning 9-11 %; after conditioning: 18-20 %

Figure 12: Changes of the acrylamide content under posttreatment conditions.

d) Technology changes in the baking process

Alternatives for the course of the baking process are seen in the reduction of energy input. Tests have shown that after the sufficient stabilization of crumb/crust which is achieved after 70 % of the baking time, the development of acrylamide was significantly lower than after the baking process at regular baking time. For the optimization of the baking process, therefore, further processes were tested which impart to the product the desired sensory properties and at the same time withdraw moisture in the desired quantity without thermal impact to the product which would activate the development of acrylamide.

e) Application of vacuum cooling

Having reduced the baking time to 75 %, brown gingerbread was subjected to a "drying process" in vacuum, after baking. First orientating tests resulted in a reduction of the development of acrylamide (Figure 13).

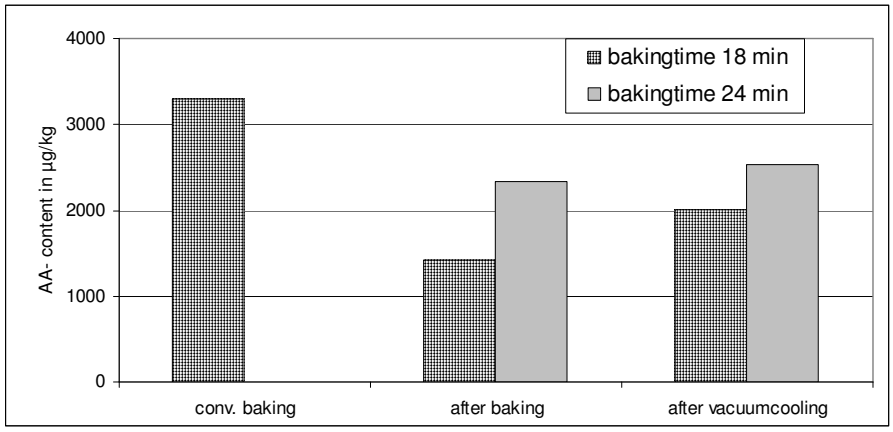


Figure 13: Acrylamide content after vacuum cooling.

The tests were performed in baking ovens of different models (storey oven, rack oven) and resulted in average reductions of 43-45 %.

f) Application of microwave energy

Microwave energy is applied after the reduced baking process. The acrylamide content also could be reduced (Figure 14).

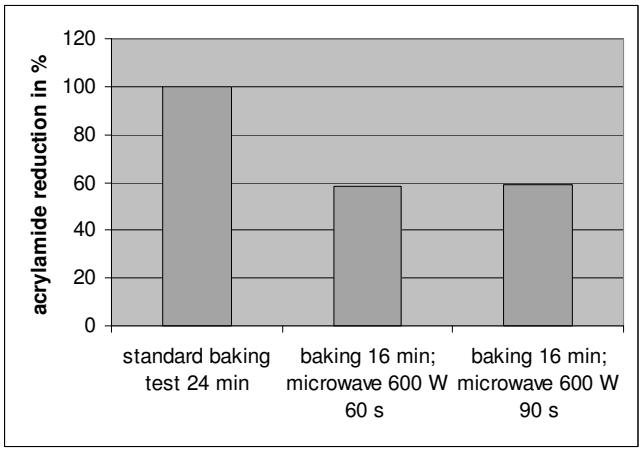


Figure 14: Acrylamide reduction by application of microwave energy baking.

5. Summary and conclusions for minimization strategies

Components of formula

Tests on the influence of various ingredients on the acrylamide content showed that the increase of the content of reducing sugars as reaction partner of asparagine will increase the development of acrylamide. This refers to added sugars (glucose>fructose>sucrose) as well as to sugar-containing ingredients (oligofructose, skim milk powder, malt flour).

The relatively high reactivity of fructose causes the partly very high acrylamide content of diabetic products. It was demonstrated that the acrylamide content can be reduced significantly (to app. 50 % of the control sample) by the use of other sweeteners (sugar alcohols, sorbite, isomalt).

The addition of salt, citric acid, ascorbic acid, or glycerol can be recommended for the reduction of the acrylamide content. The amount to be added has to be tested product specifically under sensory aspects. The acrylamide reducing effect is the better, the more of the substances can be added. A reduction to 37 % compared with the control sample was reached by the addition of 2 % salt.

The addition of thickeners, phosphates, and rework showed an increase of acrylamide in the product.

The results achieved are an important basis of the aimed development of formulas for breakfast cereals and baked goods. If the effect of different ingredients and of food relevant additives is known products can be developed which have a minimum acrylamide content.

Process operation and equipment

Acrylamide is formed especially if extruders with high thermal and mechanical energy input is used. The acrylamide content is increased drastically with increasing temperature and at low moisture. Already slight differences of the moisture by 1 % significantly influence the acrylamide content.

Due to the intensive mixing of the ingredients and the high mechanical energy input twin-screw extruders have a higher potential for acrylamide development than single-screw extruders.

In the process of gelatinisation for products like corn flakes only the post-treatment (drying, frying, roasting) has an adverse effect on the development of acrylamide. Gelatinisation itself is performed at low temperatures (90-100 °C) and does not bear the risk of development of acrylamide.

During roasting the temperature-time regime applied is of decisive importance for the development of acrylamide. Short-time roasting at higher temperatures is better for cornflakes than longer roasting at lower temperatures. The optimisation in dependence on technology, raw material and desired sensory properties is an approach to minimize acrylamide in roasted products.

Baking process

The formation of acrylamide in the baking process can be influenced by control of the process parameters temperature, baking time, humidity. A critical range is reached after 30-35 min from starting the baking process for bread. Measurement of crust temperature and crust moisture for control is recommended. New processes with reduced energy input, realized by application of microwave energy, by two-step-processes and by application of vacuum have been tested successfully.

In the production of gingerbread post-treatment with moisture app. 18-20 % in the product was found as a way for the reduction of acrylamide.

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Importance of frying fat and frying equipment conception on the acrylamide contents in fried products

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1. Introduction

1.1 Frying as a heating process of foods

Due to the focusing of the acrylamide problem on heated foods the heating equipment concepts and heating medium itself plays an important role with respect to acrylamide reduction. Optimising of heat transfer processes and related equipment contributes to the minimising strategies of acrylamide.

In the deep-fat frying process which is one of the relevant heating processes the frying fat works as a very effective heat transfer medium leading to short heating times. The fat itself becomes a part of the fried product and therefore determines its quality. Therefore, investigations were concentrated of heat transfer during frying and on the reactions at the product surface.

During frying the water in outer product layers evaporates very fast. Local temperature increases to values above 100°C after drying and the desired brown and dry crust is formed. The evaporation zone moves to the inner layers and frying fat diffuses into the crust pores resulting from water evaporation [1]. Temperature raise in the crust and the evaporation rate is determined by the heat transfer coefficient which is also influenced by the content of polar components in the fat [2,3]. Additionally to the heat transfer, also exchange of substances, e.g. triglycerides and flavour components, takes place through the product surface (interface). Water, water soluble compounds and lipids from par-frying get contact to frying fat. Lipids and lipid oxidation products react with product components during frying and contribute to flavour [4].

1.2 Frying equipment and simulation

Principally, industrial fryers, catering fryers and household fryers can be distinguished. Catering fryers have a cold zone on the bottom of the fry pot where product residuals can keep at lower temperatures. This contributes to better maintaining of frying fat quality. On the other side, the cold zone generates higher temperature gradients in the frying fat resulting in different frying conditions [5] and therefore leading to variations in acrylamide contents of fried products. Current developments are the frying at lower pressure levels which enables a lower water evaporation temperature [6] and therefore lower heat loading during drying. Also new catering fryers with a very low oil volume related to product mass are described [7], which enables higher oil refreshment rates and therefore slower oil degradation in the fryer.

The process modelling and simulation enables the estimation of local temperature and moisture distribution and can predict acrylamide formation if reliable kinetic data are available. Several approaches for frying models have been described, e.g. regression models [8], neural networks [9] and analytical models based on mass and energy balances [10, 11].

1.3 Acrylamide formation in fried products

Beginning from the first publications concerning acrylamide in foods the fried products, e.g. French fries and chips, have been in the focus of the interest due to the high values measured in theses products. The main pathway in fried potato products is the reaction of free asparagine and reducing sugars [12]. Therefore the contents of these precursors in fried products are important and have to be controlled. During the last decades, the contents of reducing sugars have been reduced in potatoes used for the manufacturing of fried products to avoid excessive browning.

In 2002 a possible formation pathway of acrylamide formation in frying fat was proposed [13] but not yet confirmed. On the other side, treatment of asparagine in hot frying fat leads to distinct formation of acrylamide [14]. The influence of additives to the frying fat has been discussed controversially. Gertz and Klostermann [13] found higher acrylamide contents in products fried in palm oil with the additive dimethyl polysiloxane (E 900) whereas results of own investigations indicate no influence [15]. Also, influence of frying oil type on acrylamide formation in fried products can be neglected [16]. Limited knowledge is available about the influence of interface processes between product and frying medium in acrylamide formation.

Regarding to the influence of frying equipment the headspace atmosphere above the fryer bath (pressure, humidity) is of interest as shown for vacuum frying [17]. However, other pressure levels and possible equipment influences has not been investigated in detail. Additionally, only few information is available about the spatial distribution of acrylamide in fried products although, especially in French fries, large differences in local distribution of temperature and moisture can be expected.

2. Aims

The purpose of this work was to describe and, if possible, to quantify the influence of frying fat and frying equipment on the acrylamide content of fried products. Resulting from these studies new concepts for frying processes minimising the contribution of frying fat and process on acrylamide contents in the products should be developed. Due to the high priority of product quality for consumer acceptance of new solutions for frying processes and the importance of product precursors on acrylamide formation an integrated approach considering all relevant variables had been applied for the investigations in this sub-project (Figure 1). This means that the established quality parameters of fried potatoes like browning or crispness have to be maintained by the new approaches however acrylamide as new quality parameter has to be reduced.

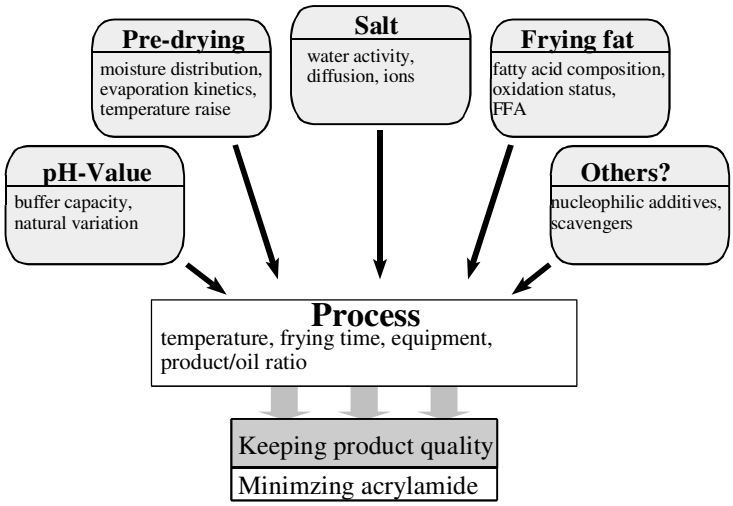

Figure 1: Integrated approach for the minimisation of acrylamide contents considering the role of frying fat.

3. Results und Discussion

3.1 Local distribution of acrylamide in fried products

During frying in the hot fat the temperature load of the product, e.g. French fries, varies locally. To determine the effect of spatial temperature differences on acrylamide formation a model food prepared from potato flakes and shaped rectangularly in pieces with the dimension of 2 x 2 x 10 cm were fried for 12

min to get the desired brown surface. After frying the pieces were cut in thin slices beginning with the outer layers. Each layer was analysed with respect to content of fat, water and acrylamide (Figure 2). The vertical lines in Figure 2 show the thickness of the truncated crust layers. The measured values are drawn up in the middle of every layer.

