# Development of new Procedures for heated Potato and Cereal Products with reduced Acrylamide Contents

# Entwicklung von neuen Verfahren für Kartoffel- und Getreideerzeugnisse mit reduzierten Gehalten an Acrylamid

BLL

FEI
FORSCHUNGSKREIS
DER ERNÄHRUNGSINDUSTRIE E.V.

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 209 ZBG

# Development and Application of new Processing Procedures for Potato and Cereal Products having reduced Contents of Acrylamide and its Following Products

## Entwicklung und Anwendung neuer Verfahrensabläufe in Produktionsanlagen für Kartoffel- und Getreideerzeugnisse mit reduzierten Gehalten an Acrylamid und dessen Folgeprodukten

BLL

FEI
FORSCHUNGSKREIS
DER ERNÄHRUNGSINDUSTRIE E.V.

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

AiF 209 ZBG

Herausgeber:

Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL)
Godesberger Allee 142-148, 53175 Bonn

Forschungskreis der Ernährungsindustrie e. V. (FEI)
Godesberger Allee 142-148, 53175 Bonn



Druck: DCM-Druck Center Meckenheim GmbH & Co. KG

ISBN: 978-3-9818611-0-3

Erstauflage 2008

# Inhaltsverzeichnis

# Introduction

Heated potato and cereal products are basic components of daily nutrition in Germany and Europe due to their nutritional value and their unique sensory properties. Due to the recurrent detection of health relevant substances in this kind of food, for example acrylamide in April 2002, food manufacturers have been forced into an increased responsibility to take all necessary actions to substantially minimise the potential risks which may result from their products.

As early as in 2002 the German food industry initiated via its associations, the German Federation of Food Law and Food Science (BLL) and the Research Association of the German Food Industry (FEI), a cooperative research project which was sponsored by the former Ministry of Economics and Labour (BMWA) via AiF from 2003 to 2005 (Project No. AiF 108 ZBG). Within the frame of this previous research project involving five research institutes extensive technological, analytical, and toxicological investigations were carried out regarding the formation of acrylamide in foods and its effects in model systems. Especially relationships between raw material properties (precursors), process conditions and the resulting acrylamide contents and product qualities were detected and validated in laboratory scale.

However, as a result of the extensive investigations novel tasks in this field emerged which extended the objectives of this previous project. These tasks covered the influence of formation pathways and reactions of acrylamide and glycidamide in foods, the further development of baking technology (optimized split baking procedures) and the manufacturing procedures for recombined potato products (better opportunities for precursor control). A special pre-treatment for French fries with lower acrylamide contents in the final product and the possibility of minimizing acrylamide formation during frying in households and restaurants were additional tasks in this field.

Systematic scientific investigations on these novel tasks were required to enable the industrial implementation of the new minimizing approaches with respect to acrylamide and the economic utilization of the approaches as well as to obtain more insight about chemical processes during heating of foods and their health relevance.

Therefore, a second project was successfully initiated by BLL and FEI involving the manufacturers of potato and cereal products as well as the manufacturers of equipment for potato processing and baking technology. This project was sponsored by the German Ministry of Economics and Technology (BMWi) via AiF in the frame of specific support for future technologies for SME (ZUTECH) from 2006 to 2008 (Project No. AiF 209 ZBG).

According to the broad scientific approach of this second research project four institutes with different and complementary scientific competences were involved:

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

The aim of the project was the supply of all data required for the development of new plants for manufacturing high quality potato and cereal products with reduced and defined acrylamide contents (DIL and ILU) according to the technological tasks explained above. Additionally, data about the bioavailability of acrylamide formed in foods were required considering the product matrix (TU KL) and formation pathways and further reactions of these substances in foods (DFA).

This consolidated project report contains the results of the investigations which were generated in the frame of the second project. Therefore, the report represents the successful continuation of the integrated and networked approach on this sophisticated problem as illustrated in the previous publication of BLL regarding acrylamide minimisation in foods (BLL, FEI (2005) Development of New Technologies to Minimize Acrylamide in Food, http://www.bll.de/download/themen/kontaminanten/acrylamid/acrylamid.pdf).