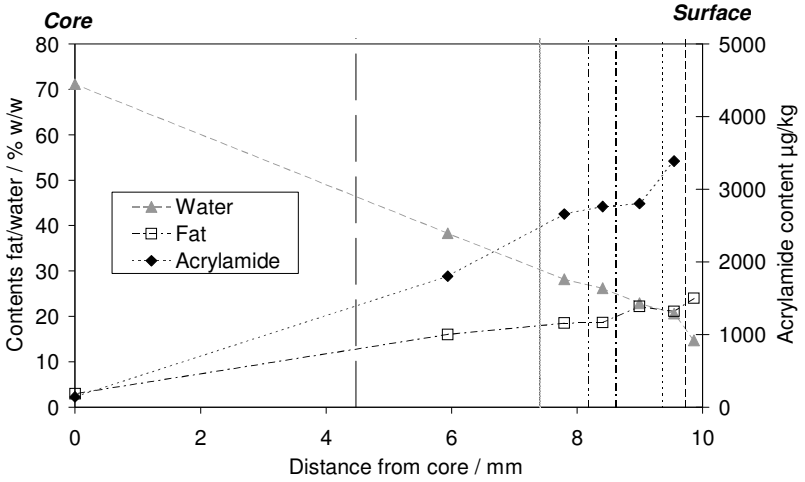

Figure 2: Spatial distribution of acrylamide, water and fat in model French fries.

As expected, the water content decreases from the core of about 70% to the outer surface of about 15%. The sharp decrease is caused by evaporation of water beginning in the outer layers. On the other side the fat content increases from the inner core to the outer surface. However, the largest changes are found in the acrylamide content. The acrylamide content in the core is very low (about 150 µg/kg) but detectable whereas the content at the outer layers is very high (about 3500 µg/kg). This is more then 20 times higher than the core content and confirms the importance of surface processes for the acrylamide formation in fried products [18]. As can be also observed in Figure 2 the layers having high acrylamide contents also contain remarkable contents of frying fat. This fact supports the approach to influence acrylamide formation by frying process and frying fat direct in the interface between product and fat.

3.2 Influence of Additives to the frying fat on acrylamide formation

To validate the possibilities to influence the acrylamide formation during frying by the frying fat different compounds were added to fat before finishing of par-fried French fries in the fryer. Whereas the addition of nucleophilic reactants, e.g. 2-mercaptobenzoic acid, and free fatty acids indicated no significance effect, the addition of w/o-emulsions as described in [3] can contribute to lower acrylamide contents maintaining the same level of browning and therefore product quality. The emulsions containing 20% inner aqueous phase and PGPR as emulsifier were added in a level of 1% to the frying fat. The inner phase consisted of binary solutions of water and citric acid or amino acids to influence the interface processes at product surface. Par-fried French fries were finished in these fats using different frying durations to achieve a broad range of quality with respect to browning. A faster frying process in fat containing emulsions as postulated in literature [18] could not be observed. Also the water content in the frying fat did not increase due to emulsion addition. This means that the main part of the added aqueous phase is evaporated due to the high temperature during frying. The relationship between surface browning of finished French fries shown as brightness (L*) and their acrylamide contents is presented in Figure 3 for the different emulsions added to the frying fat.

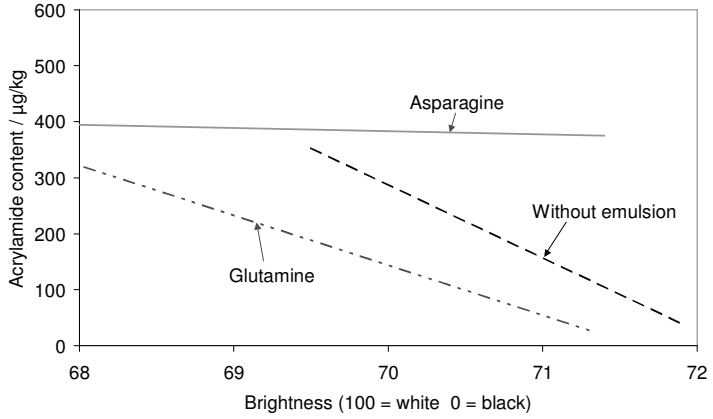

Figure 3: Calculated linear relationship between acrylamide content and brightness of French fries fried in fat with and without added w/o-emulsions (1% w/w) having different aqueous phases.

The straight line for the French fries fried without added emulsion shows a clear increase of acrylamide contents with decreasing brightness (or darker) French fries. If the emulsion with glutamine solution in the inner phase was added a comparable course can be stated, however, below the line of the pure fat. This means that lower acrylamide contents can be obtained at the same level of browning or quality of the final product. Glutamine is one of the precursors for browning in potatoes [20] and seems to shift the Maillard reaction on product surfaces away from acrylamide formation. Therefore, the providing of aqueous reaction components through the frying fat during heating of potato products influences the reaction mechanisms and contributes to lower acrylamide contents in French fries. On the other side, the addition of asparagine leads to higher acrylamide contents independent on the frying time (Figure 2).

3.3 Influence of frying conditions on acrylamide formation

A new multi-functional frying apparatus was designed and built to enable well defined frying processes and the measurement of the main process parameters i.e. product temperatures and headspace pressure (Figure 4). The new fryer allows the frying in a closed system with a full control of the headspace conditions above the frying fat. Using these features especially the water evaporation out of the product can be controlled in a broad range of temperatures. The size of the fried product batch is similar to catering fryers which enables a direct comparability of the results with traditional frying processes.

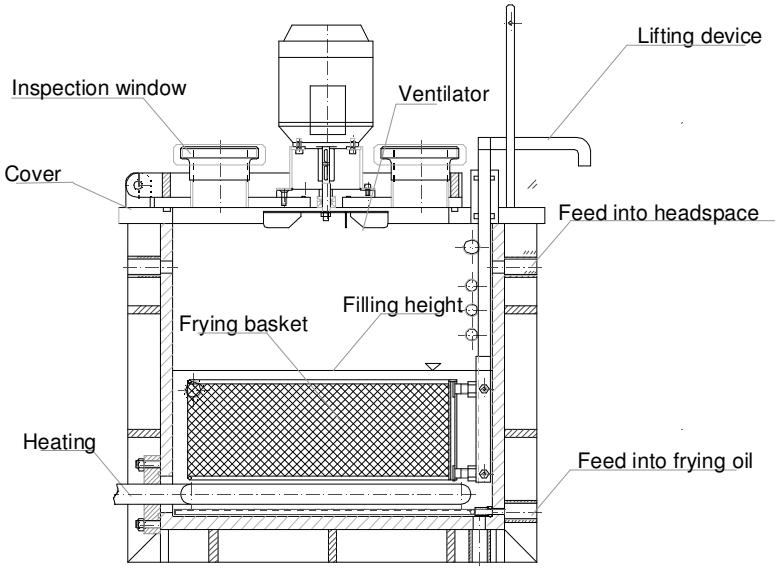

Figure 4: Multi-functional fryer for a well defined frying process.

This fryer was used to investigate the influence of headspace conditions on sensory quality (browning) and acrylamide formation.

For this purpose, par-fried French fries from the same batch were fried for different durations to get a broad range of quality levels. The pressure in the headspace was varied from very low pressure (0.1 bar absolute) to 4 bar over-pressure (fixed by compressed air). This is equivalent to water evaporation temperatures from approx. 40°C to 150°C. The resulting quality parameters as brightness (colour) of the fries and the acrylamide concentrations are shown in Figure 5.

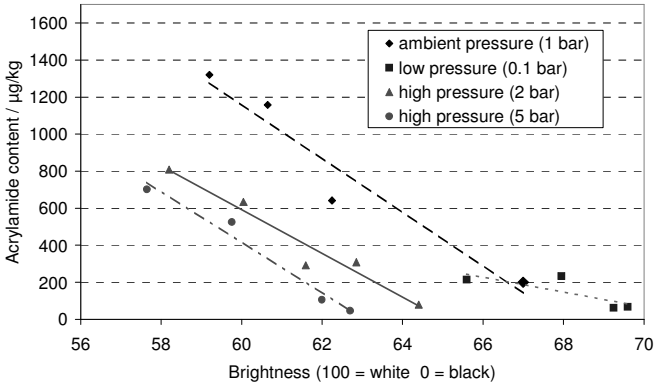

Figure 5: Acrylamide contents vs. colour (brightness) of French fries fried under different headspace conditions.

As can be observed in Figure 5 a strong correlation between the colour (brightness) and the acrylamide contents exists for all frying conditions. The darker the fries (lower brightness) the higher the acrylamide contents. This fact is well-known and integrated into the recommendations for frying operations [21, 22]. However the line position differs depending on frying conditions. Frying with higher pressures in the headspace at 2 and 5 bar absolute results in similar slopes compared to frying at ambient conditions (open fryer bath) but the lines for pressure frying runs below the line of traditional frying. This means that frying at these conditions leads to lower acrylamide contents for the same colour level or browning. Therefore, the frying at higher pressure levels contributes to lower acrylamide contents in French fries and is one opportunity to control frying processes by frying equipment with respect to acrylamide minimisation.

Frying at lower pressures (0.1 bar) results in lower increase of acrylamide contents but also in lower browning of the French fries. The slope of the line is lower compared to traditional frying.

These two examples show some of the opportunities for control frying processes by appropriate frying equipment.

3.4 Pre-treatment for influencing interface processes on product surface

According to the importance of processes on interface between product and frying fat (Figure 1) possible measures to control these processes with respect to acrylamide formation and quality changes in French fries were investigated. The investigations include the coating of the product surface before frying to influence contents of possible precursors or reactants and the pre-drying of the French fries to control the water evaporation during frying.

a) Coating with salt solutions

The French fries were coated in NaCl-solutions with concentrations between 5 and 20% NaCl. Additionally, one batch was coated with demineralised water as control. Before coating par-fried frozen potato sticks were defrosted at room temperature. After 1 hour the sticks were dipped into the solutions for 5 min, followed by dripping off for 1 min and freezing at -20°C. After 24 h storage at -20°C the frozen potato sticks were fried according to chapter 3.2 for 3 min.

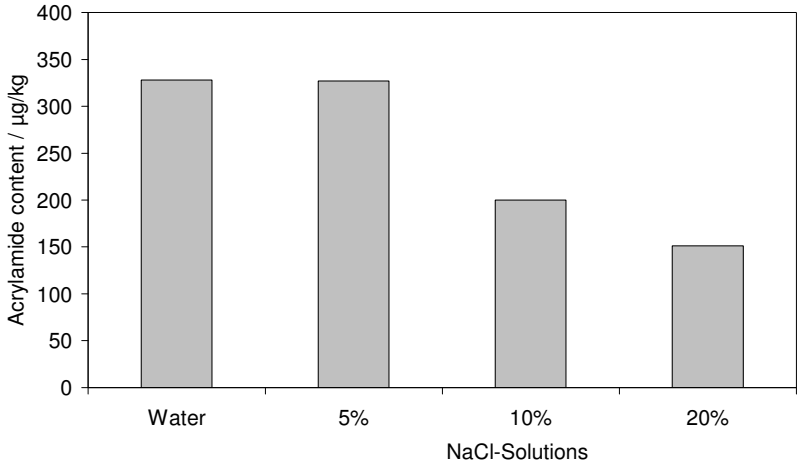

Figure 6: Acrylamide contents of French fries coated with water or salt solutions before frying.

Figure 6 presents the acrylamide contents of the coated French fries after frying. It can be observed that coating with a salt solution of 10% NaCl leads to a significant reduction in acrylamide contents of the French fries. The coating in demineralised water and in the salt solution with lower salt content has no effect on acrylamide contents after frying. Therefore a leaching effect for reducing sugars form the outer layers of the potato sticks caused by coating process can be neglected.

The higher salt contents in the potato surface lowers the water activity and increases the ionic strength. Both influence the chemical reactions during heating, whereby the precise influence mechanism is not fully understood although similar results were found for baked goods.

b) Pre-drying and coating before frying

Pre-drying is another opportunity to influence water activity and moisture in the outer layers of French fries during frying. A modified moisture and water activity shifts reaction mechanisms during heating. Therefore, the influence of a reduced initial moisture in French fries at the beginning of the frying process was investigated. Figure 7 shows the procedure used to pre-treat par-fried potato sticks before finishing in the fryer to investigate the influence of raw material modifications on acrylamide formation.

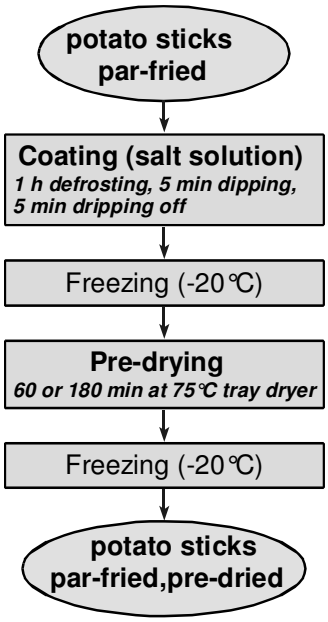

Figure 7: Procedure for the preparation of coated and pre-dried French fries.

The pre-drying of the potato sticks in the tray dryer over 60 min results in a mass reduction of about 7% whereas the drying for 180 min reduces the mass about 21%. The frying time of the pre-dried potato sticks can be reduced due to less water which has to be evaporated. The necessary frying time was adjusted to get French fries with the same colour as in the traditional process for 3 min. French fries pre-dried for 60 min require a frying time of 2 min and the longer drying reduces the frying time to 90 sec.

Figure 8 illustrates the potential of raw material pre-treatment with respect to surface processes for reducing the acrylamide contents in fried products. The pre-drying has a greater influence compared to coating in salt solutions alone. However, for medium salt contents in the coating (6 and 9%) both treatment types contribute to the acrylamide reduction in the final product.

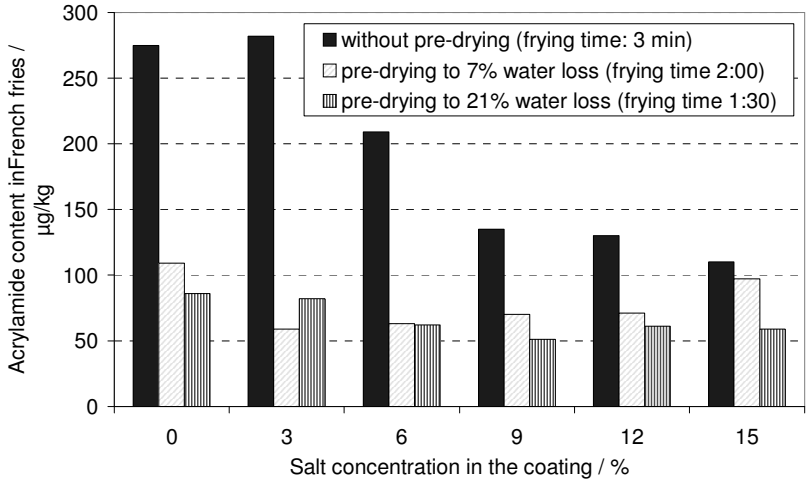

Figure 8: Acrylamide contents of par-fried French fries coated with salt solutions with different concentrations and pre-dried before frying to different water contents.

To quantify the influence of pre-drying and coating on acrylamide contents in French fries the data were applied to estimate possible acrylamide reductions in French fries using a multiple regression approach. The results of the estimations are presented in Figure 9.

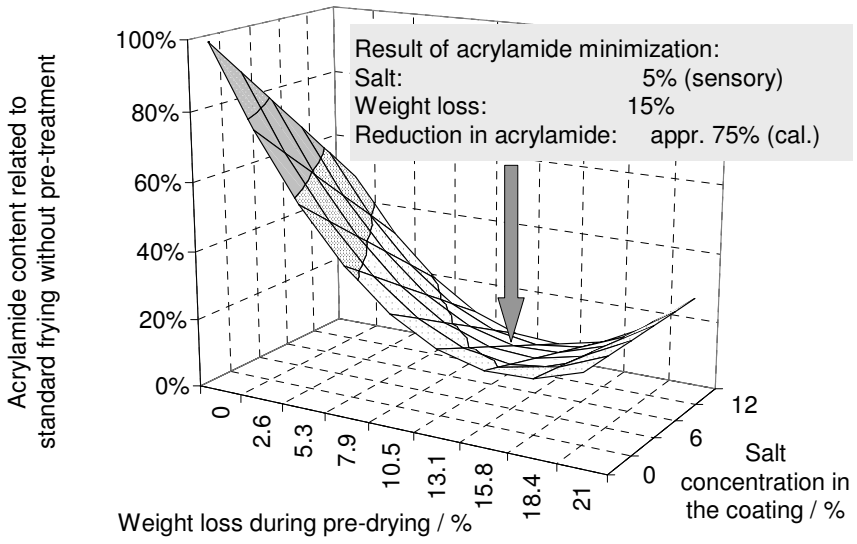

Figure 9: Estimated reduction of acrylamide contents in pre-treated French fries depending on pre-drying and salt concentration in the coating.

It could be observed that the largest reduction of acrylamide contents could be reached for medium pre-drying settings and higher salt contents. However, the maximum salt content is limited to 5% in the coating which is just acceptable for sensory reasons. Applying this salt content a pre-drying to a weight loss of about 15% an acrylamide reduction between 70 and 80% can be estimated. This calculated potential of the minimisation approach using coating and pre-drying was confirmed by further tests (results not shown).

It can be stated that the application of coating and pre-drying during industrial manufacturing results in par-fried French fries which enables much lower acrylamide contents in ready-to-eat product at the same quality level with respect to browning.

4. Summary and conclusions

The frying fat plays an important role for heat transfer to the product and for material exchange processes during deep-fat frying. Both processes determine product quality of deep-fried products including acrylamide formations. Due to the high priority of product quality for consumer acceptance of new process solutions or formulations an integrated approach was used in this sub-project considering all relevant variables. The special focus was on the interface processes between frying fat and product surface.

It could be confirmed that during frying an intensive exchange of triglycerides and minor components takes place between product surface and frying fat. Investigations on model frying foods showed the importance of these interface processes for product quality development and also formation of acrylamide. The extremely high contents of acrylamide in the outer product layers of French fries and the higher fat content demonstrate opportunities for influencing quality through the frying fat.

Additives to frying fat, e.g. w/o-emulsions with aqueous reactants, can influence the acrylamide formation in French fries and lead to lower contents at similar quality levels with respect to browning. Improvements of heat transfer during frying due to addition of emulsions as stated in literature could not be observed. Also addition of oleophilic substances, e.g. nucleophilic reactants or emulsifiers, has only negligible effects on acrylamide formation in the fried product.

To investigate the influence of frying equipment on product quality in detail a multi-functional frying apparatus was designed and built enabling the control of headspace conditions with respect to pressure, e.g. vacuum or pressure frying. Using this equipment it could be demonstrated that frying under higher pressure of 1 bar leads to lower acrylamide contents in French fries for the same level of browning (quality). Therefore, pressure shifts the reaction processes during frying to lower acrylamide formation.

Coating of product surfaces to enrich reactive components or to modify reaction conditions (water activity, pH-value) is another opportunity to influence the interface processes between product and frying fat. Lower surface pH-values and higher salt contents lower acrylamide formation. However, the application of these measures is limited due to sensory acceptance of the fried products. Pre-drying of French fries before finishing in the catering fryer lowers the water activity at the surface and shorten the required frying time for the same final quality with respect to browning. It could be verified that this measure in combination with a sensory acceptable coating in salt solutions reduces acrylamide contents in ready-to-eat French fries up to 75%.

Therefore, coating and pre-drying for influencing the product surface before frying are applicable methods for a sustainable reduction of acrylamide contents in French fries while maintaining the expected product quality. Also modification of frying conditions, e.g. pressure frying or frying at very low pressures can, contribute to lower acrylamide contents in French fries.

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Minimization strategies in potato food

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1. Introduction

The consumers demand for fried, baked, and roasted potato products is noticeable. Acrylamide findings in most of these foods gave reason for a major concern and several action plans of the industry involved were started. New knowledge of fundamental and applied research on acrylamide was collected in a relative short period, to allow an updated view upon potato products combining the overall product quality with health aspects [1].

The main pathway of acrylamide formation in potatoes is linked to the Maillard reaction, concerning the free amino acid asparagine and carbonyl groups from reducing sugars (e.g. glucose, fructose) [2-4]. The overall reaction efficiency is low [5], and the final acrylamide concentration in food is a result of concurrent formation and elimination reactions [6]. Potatoes are known to have a relative high level of free asparagine [7, 8]. Consequently, the concentration of reducing sugars will limit the acrylamide formation in potatoes and has to be controlled in particular. In this context the alternative reaction of asparagine via 3-aminopropionamide [9] should not be neglected.

Potato food manufacturing is directly linked to the raw material, because several factors have an influence upon the final quality profile of the potato (e.g. cultivar, plant treatment, water availability during growth, growing site, local weather, storage). As a consequence, a contracted potato production with specified potato cultivars was established at most companies to increase the quality of the raw material [10-12]. Also processing itself was optimised to offer a wide assortment of high quality potato food [13], not taking into consideration the acrylamide forming potential until 2002.

Crisps and French fries processing present well established techniques. Principal descriptions [14, 15] point out basic treatments, individually modified by the companies. In case of potato crisps, thin sliced potatoes are dehydrated in a hot oil bath (170 - 190 °C) to achieve a final moisture below 2 %. A blanching step may lower the level of reducing sugars to prevent a dark coloured product. French fries are produced in a dual-stage process. Cut and blanched potato stripes are par-fried and cooled or frozen on an industrial scale. Finishing of the French fries takes place in a small scale fryer or in an oven at restaurants and in the domestic sector.

A reduction of any acrylamide formation relates to two main principles. One is a lowering of the precursor concentration in the raw material or in the prepared food to reduce the absolute potential. The other one relates to less heat treatment for a lower acrylamide value. Both approaches have to be considered carefully, because a reduction of acrylamide should maintain the overall organoleptic and nutritional quality of the products as far as possible [1].

2. Material and Methods

Several potato samples from different origin (cultivars, growing sites) were processed to potato crisps and French fries on a semi-technical pilot scale. Frying medium was peanut oil or hydrogenated fat. Most of experiments were carried out with stored potatoes (8 °C, 96% rel. humidity, chemical sprout suppression with CIPC).

Before processing the tubers were cleaned with tap water. Specific gravity was determined with a special balance (KUV 2000, Fischer, Bielefeld) to calculate dry matter and starch content with a new regression

formula [16]. A special peeling unit (type 16 K, Flottwerk, Rotenburg a.d. Fulda) was implemented when required.

Processing details were according to the BFEL standard procedure. Differences are described in context to the concrete experiment. Potato crisps production was carried out with cleaned tubers. After slicing (1.3 mm), free starch and other components were washed off. In some cases, the crisps-slices were blanched for 2 min at 80 °C in a continuous blancheur. Frying temperature and frying time was adjusted, too. Par-fried deep frozen French fries were produced similar. Small cuts were removed just before washing and blanching. After frying, the stripes were cooled and frozen (-20 °C) until final preparation in a restaurant fryer at 175°C and 2.5 min.

Residual moisture and fat content of the products were measured according to standardized protocols [17, 18]. Acrylamide was analyzed with a GC-MS technique after defatting and derivatisation (EPA 8032 protocol [19]; carried out by Dr. Weßling Laboratories, Berlin).