# Einleitung

Erhitzte Kartoffel- und Getreideerzeugnisse sind aufgrund ihres Nährwertes und ihrer einzigartigen sensorischen Eigenschaften grundlegende Bestandteile der Ernährung in Deutschland und Europa. Durch den Nachweis von gesundheitlich relevanten Verbindungen in dieser Produktgruppe, wie z. B. Acrylamid im April des Jahres 2002, sind die Lebensmittelhersteller aber nach wie vor in der Verantwortung, alles zu unternehmen, damit ein mögliches Risiko durch ihre Produkte langfristig minimiert wird.

Die deutsche Lebensmittelindustrie initiierte daher bereits im Jahre 2002 über den Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL) und den Forschungskreis der Ernährungsindustrie e.V. (FEI) ein entsprechendes Kooperationsforschungsprojekt, das im Zeitraum von 2003 bis 2005 durch das damalige Bundesministerium für Wirtschaft und Arbeit (BMWA) und die Arbeitsgemeinschaft industrieller Forschungsvereinigungen (AiF) gefördert wurde (Projekt-Nr. AiF 108 ZBG). Im Rahmen dieses Forschungsprojektes, an dem fünf Forschungsstellen beteiligt waren, wurden technologische, analytische und toxikologische Untersuchungen zur Entstehung von Acrylamid im Lebensmittel und dessen Wirkung durchgeführt, wobei insbesondere Zusammenhänge zwischen Rohstoffeigenschaften (Precursoren), Prozessbedingungen sowie dem resultierenden Acrylamidgehalt und der Produktqualität ermittelt und unter Laborbedingungen validiert wurden.

Aus den Untersuchungen resultierten jedoch auch weitergehende Aufgabenstellungen, die über die Ziele dieses ersten Projektes hinausgingen. Das betraf u. a. die Beeinflussung von Bildungswegen und die weiteren Reaktionen des Acrylamids und Glycidamids, die Weiterentwicklung der Backtechnik (optimierte gesplittete Backverfahren), die Herstellungsverfahren für rekombinierte Kartoffelprodukte (erweiterte Precursorbeeinflussung) und für vorfrittierte Pommes frites mit reduzierten Acrylamidgehalten im Fertigprodukt (Vorbehandlung) sowie die Möglichkeit der Minimierung der Acrylamidbildung beim Frittieren in Haushalt und Gastronomie. Hinzu kamen Fragen der Bioverfügbarkeit des im Lebensmittel gebildeten Acrylamids im Vergleich zu den bisher vorliegenden toxikologischen Untersuchungen für die Zugabe im Trinkwasser.

Die systematische wissenschaftliche Bearbeitung dieser neuen Aufgabenstellung war für die anstehende industrielle Umsetzung der Minimierungsansätze bezüglich Acrylamid und damit deren Verwertung in den einzelnen Unternehmen zwingend erforderlich. Zudem sollte ein zusätzlicher Beitrag für das verbesserte Verständnis der Vorgänge beim Erhitzen dieser Produkte und deren gesundheitliche Relevanz geleitet werden. Daher wurde wiederum über den BLL und den FEI ein Nachfolgeprojekt unter Mitwirkung der Lebensmittelindustrie, insbesondere der Hersteller von Kartoffel- und Getreideerzeugnissen sowie dem Anlagenbau (Kartoffelverarbeitungsanlagen und Backofenbau), erfolgreich auf den Weg gebracht. Dieses Nachfolgeprojekt wurde im Zeitraum von 2006 bis 2008 im Rahmen der speziellen Förderung von Zukunftstechnologien für KMU (ZUTECH) durch das Bundesministerium für Wirtschaft und Technologie (BMWi) via AiF gefördert (Projekt-Nr. AiF 209 ZBG).

Bedingt durch den breiten wissenschaftlichen Ansatz des Nachfolgeprojektes waren wiederum Forschungsstellen unterschiedlicher Kompetenz an dem Projekt beteiligt:

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

Ziel des Projektes war die Bereitstellung aller notwendigen Daten für die Entwicklung von Anlagen auf der Basis der Ergebnisse aus dem Vorläuferprojekt zur Herstellung von qualitativ hochwertigen Kartoffel- und Getreideerzeugnissen mit reduzierten und definierten Gehalten an Acrylamid entsprechend den oben angeführten Aufgabenstellungen (DIL und ILU). Hinzu kam die Gewinnung von Daten zur Bioverfügbarkeit des im Lebensmittel gebildeten Acrylamids in Abhängigkeit von der Produktmatrix (TU LK) sowie zu Bildungswegen und Weiterreaktionen im Lebensmittel (DFA).