Crisps colour was measured in the L*a*b* modus with a special unit (Chroma-Meter 310, Konica Minolta, Langenhagen) [20]. Crispiness of 15 single crisps-slices was determined by measuring the fracture with a 3 mm tool kit (texture analyzer TA-XT2i, Stable Micro Systems, Godalming, Surrey, England).

Reducing sugars and free asparagine were determined in cut or sliced potatoes just before frying. Water content in the homogenized samples (Combimax, Braun, Kronberg) was determined as mass loss in an oven (105°C) [21]. The samples were lyophilised and residual moisture was determined [17]. Reducing sugar content was measured with an enzymatic test combination (Roche, Darmstadt) [22]. Free asparagine was determined by an hplc procedure according to Hippe [23] with some modifications. After wet extraction of asparagine (aqua dest./dichloromethane, 36/10 v/v) interfering substances were removed with 0.01 N hydrochloric acid by means of solid phase extraction cartridges (Strata SCX 500 mg, Phenomenex, Aschaffenburg). Activation took place with 5 ml methanol and 5 ml aqua dest.. Asparagine was eluted with 2 x 5 ml ammoniumhydroxide in methanol. After evaporation, the residue was resolved in 5 ml of the mobile phase (25 mM phosphate buffer (1.7 g KH_2PO_4/l, pH 2.1 with H_3PO_4) with 0.25 % methanol and 5 mM heptylsulphate) and 20 µl were injected after filtration (45 µm mesh) on a Synergi-RP column (150 x 4.6 mm; 4 µm; 80 A; Phenonmenex, Aschaffenburg) with a flow rate of 0.8 ml/min and UV detection at 200 nm. Calibration was performed with pure asparagine.

A sensoric description of the products was made with a panel of trained individuals (3 to 5 individuals each). French fries rating was according to the specifications of the German Federal Office of Varieties [24]. Crisps scores were detected with special descriptors and averaged to a sensoric quality score. Most perfect samples were rated with „five". Uneatable samples were rated with „zero".

Statistical calculations were performed with STATISTICA V. 6.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

Minimization concepts for potato food could be identified at several points of processing, but factory- and product-specific adaptations have to be considered to meet all relevant details. Therefore, the following description of principles to reduce the acrylamide level represents a common validity and have to be adopted to specific conditions.

3.1 Raw material

Potato tuber composition is under the influence of several factors. To get an impression on that variability, a number of 10 cultivars, grown at 5 locations, were analysed according to the concentration of relevant acrylamide precursors reducing sugars and free asparagine. The reducing sugars concentration varied significantly ($p < 0.05$) between cultivars, but on a non-significant level between growing locations. Variation between locations was not the same for all cultivars, as to be shown by the min-max range of

data. Asparagine concentration differed between cultivars and growing locations on a significant level ($p < 0.05$), but again a cultivar dependent variation could be observed (Figure 1).

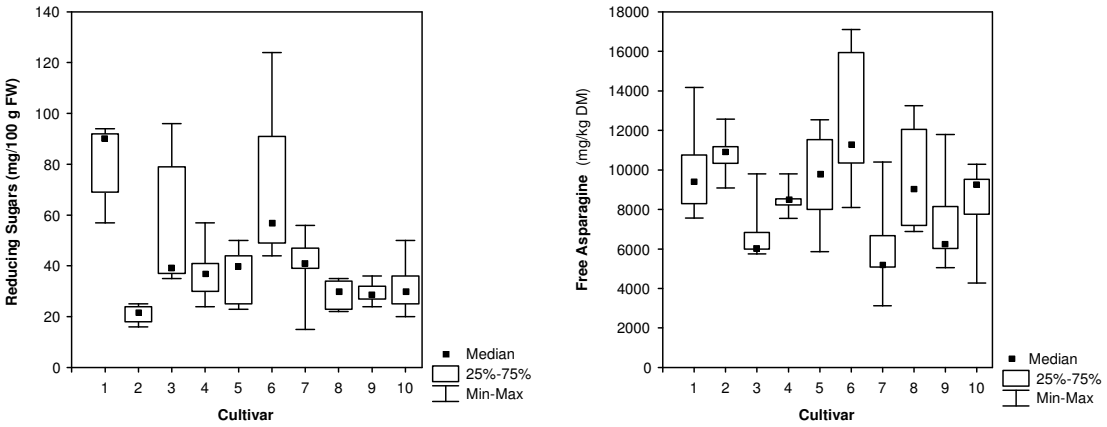

Figure 1: Reducing sugars and free asparagine in 10 cultivars, grown at 5 locations.

Acrylamide data of industrial potato processing indicate a high variability even within one cultivar [1]. To demonstrate such inhomogenities, a representative aliquot of 30 tubers of a single lot was randomly divided into 6 sub-samples of 5 tubers each and analysed according to the main acrylamide precursors (Figure 2).

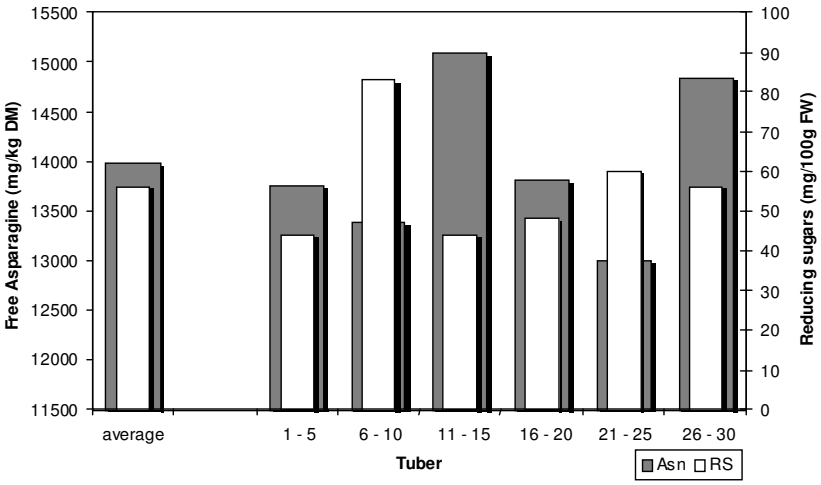

Figure 2: Free asparagine and reducing sugars in 6 sub-samples of a representative potato sample.

The results confirmed the above mentioned variability. The level of reducing sugars was between 44 and 83 mg/100 g FW, whereas the content of free asparagine was between 13,000 and 15,000 mg/kg DM. A correlation of both criteria according to the sub-sample was not possible.

In developing a long term perspective of the acrylamide potential of the raw material an retrospect of several years points out year-dependencies. Four cultivars, grown at three different locations, were analysed according to their content of reducing sugars, both after harvest and after a half year of storage at 8 °C (Figure 3).

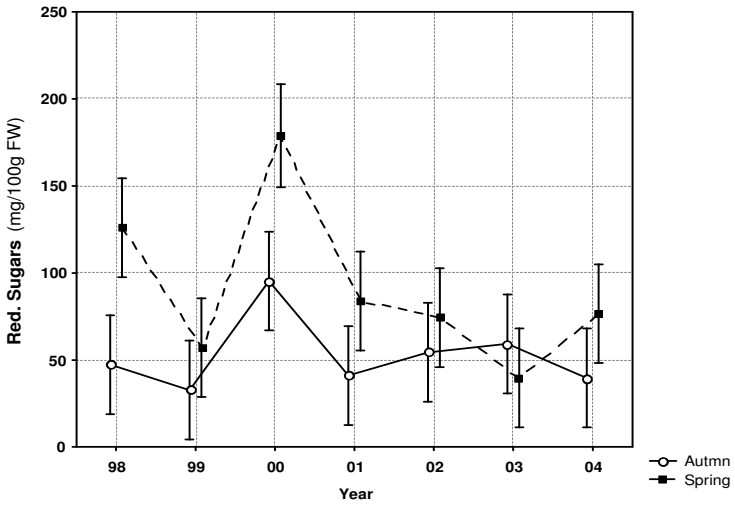

Figure 3: Reducing sugars in four cultivars, suitable for processing, between 1998 and 2004 (data of harvest and storage time); vertical bars indicate a 0.95 confidence interval.

Next to year dependent reducing sugar concentrations at harvest time, also the increases after a long term storage were different. 2003 was noticeable as a growing season with lower reducing sugar values after storage than at harvest time.

Absolutely, the concentration of free asparagine exceeded RS values in all investigated samples, indicating the relevance of reducing sugars in minimization concepts for acrylamide. Since many years, reducing sugars are discussed critically with heated potato products, because colour formation within the Maillard reaction has been found to be closely connected with reducing sugars. Variability of RS data is confirmed in relation to cultivars [25], soil type, weather [26-28], specific growing conditions with individual plant treatments [28, 29], harvest [30] and storage [31]. Summarizing these aspects, several reduction possibilities are offered, but it has to be proven which factor is of relevance in each concrete situation.

Asparagine as the most relevant amino acid for acrylamide formation was found to be 39 % of total free amino acids [32]. On the other hand, around 90 % of the colour variation of fried potato food could be explained by reducing sugars [33], while free asparagine was not correlated with the Maillard derived colour [34]. With respect to the dimension of asparagine values, the effect of an reduction is still on debate. Heuser et al. [35] have shown some principal aspects in relation to fertilisation, which have to be proven in relation to the overall processing suitability of those potato tubers.

3.2 Processing of potato food

a) Semi-finished potato food (French fries)

Industrially produced par-fried French fries contain only traces of acrylamide. Nevertheless, it seems to be possible to reduce the overall potential by a prolonged leaching period, especially in case of potato lots with high sugar level. An experiment with samples from 2 cultivars with an additional leaching step after the typical blanching procedure reduced the concentration of reducing sugars. As a consequence the acrylamide formation at final preparation dropped down drastically (Table 1).

Table 1: Acrylamide concentration in French fries as well as precursor concentration in the raw material; results of a 50 min leaching with 2 cultivars, grown at 3 locations; autumn and spring data

Leaching		Acrylamide [µg/kg]		Red. Sugars [mg/100 g FW]		Free Asn [mg/kg DM]	
	N	average	Stddev.	average	Stddev.	average	Stddev.
All	24	110	67.1	54.5	54.2	9958	5035
No	12	138	82.1	67.5	64.2	12495	5616
Yes	12	81.9	31.3	41.5	40.7	7420	2724
	Autumn						
No	6	103	26.9	30.8	19.3	8574	3149
Yes	6	68.7	23.2	19.2	9.7	5772	2073
	Spring						
No	6	172	106	104	73.9	16416	4751
Yes	6	95.0	34.7	63.8	48.4	9069	2345

Cooking recommendations for French fries finishing were changed to reduce the acrylamide formation [1, 36]. As data have shown (see Table 1), the overall potential may also be reduced by an intensified leaching at par-frying. The finished French fries had an acceptable organoleptic quality with less browning and with a bit firm texture (enzymatic hardening of the pectin network [37]).

Otherwise, also a simple renouncement of fine cuts (6 x 6 mm; 8 x 8 mm) and a preference of normal or thick cuts reduced the acrylamide values significantly [38] because of the changed surface to volume ratio.

b) Finished potato food (Potato crisps)

Processing of potato crisps represents a dehydration of thin sliced potatoes in hot oil. An acceptable level of crispiness needs an residual moisture level below 5 %. Under the guideline of acrylamide minimization tools, industry has increased the moisture level. To follow that procedure, a set of potato samples was crisped under standardized conditions. As a result, the final moisture level was not correlated with the acrylamide concentration (Figure 4), indicating that rather a change of the individual heat treatment will have an influence upon the concrete acrylamide formation. On the same subject, Biedermann et al. [6] reported higher acrylamide levels in case of longer heating but without any change in residual moisture.

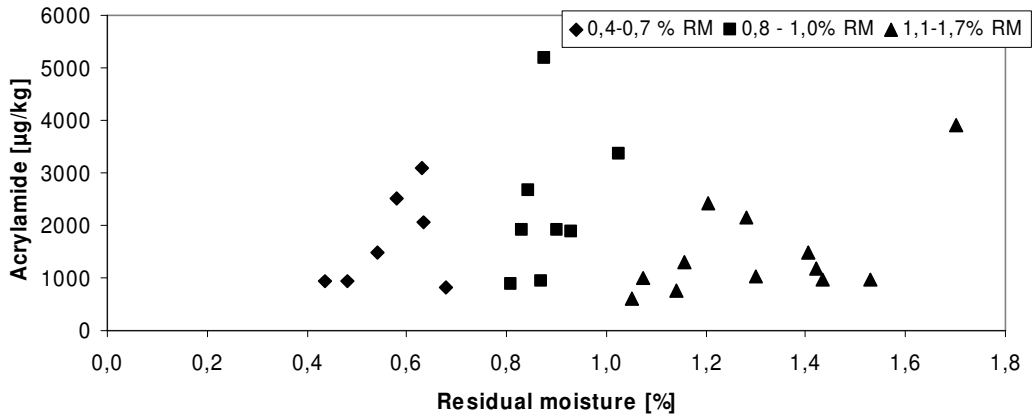

Figure 4: Relation between residual moisture of crisps and acrylamide level.

A reduction of low molecular weight components including sugars and free asparagine in crisps potatoes by leaching or blanching is possible [39]. Therefore, that effect was tested in relation to a potential acrylamide reduction. Calcium ions were added to the water (2 % $CaCl_2$), because some negative textural changes of the pectin network are reported [37]. Also the influence of a simple pH-lowering by citric acid (0.1 %) was tested at different temperature regimes (30°, 50°, 70° and 90°C, respectively; 2.5 min each) (Table 2).

Table 2: Efficiency of different leaching conditions (water, calciumchloride (2 % w/v), citric acid (0.1 % w/v), temperature) on several quality aspects

Variant	Temp. [°C]	RS [mg/100g FW]	Free Asn [mg/kg DM]	Colour [L-value]	Texture [N]	Acrylamide [µg/kg]
	30	73	11335	64.2	2.56	n.d.
	50	45	14642	63.9	2.3	1770
	70	34	8405	63.5	2.09	627
Water	90	31	6425	66.7	2.55	371
	30	73	11753	59.4	3.78	n.d.
	50	53	5322	58.1	3.82	1260
	70	55	6766	59.4	2.76	54
$CaCl_2$	90	31	6555	58.1	3.09	46
	30	67	12263	61.0	2.29	n.d.
	50	50	11583	62.8	2.60	1030
	70	36	8015	61.5	2.2	979
Citric acid	90	26	7639	65.3	2.35	558

A short water contact at 50 °C caused a noticeable decrease in sugars, even continued above the gelatinization range of potato starch (55 - 65 °C). The same effect could be observed for free asparagine. Pedreschi et al. [40] reported about a 76% reduction of glucose and a 68 % reduction of asparagine by blanching of sliced potatoes. Colour of the final product became brighter, crispiness was not affected, and acrylamide concentration dropped down drastically, reported also by Haase et al. [41]. An addition of calciumchloride to the water produced no further reduction of low molecular weight compounds, but breaking force was a bit higher in relation to water leached samples. As a main result, the acrylamide concentration was almost zero at water temperatures beyond the gelatinization temperature. Lowering the pH of the water down to pH 4.2, no additional effect of the pH was detected. On the same subject, Pedreschi et al. [40] described a reduced acrylamide formation by almost 70 % against a control for slices fried at 150 °C. Surprisingly this effect was not present at higher frying temperatures.

The crisps samples of the calciumchloride variants had a bitter taste. Therefore, the experiment was repeated with a reduced calciumchloride concentration (0.1 - 1.0 % w/v). A separate washing step after leaving the blancheur was implemented to clean the surface of the slices (Table 3). The additional cleaning of the blanched slices removed most of the gelatinized starch from the surface, and the concentration of water soluble compounds was reduced, e.g. RS. As a result, the acrylamide formation declined. A low calciumchloride supplement to the blanching medium (up to 0.2 % w/v) had no further reduction potential for acrylamide compared with water treatments. Also a substantial influence upon crisps hardness (texture) could not be detected. A negative bitter taste of the crisps was not described below a

0.5 % $CaCl_2$ supplement. This indicates a small effective concentration range of an acrylamide reduction potential in combination with a most perfect organoleptic behaviour.

Table 3: Salt effect at blanching (80 °C, 2 min) upon several quality parameters without and with additional washing

Variant	Salt	Washing	RS [mg/100 g FW]	Texture [N]	Sensoric quality [Score]	Acrylamide [µg/kg]
Control		No	108	2.88	4.4	1874
		No	51	1.97	5.0	867
Water	0	Yes	42	2.30	4.4	446
		No	60	2.39	4.8	1185
	0.1	Yes	65	2.44	4.5	676
		No	54	2.61	4.5	751
	0.2	Yes	41	3.07	4.5	681
		No	43	3.14	4.0	260
	0.5	Yes	34	2.84	3.8	441
		No	53	2.78	3.5	158
$CaCl_2$	1.0	Yes	39	2.45	3.8	214

The formation of acrylamide in relation to the frying temperature itself was investigated in a temperature range between 140 and 220°C. Frying time was adjusted to get crispy potato slices (Figure 5).

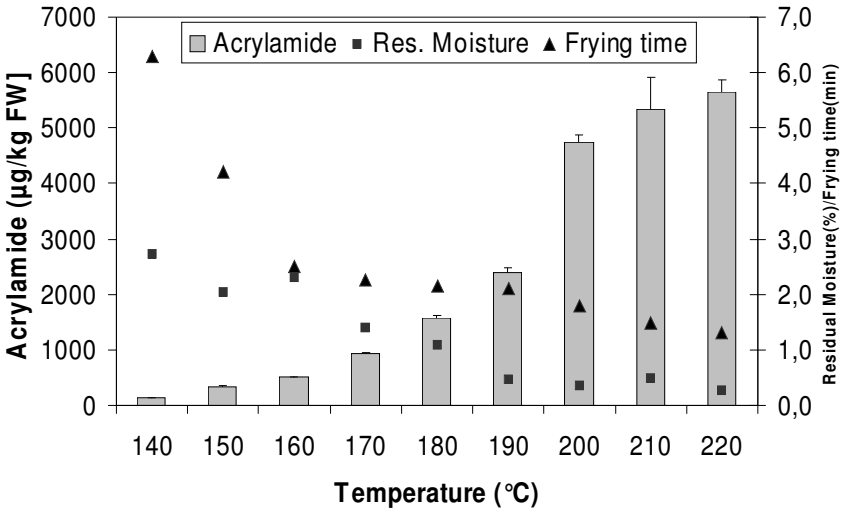

Figure 5: Acrylamide formation in relation to the frying temperature.

The acrylamide increase followed a non-linear progression line with a correlation coefficient of r = 0.92 against frying temperature. The highest value was measured at highest temperature. At rising temperatures the calculated temperature load (temp. x time) went down from 52,920 (° Cs) at 140 °C to 17,160 (° Cs) at 220 °C. The topmost sample still required a relatively long frying time of 80 s. As a consequence the present advice toward moderate frying temperatures [1] was verified. A temperature decrease from 180 to 165 °C resulted in an acrylamide reduction of 51 % during traditional frying [42].

Another subject related to an overfrying of the samples, being present especially in inhomogeneous potato lots with different dry matter contents. A first sample set was heated until no large bubbles were formed anymore, and a second set of samples for one more minute each (Figure 6).

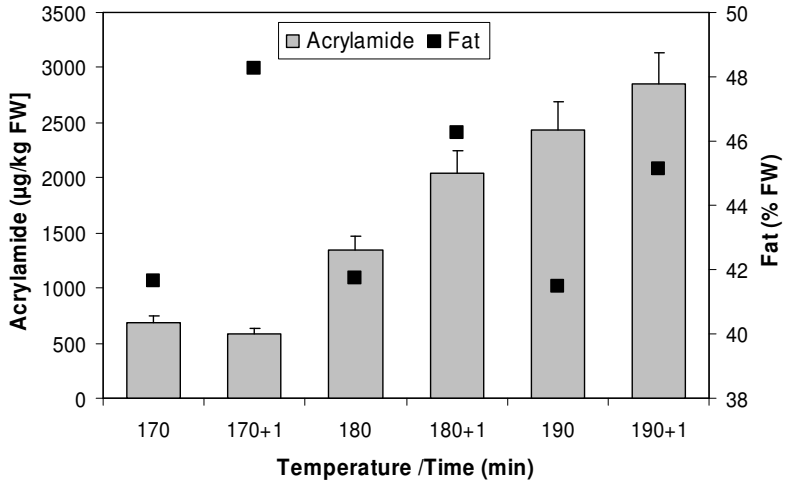

Figure 6: Effect of a prolonged frying time upon acrylamide concentration and fat uptake.

At frying temperatures above 170 °C, a prolonged frying resulted in increased acrylamide levels. This would indicate a need for specified frying times, if the actual preparation advice of moderate frying [1] would not exist. The fat content was the same at all three temperatures independent to the different frying times, but increased substantially at prolonged frying.

The above discussed frying experiments were conducted with a most stable temperature of the oil bath. Within the following experiment, temperature was lowered after a first frying at 170 °C (Figure 7).

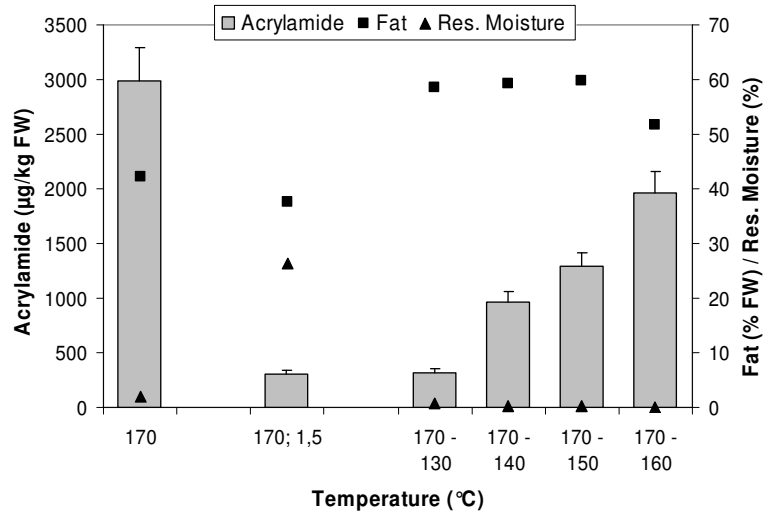

Figure 7: Effect of a temperature gradient during frying upon acrylamide and fat concentration.

Temperature was changed after 1.5 min initial frying at 170 °C. At that time, the water content was about 26 %, and fat content was about 37 %. An acrylamide formation was already present (300 µg/kg). The gradient (Δ 10°C to 40°C) had no effect upon colour and texture, whereas fat concentration increased up to 60 %. The acrylamide formation was reduced substantially, especially at an intensified temperature decline.

A clear temperature decline resulted in quality losses (fat uptake). To overcome these negative product qualities at low frying temperatures, samples were finished fried at reduced atmospheric pressure in a special fryer of BMA Florigo B.V. (Woerden, NL). Initial frying was with the atmospheric fryer of BFEL prior to switching over to the closed system (at that time sample moisture was between 5 and 10 %) (Table 4).