Dieser Bericht enthält die Zusammenstellung der Ergebnisse von allen beteiligten Forschungsstellen, die im Rahmen dieses Folgeprojektes erarbeitet wurden, und stellt damit die erfolgreiche Weiterführung der bereits in der Vorgängerpublikation des BLL(BLL, FEI (2005) Development of New Technologies to Minimize Acrylamide in Food, http://www.bll.de/download/themen/kontaminanten/acrylamid/acrylamid.pdf) dargestellten integrierten und vernetzten Herangehensweise an diese sehr komplexe Problematik dar.

# Mechanisms of Acrylamide Formation and Quantification of the Acrylamide Reaction Products Glycidamide and Cystein-Acrylamide in Food

Deutsche Forschungsanstalt für Lebensmittelchemie (DFA), Garching

Michael Granvogl, Larissa Latzer, Susanne Gradl, Peter Köhler, Peter Schieberle

## 1. Introduction

### 1.1 Mechanisms of Acrylamide Formation

Many papers are available on the mechanism of acrylamide (AA) formation [1-5]. It is generally accepted that free asparagine is the most important precursor, however, the pathway of acrylamide formation is still not fully understood. In particular, the role of 3-aminopropionamide (3-APA), the biogenic amine of asparagine, remains unclear. Up to now there is only evidence of the biochemical formation of this compound [6]. However, a direct thermal formation by decarboxylation/deamidation of asparagine as shown in Figure 1 could also be possible, but there is no experimental proof whether this pathway occurs during heat-processing of foods.

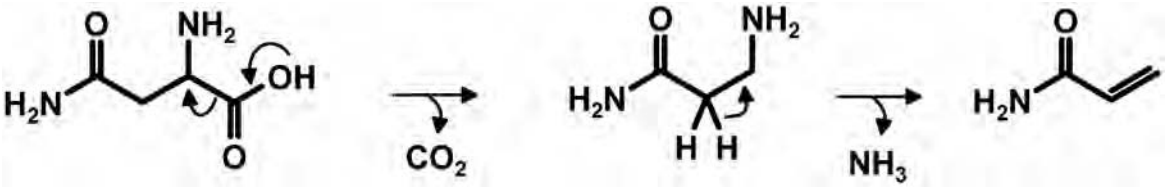


**Figure 1: Formation of acrylamide by direct thermal decarboxylation/deamidation of asparagine via 3-aminopropionamide.**

AA formation is also affected by environmental factors such as climate, soil, and fertilisation. In the beginning of this project, there was no information that S-deficiency affects the acrylamide formation potential of flours [7,8]. Furthermore, no information was available whether S-deficient flours can bee used in food processing because no quality parameters of these flours had been determined.

### 1.2 Reactions of Acrylamide and Glycidamide in Food

Due to the double bond in the structure, AA is a quite reactive compound. In particular, nucleophils such as compounds bearing a thiol or amino group can be added to the double bond. This reaction, using 2-mercaptobenzoic acid as the nucleophil, has been used to establish an analytical method for AA quantification [9]. The same type of reaction might also occur in food leading to covalently linked AA adducts and thus to a decrease of the concentration of free AA in food. However, little information is available on the reactions of AA with food constituents.

Furthermore it has been shown that acrylamide is converted into glycidamide (GA) *in vivo* by cytochrome P450 catalysis [10]. There is no information on whether this reaction already occurs in food, too. Oxidising conditions or the presence of peroxides could favour the conversion of AA into GA in food. However, a prerequisite to study GA formation is a method to identify and quantify GA in model systems as well as in food. In the beginning of the project no method was available for this purpose.

### 1.3 Aims

The aims of this part of the study were as follows:

- To investigate the role of the *Strecker* reaction in the formation of 3-APA and AA,
- to study the effects of sulphur fertilisation during wheat growth on AA formation potential of flours,
- to develop an analytical method for the quantitative determination of GA in model systems and in food
- to elucidate the conversion of AA into GA by fatty acid hydroperoxides in model systems and in food, as well as
- to study AA reactions with amino acids and peptides (adduct formation) in food.

## 2. Results and Discussion

### 2.1 Studies on the role of the *Strecker* reaction in the formation of 3-aminopropionamide and acrylamide

On the basis of the recent findings that "biogenic amines" can also be formed during thermal food processing from their parent amino acids in a *Strecker*-type reaction [11], the formation of 3-APA, the biogenic amine of asparagine, was investigated in model systems as well as in thermally processed Gouda cheese. The results of model studies revealed that, besides AA, 3-APA was also formed in amounts of 0.1-0.4 mol% when asparagine (Asn) was reacted in the presence of either glucose or 2-oxopropionic acid. Results of a second series of model experiments in which [$^{13}C_4{}^{15}N_2$]-asparagine ([$^{13}C_4{}^{15}N_2$]-Asn) and unlabeled 3-APA were reacted together in the presence of glucose revealed a >12-fold higher efficacy of 3-APA in AA generation as compared to Asn. Both [$^{13}C_3{}^{15}N_2$]-3-aminopropionamide ([$^{13}C_3{}^{15}N_2$]-3-APA) and [$^{13}C_3{}^{15}N_1$]-acrylamide ([$^{13}C_3{}^{15}N_1$]-AA) were formed during [$^{13}C_4{}^{15}N_2$]-Asn degradation in a ratio of about 1:4, supporting the idea that 3-APA is a transient intermediate in acrylamide formation (data not shown). In this study, 3-APA was also identified in Gouda cheese (Table 1). Although the fresh cheese contained low amounts of 3-APA, its concentration was much increased to >1300 µg/kg after thermal processing. In isotopically labelled studies, performed by administering [$^{13}C_4{}^{15}N_2$]-Asn to the cheese in a similar ratio of the "natural" Asn concentration, nearly the same ratios of unlabelled/labelled 3-APA as well as of unlabelled/labelled AA were determined (Table 2). Thus 3-APA could be confirmed as a transient intermediate of AA formation during food processing as outlined in Figure 2.