Table 4: Effect of a vacuum frying upon potato crisps quality

Atmospheric		Vacuum		Product			
Temp. [°C]	Res. moisture [%]	Pressure [mbar]	Temp. [°C]	Res. moisture [%]	Fat [% lufttr.]	Colour [L-value]	Acrylamide [µg/kg]
160	2.5	-	-	2.5	36	67	2140
160	9	200	100	2.7	43.8	64	610
160	4.5	100	90	3.2	44.9	66	486
160	4.5	100 -->500	90	4.9	47.1	63	468
160	9	100	100	3.3	41.7	66	279

A significant decrease of the frying temperature at the final frying stage resulted in a considerable reduction of acrylamide. Granda et al. [42] reported a significant acrylamide reduction of 63 % when the temperature decreased from 140 to 125 °C in vacuum frying. The overall product quality was different with respect to specific processing parameters (e.g. pressure) indicating the need for further optimization of chemical and organoleptic quality of the product. Other experiments in this test bed have shown, that the organoleptic quality of the vacuum fried crisps was not significantly different in relation to the frying method for texture and flavour characteristics (mouth feeling; flavour). Otherwise colour (b-value) was significantly different between frying methods [42].

The inverse technique with a pressurised frying system is used in some special cases to reduce frying time (especially for fabricated crisps) [1]. In future experiments it must be examined whether the anticipated reduction in temperature load will break the inverse relationship with acrylamide formation (see above).

4. Conclusion

The research project dealing with acrylamide formation in potato food has identified several minimization tools for a systematic and permanent reduction. Raw material and processing are successful starting points. Variation of single steps may be effective, but in view of a high organoleptic and chemical product quality a combination of several modifications with slight changes each will be the best strategy.

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Toxicology of acrylamide: Concentration-response relationships of acrylamide and glycidamide in human blood

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1. Introduction

Acrylamide is a carcinogen as demonstrated in animal experiments with rats after oral application [8, 12]. These studies indicate that the carcinogenic potential of acrylamide is relatively low compared to other carcinogens for which there is also evidence for carcinogenicity in humans like some mycotoxins, polycyclic aromatic hydrocarbons or N-nitrosamines, many of them being genotoxic by direct interaction with the DNA. For acrylamide, the mechanism of carcinogenicity and its relevance for the human situation is still under debate. Several studies demonstrated the ability of acrylamide to induce chromosomal mutations in rats and mice, but doses applied to animals in most cases were relatively high, between 30 and 150 mg/kg body weight [1, 6, 27, 14]. In in vitro experiments with mammalian cells, acrylamide induces sister chromatid exchanges and chromosomal aberrations [1, 27], but in most of the studies millimolar to molar acrylamide concentrations were used. Moreover, acrylamide is inactive in mutagenicity tests with bacteria in the presence or absence of activating systems [13, 26, 28]. In total, there is limited evidence for a direct mutagenic and genotoxic potential of acrylamide. Acrylamide under physiological conditions does not show direct reactivity towards DNA.

In biological systems, the epoxide glycidamide (2, 3-epoxypropanamide) is generated metabolically from acrylamide by the cytochrome P450 enzyme 2E1, oxidizing the double bond of acrylamide (Figure 1). Adducts formed by interaction of acrylamide and glycidamide with hemoglobin in the body are used to investigate exposure with acrylamide or glycidamide. Glycidamide induces mutations in the Ames-Test [11]. In animal experiments, application of acrylamide resulted in glycidamide-DNA adducts. Glycidamide adducts with N-7 of guanine and N-3 of adenosine were formed as major products [5]. Glycidamide applied intraperitoneally in doses between 16 and 122 mg/kg body weight also induces micronuclei in bone marrow from mice and rats [15]. Therefore glycidamide is considered as the ultimate carcinogen generated from acrylamide that induces mutations by covalently binding to DNA . Acrylamide has been found to be conjugated with glutathione [20]. In vivo, glutathione adducts follow the degradation pathway to mercapturic acids to be excreted via urine. In blood, binding to proteins like hemoglobin (Hb) or albumin [24] and other plasma proteins with nucleophilic centres may as well contribute to the deactivation of acrylamide and glycidamide.

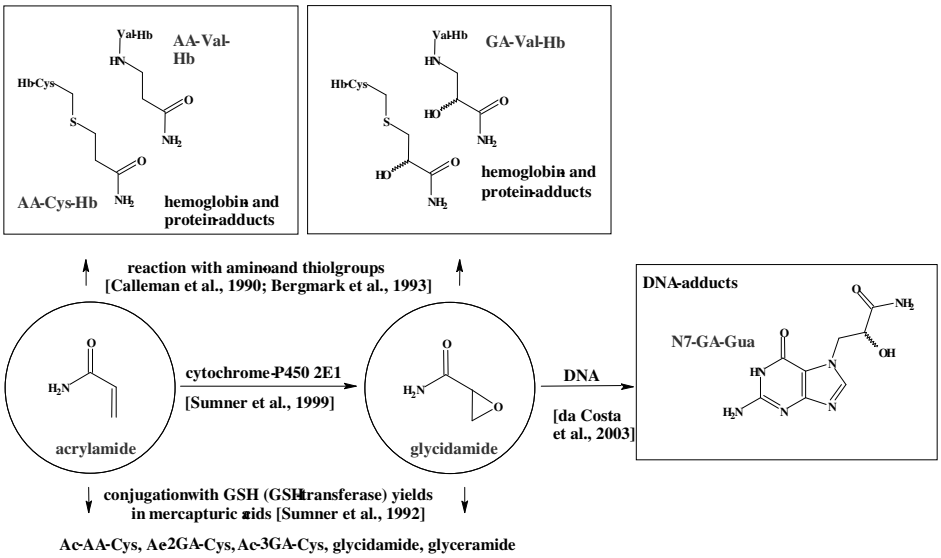

Figure 1: Metabolism of acrylamide.

2. Aim of this study

The biological activity of acrylamide strongly depends on the balance between toxifying and detoxifying mechanisms in the organism. In this study we used human blood as a model to study concentration dependent interactions with DNA in lymphocytes, binding to hemoglobin in erythrocytes and interactions with other blood components. Blood is the first target following the absorption of AA through the gut and is an easily accessible substrate to measure genotoxicity. Concentration response relationships of genotoxic effects served to explore the blood concentration range at which DNA damage in lymphocytes is significantly induced as monitored by single cell gel electrophoresis ("comet assay"). The potential to induce chromosomal mutations was investigated by micronucleus induction, structural chromosomal aberrations and sister chromatid exchange. Genotoxic activity of the known carcinogens benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide and α-acetoxy-N-nitrosodiethanolamine was measured in comparison.

Acrylamide and glycidamide react with other "non-critical" nucleophiles in the blood, such as blood proteins or glutathione. We therefore determined the formation of acrylamide/glycidamide adducts at the N-terminal valine of hemoglobin in concentration ranges derived from the genotoxicity studies. Overall binding of acrylamide to blood components was investigated by incubating blood with ^{14}C-acrylamide and determining radiolabel in different blood compartments.

To approach a biomarker guided dosimetry of genotoxic effects in blood, concentration dependent formation of Hb adducts was compared to DNA damage in lymphocytes.

These studies were further complemented by studying the induction of mutations at the hprt-gene in V79 mammalian cells by acrylamide and glycidamide in comparison, using N-methyl-N-nitro-N-nitroso-guanidine (MNNG) as a reference mutagen.

3. Results

3.1 DNA damage in lymphocytes (monitored by single cell gel electrophoresis)

DNA-damage in lymphocytes was monitored by single cell gel electrophoresis and expressed as tail intensity (TI). Blood was freshly collected from donors, heparinized and incubated for 1, 2, or 4 hours with acrylamide in concentrations between 1000 and 6000 μM) or glycidamide (100-3000 μM). Blood aliquots in low melting agarose were put onto slides. After alkaline DNA unwinding and electrophoresis, DNA was

stained and the tail intensity of the cells was quantified by computer based microscopy [10, 18, 23]. Acrylamide did not induce significant DNA-damage at all concentrations tested (Figure 2a), whereas glycidamide induced significant DNA-damage, beginning at 300 µM after 4h (Figure 3a).

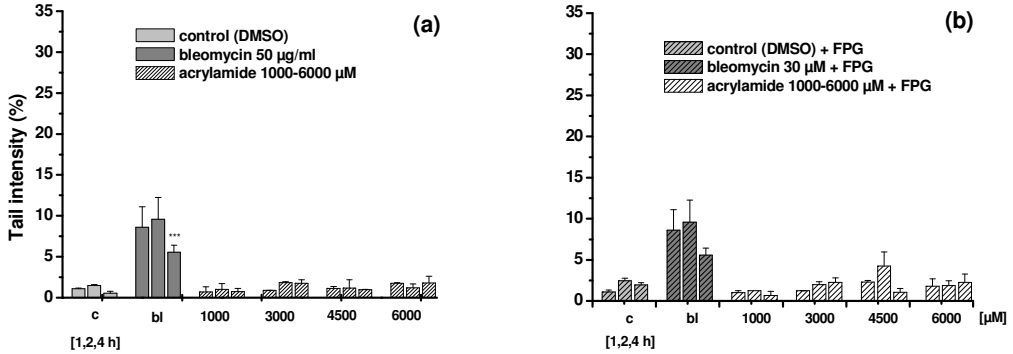

Figure 2: DNA damage in lymphocytes after incubation of human blood (1-4h) with acrylamide (1000-6000 µM) without (a) and with FPG-treatment (b) (c: DMSO as negative control; bl: bleomycin as positive control; ***p<0,001).

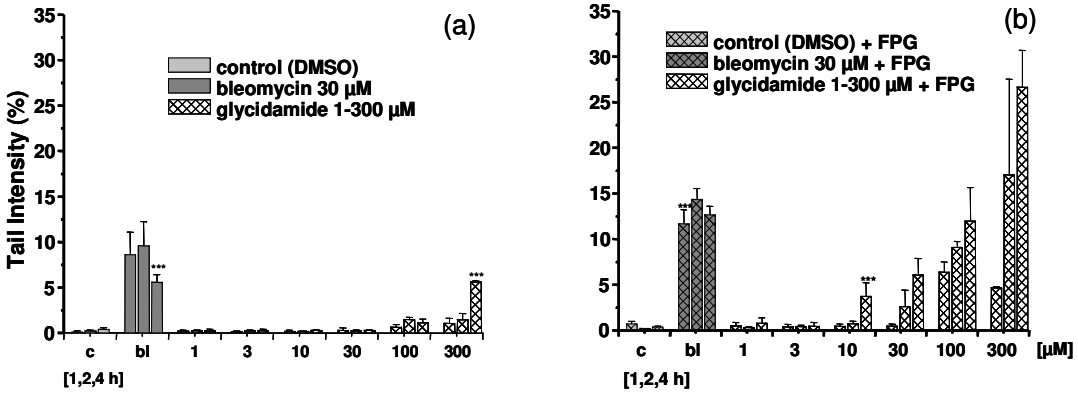

Figure 3: DNA damage in lymphocytes after incubation of human blood (1-4h) with glycidamide (1-300 µM) without (a) and with FPG-treatment (b) (c: DMSO as negative control; bl: bleomycin as positive control; ***p<0,001).

Acrylamide/glycidamide treated and agarose embedded lymphocytes were additionally incubated with the DNA repair enzyme formamidopyrimidine-glycosylase (FPG). FPG recognizes specific DNA damage like apurinic and apyrimidinic sites as well as oxidized and ring-opened purines that became detectable as additional strand breaks. With FPG, acrylamide remained inactive (Figure 2b) while glycidamide induced genotoxicity already at 10 µmolar concentration (Figure 3b). For comparison, under our test conditions, the potent carcinogens benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (ultimate carcinogenic metabolite of benzo[a]pyrene) and α-acetoxy-N-Nitroso-diethanolamine induced genotoxicity as detected by comet assay at similar concentrations.