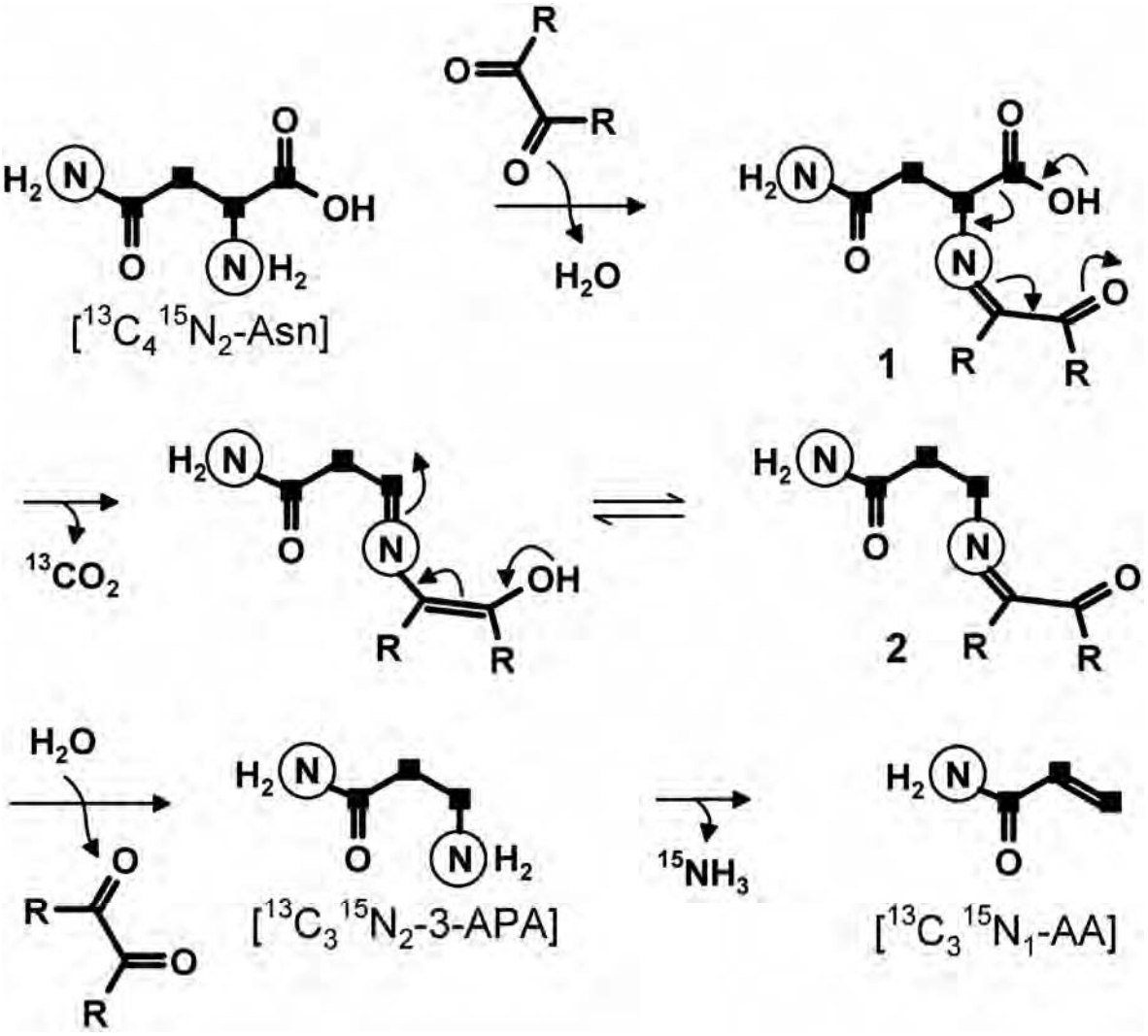


**Figure 2: Reaction pathway of the formation of acrylamide (AA) in food via the transient intermediate 3-aminopropionamide (3-APA) by a *Strecker*-type reaction of asparagine (Asn) with an α-dicarbony compound.■: [$^{13}$C]-label; Ⓝ: [$^{15}$N]-label.**

**Table 1: Concentrations of asparagine, 3-aminopropionamide, and acrylamide in Gouda cheese before and after heat treatment**

| Sample | Asparagine | | 3-Aminopropionamide | | Acrylamide | |
|---|---|---|---|---|---|---|
| | Conc. (mg/kg) | RSD (%)[a] | Conc. (µg/kg) | RSD (%)[a] | Conc. (µg/kg) | RSD (%)[a] |
| unheated | 1790 | 5.2 | 4.4 | 3.6 | 10.8 | 9.1 |
| heated | 861 | 6.1 | 1324 | 2.7 | 189 | 8.9 |

[a] Relative standard deviation of triplicates.

**Table 2: Concentrations of 3-aminopropionamide (3-APA), labelled 3-aminopropionamide ([$^{13}C_3{}^{15}N_2$]-3-APA), acrylamide (AA), and labelled acrylamide ([$^{13}C_3{}^{15}N_1$]-AA) in heated Gouda cheese administered with [$^{13}C_4{}^{15}N_2$]-asparagine[a] (860 mg/kg)**

| Reaction product | Conc. (µg/kg)[b] | Conc. (mmol/mol Asn or [$^{13}C_4{}^{15}N_2$]-Asn) | RSD (%)[c] |
|---|---|---|---|
| [$^{13}C_3{}^{15}N_2$]-3-APA | 2106 | 4.12 | 3.6 |
| [$^{13}C_3{}^{15}N_1$]-AA | 90 | 0.22 | 12.4 |
| 3-APA | 7165 | 6.00 | 2.0 |
| AA | 420 | 0.44 | 12.2 |

[a] Ratio Asn/[13C415N2]-Asn: 3:1.

[b] Based on fresh weight.

[c] Relative standard deviation of triplicates.

3-APA was also quantified in cocoa masses, cocoa beans, coffee, and cereal products. Cocoa masses contained >3000 µg 3-APA/kg, but varied significantly in its concentration depending on the respective sample. Therefore a good possibility is given for companies to minimise AA by a selective choice of the right raw material. For the quantification of AA in cocoa and coffee, an improved isolation procedure using charcoal was developed. In various samples of unroasted and roasted cocoa beans, the AA as well as the 3-APA concentrations were determined always showing a good correlation, which was much better than the correlation of the AA and the Asn concentrations. Experiments on authentic cocoa beans from Ghana and Sulawesi indicated that the thermal generation of 3-APA during roasting was much more pronounced as compared to its biochemical formation (Table 3). By administering fermented cocoa beans with [$^{13}C_4{}^{15}N_2$]-Asn before roasting, 3-APA was again confirmed as key intermediate in AA formation during food processing (Table 4). Among the cereal products analysed, popcorn particularly contained quite high amounts of 3-APA, which were also well correlated with the AA concentration.

**Table 3: Influence of fermentation and roasting on the concentrations of asparagine (Asn), 3-aminopropionamide (3-APA), and acrylamide (AA) in cocoa beans from Ghana**

| Sample | Asn | | 3-APA | | AA | |
|---|---|---|---|---|---|---|
| | Conc. (mg/kg)[a] | RSD (%)[b] | Conc. (µg/kg)[a] | RSD (%)[b] | Conc. (µg/kg)[a] | RSD (%)[b] |
| Unfermented | 721 | 2.9 | 313 | 8.1 | 57 | 5.1 |
| 7 days fermented | 655 | 0.8 | 792 | 5.9 | 64 | 4.8 |
| Unfermented, roasted | 584 | 7.7 | 2078 | 3.1 | 223 | 8.4 |
| 7 days fermented, roasted | 472 | 4.8 | 3476 | 3.1 | 922 | 1.3 |

[a] In dry mass.

[b] Relative standard deviation of triplicates.

**Table 4: Comparison of the concentrations of 3-aminopropionamide (3-APA), [$^{13}C_3{}^{15}N_2$]-3-aminopropionamide ([$^{13}C_3{}^{15}N_2$]-3-APA), acrylamide (AA), and [$^{13}C_3{}^{15}N_1$]-acrylamide ([$^{13}C_3{}^{15}N_1$]-AA) formed during roasting of fermented, unroasted cocoa beans administered with 430 mg/kg of [$^{13}C_4{}^{15}N_2$]-asparagine[a]**

| | Sulawesi | | Ghana | |
|---|---|---|---|---|
| Compound | Conc. (µg/kg)[b] | RSD (%)[c] | Conc. (µg/kg)[b] | RSD (%)[c] |
| 3-APA | 1095.4 | 5.1 | 1682.8 | 0.7 |
| [$^{13}C_3{}^{15}N_2$]-3-APA | 660.2 | 7.1 | 650.5 | 5.0 |
| AA | 346.4 | 2.4 | 543.5 | 3.7 |
| [$^{13}C_3{}^{15}N_1$]-AA | 180.9 | 2.0 | 184.4 | 2.6 |

[a] The ratio of Asn to [$^{13}C_4{}^{15}N_2$]-Asn was 1:1 for the Sulawesian beans and 1.5:1 for the Ghanaan beans, respectively.

[b] In dry mass.

[c] Relative standard deviation of triplicates.

## 2.2 Effects of sulphur fertilisation during wheat growth on the acrylamide formation potential of flours

The German spring wheat cultivar 'Star' was grown in five pots (5 L) supplied with either 30 mg (= flour 1), 60 mg (= flour 2) or 90 mg (= flour 3) of sulphur (S; applied as an aqueous solution of $K_2SO_4$) as well as with a combination of 60 mg (= flour 4) or 90 mg (= flour 5) of S before sowings and an additional 60 mg of S during growing. The supply of nitrogen (N), phosphorus, potassium, and magnesium was optimal for normal development. The mature grains were milled into white flours with an ash content of 0.55 % in dry mass. Additionally, 15 commercial flours of wheat, spelt, and oats were obtained from a German manufacturer or were purchased in a local supermarket.

### Wheat flours

The total S content in the model wheat flours rose with increasing amounts of S administered to the soil (Table 5). Low S dosages (flours 1 and 2) also affected the total N content, but when amounts of ≥ 90 mg of S were applied, the N content remained constant. Wheat with an S content of less than 0.120 % is regarded as S deficient [12], and, as to be expected, S dosages < 90 mg/pot resulted in S depletion. The ratio of N to S, which is another indicator for S deficiency [12], indicated that only flour 5 was provided with an optimal amount of S (ratio of N/S ≤ 17). Except one sample, all commercial wheat flours had an S content > 0.120 %. On the basis of the N/S ratio three flours resulted in a value scarcely > 17 indicating a slight S deficiency in these flours.