3.2 Induction of chromosomal mutations

Induction of micronuclei (MN) and structural chromosomal aberrations

Micronuclei are DNA fragments resulting from DNA strandbreaks (clastogenic activity) or aberrantly distributed chromosomes (aneugenic activity). MN induction in lymphocytes was investigated by the cytokinesis block micronucleus assay. After incubation with acrylamide or glycidamide, blood was treated with phytohemagglutinine to stimulate mitosis and then incubated with acrylamide (500-5000 µM) or

glycidamide (50-1000 μM) for 23 hours. Lymphocytes were treated with cytochalasin B to block cytokinesis. After Giemsa staining, 1000 binucleated lymphocytes were investigated for MN-formation [7]. In addition, the potential of acrylamide and glycidamide to induce structural chromosomal aberrations, was also investigated. In both test systems, acrylamide and glycidamide were inactive up to the highest concentrations tested (acrylamide: 2500 μM; glycidamide: 1000 μM)

3.3 Induction of sisterchromatid exchange (SCE)

Enhanced SCE frequency is indicative for enhanced DNA repair following genotoxic damage. Treatment of blood with acrylamide had no effect on SCE frequency. In contrast with glycidamide (Figure 4), SCE frequency was significantly enhanced already at the lowest concentration tested (50 μM).

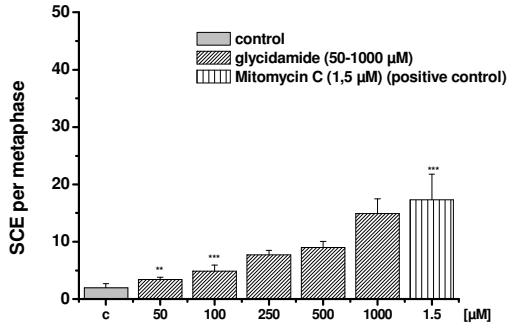

Figure 4: Induction of sisterchromatid-exchange in lymphocytes from human blood incubated with glycidamide (23h); MMC: positive control mitomycin C).

3.4 Induction of HPRT mutations in V79-mammalian cells

Determination of HPRT mutations in V79 cells (lung fibroblasts of the syrian hamster) is a well established test system to investigate mutagenic activity of xenobiotics (Figure 5). After 24 hours, the positive control N-methyl-N´-nitro-N-nitroso-guanidine (MNNG) induced hprt mutations dose dependently. The lowest concentration tested (0.5 μM) was already positive. A concentration of 10 μM resulted in ~ 200 mutants per 10^6 cells. With acrylamide (100-10 000 μM) no significant induction of HPRT mutations was observed. In contrast, with glycidamide, significantly elevated mutation frequencies (MF) became detectable from 800 μM upwards.

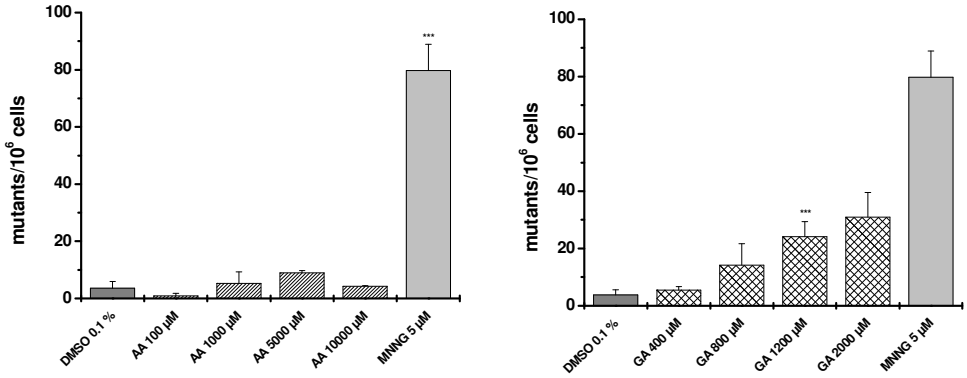

Figure 5: Induction of hprt mutations after incubation of V79 cells (24h) with acrylamide and glycidamide (DMSO as negative control; MNNG as positive control; ***p<0,001; values represent means and SD of 3 experiments).

3.5 Binding of acrylamide and glycidamide to blood proteins

Acrylamide and glycidamide are reactive towards nucleophilic regions in peptides and proteins (Figure 6). In studies measuring occupational exposure, adducts formed with the N-terminal valine in hemoglobin from erythrocytes have been exploited as a sensitive biomarker for quantification of the internal acrylamide or glycidamide doses in exposed persons [2-4, 9, 17, 25]. Hb-adducts might also be of promise to assess nutritional exposure to AA. The finding of acrylamide-Val background level in the range of 30 pmol/g Hb in otherwise unexposed persons [3] now corroborated by a wealth of analytical data on its occurrence in certain foods had been the pivotal observation leading to the assumption, that food might be a source of acrylamide [22].

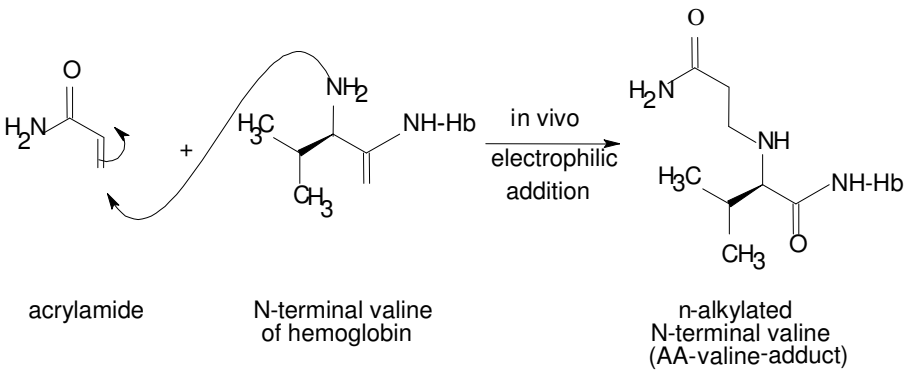

Figure 6: Formation of the acrylamide-adduct to hemoglobin at the N-terminal valine.

Blood was incubated with acrylamide in a concentration range of 0.3 µM-30 µM (1-6 hours) or glycidamide from 0.3 to 10 µM (4h). Hemoglobin was spun down and extracted as described elsewhere. For analysis of the adducts by gaschromatography/mass spectrometry, pentafluorphenyl thiohydantoin (PFPTH) derivatives of valine adducts were generated by treatment of hemoglobin with pentafluorphenyl isothiocyanate [3, 17, 25]. In the case of glycidamide, PFPTH-derivatives were further modified by acetonisation to improve GC/MS quantification. With acrylamide significant adduct formation has been observed at 0.3µM (4h), 3 µM (1h) and beyond (Figure 7). Adducts with glycidamide became significant at 3 µM after 4h incubation time (Figure 8). Glycidamide reacted more slowly with hemoglobin as compared to acrylamide.

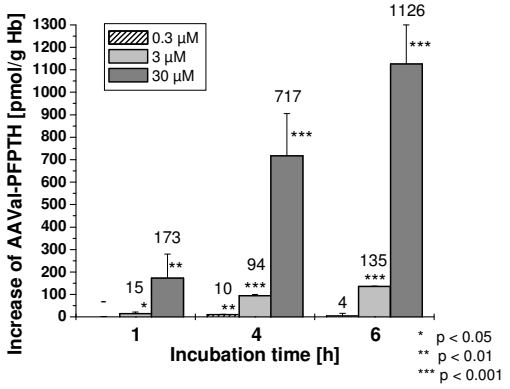

Figure 7: Concentration- and time dependent formation of acrylamide-valine-adducts in hemoglobin (background levels subtracted).

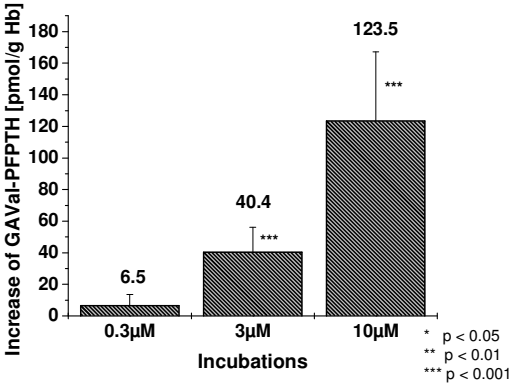

Figure 8: Concentration dependent formation of glycidamide-valine-adducts in hemoglobin after 4h (background levels subtracted).

3.6 Distribution and binding of acrylamide in human blood

Distribution and binding of acrylamide in human blood was investigated in blood incubated with ^{14}C-radiolabeled acrylamide. Blood components were isolated by fractionated centrifugation. After 4h overall about 30-40 % radioactivity was bound to erythrocytes or plasma proteins. Whether the soluble radioactivity found in plasma is ascribable to unreacted acrylamide and/or to acrylamide reaction products with low molecular weight blood compounds needs further investigation.

4. Summary

Concentration dependent induction of genotoxic effects by acrylamide and its metabolite glycidamide in human blood as a model system was studied. Significant binding of acrylamide and glycidamide to the N-terminal valine in hemoglobin was observed for acrylamide already at 0.3μM (4 h incubation time) and for glycidamide at 3μM (4h). Measurement of total binding of ^{14}C radiolabelled acrylamide to human blood compartments showed after 4 hours a total binding of the radiolabel to erythrocytes and plasma proteins of about 30-40%.

Acrylamide did not show genotoxic activity in blood cells under our test conditions. Glycidamide was genotoxic and mutagenic in V79-cells at 800 μM. In human blood, DNA damage became detectable at 300 μM glycidamide. After additional treatment with the DNA repair enzyme FPG, genotoxicity became significant at 10 μM glycidamide. Under these test conditions, benzo[a]pyrene-7,8-dihydrodiol-epoxide and α-acetoxy-N-Nitroso-diethanolamine were active in the same concentration range. Glycidamide also was found to induce sister chromatid-exchange at the lowest glycidamide concentration tested (50 μM).

5. Conclusion

The results of the study show that sensitive biomarkers for exposure to acrylamide are available, becoming significantly positive under our experimental conditions at blood concentrations as low as 300 nM. The study also shows clearly that AA itself is not genotoxic in human blood compartments even at very high concentrations. Up to about 35% of a given concentration of AA in the blood will be consumed by reaction with noncritical components. Whether the soluble radioactivity found in plasma is ascribable to unreacted acrylamide and/or to acrylamide reaction products with low molecular weight blood compounds needs further investigation.

The situation is different for glycidamide, the ultimate genotoxic metabolite of acrylamide. For glycidamide, reaction with hemoglobin became detectable at 3 μM, thus reflecting its lower reactivity to hemoglobin compared to acrylamide. Genotoxicity was detectable at 10 μM as lowest concentration, as evidenced by the comet assay. Under our test conditions in human blood the comet assay was the biomarker for genotoxicity with the highest sensitivity. Blood concentrations resulting from dietary acrylamide uptake are highly improbable to exceed the nanomolar concentration range. This range can be covered by measuring the most sensitive biomarker for exposure, the acrylamide-N-terminal valine-adduct of hemoglobin. Hb-adduct levels in otherwise unexposed persons measured by our group appear to reflect such nanomolar blood concentrations. However, at these concentrations, we were unable to detect genotoxic effects in lymphocyte DNA by the most sensitive technique in our hands, the comet assay.

The comet assay with FPG-treatment is a widely accepted high sensitivity tool for measuring genotoxicity. However there is a need for more sensitive biomarkers of genotoxicity to finally achieve biomarker-guided risk assessment of acrylamide exposure from food. An intrinsic limitation of the here described experimental approach is the maximum time period applicable to ex-vivo exposure of blood. This obviously does not reflect pharmacokinetics after dietary uptake of acrylamide under conditions of potential long term exposure.

With these limitations in mind, this concentration/effect study still indicates that acrylamide in blood is not relevant as a genotoxic agent by itself. Genotoxic effects of glycidamide become detectable at concentrations about 3-fold higher than those inducing significant Hb adduct formation.

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Approaches for industrial implementation of project results

As stated in the introduction, the resulting synergies of the related research institutes including analytical and toxicological data are used to generate substantial proposals for reducing acrylamide in cereal and potato products.

All opportunities for acrylamide reduction in cereal and potato products are related to the following general approaches:

- control of precursor situation in raw materials and intermediate products including recipe adaptations
- special pre-treatment of raw materials or intermediates
- adaptation of processes with respect to heat transfer and moisture control
- development of new procedures or equipment for food manufacturing.

These more general approaches can be transformed with respect to **deep-fried products including potato products** where the following opportunities were tested successfully in laboratory scale to reduce or control acrylamide formation:

Raw material and recipe:

- Optimal use of resources with respect to minimising content of reducing sugars in potato tubers
- Continuous water supply supported by irrigation techniques to minimize drought stress during farming; optimised nutrient supply in spring with a decreased availability before lifting
- Minimization of mechanical impact during lifting and transport during harvest and storage; optimum regulation of temperature, humidity and carbondioxide to prevent sugar enrichment
- Control with optimised test procedures (e.g. under-water balance; near infrared technique, frying test).

Pre-treatment of raw material of intermediates:

- Coating of par-fried French fries before finishing to reduce the surface pH-value or increase salt concentration in the product surface (instead of salting after frying)
- Pre-drying of French fries before finishing.

Process:

- Removal of solids within blanching medium to prevent enrichments and reduced efficiency of blanching
- Optimisation of temperature profiles; temperature gradient; adaptation of heat treatment to the raw material used
- Additives to frying fat to control reaction processes at product surface, e.g. amino acid glutamine for a faster colour development at reduced acrylamide contents.

Equipment and measurement techniques:

- Special fryers for potato food (atmospheric, pressure, and vacuum) with optimised control and extended measurement facilities
- Small scale fryers for restaurants, caterers, and domestic sector with improved temperature control facilities or possibilities for an improved control of frying time based on raw material properties
- Opto-electronic sorting for removal of dark coloured products
- Education campaigns for consumers to change the overall thermal load of potato food preparation.

In a similar way the following opportunities for **baked and extruded cereal products** which were tested successfully in laboratory scale to reduce or control acrylamide formation can be described:

Raw material and recipe:

- Avoiding higher contents of reducing sugars, e.g. replacement of fructose in diet products by other sweeteners; application of flour with low free aspargine content
- Addition of salt, citric acid, ascorbic acid or glycerol may reduce the acrylamide contents in baked goods and extruded products
- Avoiding of additives as thickeners, phosphates and also rework.

Process:

- Application of lower temperatures and higher moistures during extrusion of breakfast cereals
- Use of single screw extruders instead of twin screw extruders to lower thermal and mechanical energy input
- Short-time roasting of cornflakes at higher temperatures instead of longer roasting at lower temperatures
- Post-treatment of gingerbread with product moisture of app. 18-20 %.

Equipment and measurement techniques:

- Control of crust temperature and moisture and control of oven climate is recommended during baking
- Reduction of energy input realised by two-step baking processes using microwave energy
- Possibilities for control and influence product moisture in the extruder
- Development of short time roasting processes for cornflakes.

However, it should be pointed out that all these opportunities have to be adapted to each process, formulation and raw material to enable the manufacturing of products both having the reduced acrylamide contents and the desired and expected quality for the consumer. The latter is the main condition for a successful marketing of these products. Due to the well-known complexity of foodstuffs and the biological variations of raw materials, in some cases unpredictable interactions of food ingredients may result in reverse effects with respect to acrylamide reduction, e.g. no effect or even an increase of acrylamide contents. Often a multiple approach with slight modifications of different parameters (product, process, equipment) can be preferred.

Summary

As stated in the introduction to the report, the integrated approach for the research project including chemical-analytical research, toxicological and technological investigations resulted in scientific synergies which contributed to many opportunities for reduction of acrylamide in the related foodstuffs. All these opportunities were evaluated with respect to the final product quality which has to be maintained at a level the consumer expect. Or, any modifications in recipes and technologies which result in lower product quality, e.g. less browning due to shorter frying processes, will not be accepted by the market and, therefore, have hardly a chance to be implemented in the industrial production.

The main scientific-technical results of the project can be summarised as follows:

Methods with new sample preparations and derivatisation procedures for the determination of acrylamide were developed and applied successfully in international proficiency tests. Especially a method using LC/MS-MS together with an optimised sample workup can be recommended for difficult food matrices like coffee or cocoa. 3-Aminopropionamide could be identified and quantified for the first time in potatoes and even in foods, respectively, showing acrylamide yields upon heating which are by far the highest ever reported for a precursor of acrylamide in the literature. On the one hand, 3-aminopropionamide is formed enzymatically, e.g. during storage of potatoes, on the other hand it can also generated by a thermal formation pathway. This precursor is able to react to acrylamide without present reducing sugars. Therefore, this formation pathway may contribute to a better understanding of acrylamide findings in foods which can not be explained with the well-known precursors reducing sugars and free amino acids.

Toxicological investigations concerned the effects of acrylamide exposure for the consumer. Studies in human blood as model confirmed that acrylamide itself is not genotoxic. However, glycidamide generated metabolically from acrylamide shows genotoxic effects in the system 'Comet assay' at a blood concentrations of 10 µM. Other known carcinogens show similar concentration response relationships under the used conditions, indicating that acrylamide remains a health concerning substance due to its metabolite glycidamide. Further investigations are necessary for a sustainable risk evaluation of acrylamide exposures considering acrylamide doses approaching those from dietary acrylamide uptake.

The technological approach of the project is subdivided into three topics: cereal products, potato products and influence of process and equipment.

Acrylamide formation in carbohydrate rich foods could be characterised with respect to process parameters and raw material properties for traditional manufacturing technologies. This was carried out for cereal products (baking goods and extruded products) and for potato products (French fries and crisps). Some of these interactions could be quantified to enable an improved control of deep-frying and baking processes with respect to acrylamide formation. Additionally, the influence of recipe components and their pre-treatment could be verified or excluded. The influence of the heating medium fat including suitable additives and the plant concepts were characterised for the frying process. Based on these new findings minimisation strategies for existing machinery and processes were proposed and opportunities for new processes were developed.

The results illustrate the complexity of the acrylamide problem in food which is valid both for the industrial food manufacturing and for preparation of foods in household. This complexcity also implies that each process has to be verified and adapted to achieve high quality products with reduced acrylamide contents.

It could be summarised that the immediate initiative of the German food industry and the integrated approach realised by this cooperation project provided very positve results with respect to product quality and consumer protection.

Zusammenfassung

Wie aus dem vorliegenden Bericht deutlich wird, ergaben sich aus dem integrierten Ansatz für dieses Forschungsprojekt durch die Vernetzung von chemisch-analytischer, toxikologischer und technologischer Forschung erhebliche wissenschaftliche Synergien, auf deren Grundlage verschiedene Möglichkeiten für die Reduzierung der Acrylamidgehalte in den betroffenen Lebensmitteln erarbeitet wurden. Alle diese Ansätze wurden bezüglich der Erhaltung der vom Verbraucher erwarteten Qualität durch die vorgeschlagenen Maßnahmen im Labor- bzw. Technikumsmaßstab überprüft. Veränderungen an Rezepturen oder Prozessen, die zu Qualitätsbeeinträchtigungen führen könnten, z. B. weniger Bräunung durch kürzeres Frittieren, werden vom Markt nicht akzeptiert und haben daher kaum eine Chance für eine schnelle Überführung in die industrielle Produktion.

Die wesentlichen wissenschaftlich-technischen Ergebnisse aus den einzelnen Teilprojekten können wie folgt zusammengefasst werden:

Für die sichere und einfachere Analytik der Acrylamidgehalte in Lebensmitteln wurden Methoden mit neuen Probenaufarbeitungen und Derivatisierungen entwickelt und bei internationalen Ringtests erfolgreich angewandt. Insbesondere die Methode auf Basis LC/MS-MS mit optimierter Probenvorbereitung kann für schwierige Matrizes, z. B. Kaffee oder Kakao, empfohlen werden. 3-Aminopropionamid konnte zum ersten Mal in Kartoffeln bzw. generell in Lebensmitteln identifiziert und quantifiziert werden. Diese Verbindung zeigt bei thermischer Behandlung die mit Abstand höchsten Ausbeuten an Acrylamid, die jemals für einen Acrylamid-Prekursor in der Literatur beschrieben wurden. Einerseits wird 3-Aminopropionamid enzymatisch gebildet, zum Beispiel während der Kartoffellagerung, andererseits kann es ebenfalls auf thermischem Weg generiert werden. Dieser Prekursor kann auch ohne Vorhandensein von reduzierenden Zuckern zu Acrylamid weiter reagieren. Daher kann ein solcher Bildungsweg bei Lebensmitteln in Betracht kommen, bei denen die bekannten Precursoren reduzierende Zucker und freie Aminosäuren nicht zur Erklärung der gebildeten Acrylamidgehalte ausreichen.

Die toxikologischen Studien betrafen die Auswirkungen der Exposition der Verbraucher mit Acrylamid aus Lebensmitteln. Anhand von Studien im Modell Humanblut wurde gezeigt, dass Acrylamid selbst keine Genotoxizität aufweist. Hingegen lassen sich bei dem Metaboliten Glycidamid, der im Körper aus Acrylamid gebildet wird, in vitro im System „Comet Assay" ab Blutkonzentrationen von 10 µM genotoxische Wirkungen nachweisen. Vergleichbare Konzentrations-Wirkungs-Beziehungen zeigen auch andere bekannte Kanzerogene, so dass das Acrylamid über den Metaboliten Glycidamid eine Verbindung mit möglicherweise gesundheitlicher Relevanz bleibt. Für eine tragfähige Risikobewertung der Acrylamidexposition sind weitere Untersuchungen notwendig, wobei die Dosis/Konzentrationsbereiche aus den nach Verzehr Acrylamid-haltiger Lebensmittel zu erwartenden Aufnahmemengen abzuleiten sind.

Die technologische Forschung des Projekts war in drei Teile gegliedert: Getreideprodukte, Kartoffelprodukte sowie der Einfluss von Verfahren und Anlage.

Für die Verfahren zur Herstellung und Verarbeitung kohlenhydratreicher Lebensmittel konnte die Acrylamidbildung in den herkömmlichen Produktionstechnologien in Abhängigkeit von Prozessparametern und Rohstoffeigenschaften charakterisiert werden. Das betrifft sowohl Getreideerzeugnisse (Backwaren und extrudierte Produkte) als auch Kartoffelerzeugnisse (Pommes frites und Kartoffelchips). Teilweise konnten die Zusammenhänge auch quantifiziert werden, so dass eine bessere Steuerung z. B. von Back- und Frittierprozessen unter dem Aspekt Acrylamidminimierung möglich wird. Darüber hinaus wurde der Einfluss von Rezepturkomponenten und deren Vorbehandlung auf die Acrylamidgehalte im Fertigprodukt nachgewiesen bzw. auch ausgeschlossen. Der Einfluss des Erhitzungsmediums Fett einschließlich geeigneter Zusätze und der Anlagenkonzeption wurde anhand des Frittierprozesses charakterisiert. Basierend auf diesen Ergebnissen wurden Vorschläge für Minimierungsstrategien für vorhandene Anlagen und Prozesse sowie Möglichkeiten zur Neugestaltung von Verfahren erarbeitet.

Die Ergebnisse verdeutlichen aber auch die Komplexität des Acrylamidproblems, das sowohl die industrielle Lebensmittelherstellung als auch die Zubereitung im Haushalt betrifft. Diese Komplexität bedingt auch, dass jeder Prozess einzeln geprüft und angepasst werden muss, um bei reduzierten Acrylamidgehalten der Produkte die erforderliche Qualität zu erreichen.

Zusammenfassend bleibt festzustellen, dass durch die schnelle Reaktion der deutschen Lebensmittelindustrie auf das Problem Acrylamid und den vernetzten Forschungsansatz im Kooperationsprojekt sehr positive Ergebnisse bezüglich der Verbesserung des Verbraucherschutzes und der Erhaltung der Produktqualität erreicht werden konnten.

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