In the model wheat flours a clear correlation was found between the S supply and free Asn, 3-APA, and AA. The lower the S supply the higher were the concentrations of free Asn (unheated flours) as well as 3-APA and AA (heated flours), respectively (Table5). This correlation was less pronounced in the commercial wheat flours. Except one whole meal flour, all commercial wheat flours produced AA concentrations in the range of the model flours 4 and 5, which were sufficiently supplied with S. The sample that produced the highest AA concentration also had the highest Asn and 3-APA amounts. The higher variability of the data of the commercial flours may result from the fact that the model flours were from the same wheat cultivar and were grown under identical environmental conditions whereas neither the cultivar nor the growth conditions of the commercial samples were known.

### Flours of Other Cereals

All commercial cereal flours had an S content > 0.130 % and an N/S ratio ≤ 15.7 indicating sufficient S supply. However, in particular the oatmeals were high in Asn and produced elevated concentrations of 3-APA and AA during heating, indicating that not only the S fertilisation but also the cereal species influence the AA formation potential of cereal flours. Furthermore, it can be assumed that the criteria of Randall et al. [12] for sufficient S supply of wheat are not valid for other cereal species. In contrast to the oatmeals, the spelt flours revealed the same results as the wheat flours pointing to the close botanical relationship between these two species.

**Table 5: Concentrations of nitrogen (N), sulphur (S), free asparagine (Asn) in unheated flours as well as 3-aminopropionamide (3-APA) and acrylamide (AA) in heated flours**

| Cereal flours | N (%)[a,b] | S (%)[a,b] | Ratio (N/S) | *Asn (mg/kg)*[c] | 3-APA (mg/kg)[c] | AA (µg/kg)[c] |
|---|---|---|---|---|---|---|
| Model wheat flours (S (mg/pot)) | | | | | | |
| 1 (30) | 2.09 | 0.066 | 31.7 | 5688 | 76.01 | 3124 |
| 2 (60) | 2.44 | 0.084 | 29.0 | 3920 | 39.58 | 1703 |
| 3 (90) | 2.75 | 0.128 | 21.5 | 426 | 4.84 | 460 |
| 4 (60 + 60) | 2.79 | 0.143 | 19.5 | 142 | 1.42 | 155 |
| 5 (90 + 60) | 2.74 | 0.158 | 17.3 | 35 | 0.38 | 94 |
| Commercial flours | | | | | | |
| Wheat 1 | 2.09 | 0.135 | 15.5 | 112 | 1.47 | 157 |
| Wheat 2 | 2.19 | 0.142 | 15.4 | 130 | 1.74 | 213 |
| Wheat 3 | 2.31 | 0.130 | 17.7 | 114 | 1.50 | 130 |
| Wheat 4 | 2.19 | 0.127 | 17.3 | 121 | 1.38 | 126 |
| Wheat 5 | 1.84 | 0.105 | 17.6 | 205 | 2.35 | 222 |
| Wheat 6 | 2.04 | 0.124 | 16.5 | 220 | 4.20 | 223 |
| Whole meal wheat 1 | 2.38 | 0.141 | 16.9 | 237 | 4.68 | 412 |
| Whole meal wheat 2 | 1.95 | 0.125 | 15.6 | 179 | 4.25 | 260 |
| Spelt 1 | 2.55 | 0.163 | 15.7 | 111 | 1.50 | 259 |
| Spelt 2 | 2.07 | 0.139 | 14.9 | 208 | 1.63 | 431 |
| Whole meal spelt 1 | 2.48 | 0.163 | 15.2 | 209 | 3.90 | 236 |
| Whole meal spelt 2 | 2.41 | 0.157 | 15.3 | 212 | 3.08 | 222 |
| Oats 1 | 2.43 | 0.185 | 13.1 | 441 | 7.20 | 708 |
| Oats 2 | 2.66 | 0.191 | 14.0 | 554 | 9.54 | 663 |
| Whole meal oats | 2.56 | 0.190 | 13.5 | 627 | 7.23 | 723 |

[a] Analyses were performed in duplicates

[b] Amount based on dry mass

[c] Analyses were performed in triplicates.

An S content of at least 0.130% and an N/S ratio < 20 in wheat flours is recommended for a low concentration of free Asn and, as a result, a low AA formation potential. However, besides a sufficient S supply, also the cultivar and the environmental conditions during growth seem to have a significant effect on the AA formation potential. This is even more important for flours of other cereal species, which show a high content of free Asn despite an apparently sufficient S supply, thus providing elevated amounts of 3-APA and AA during heat processing.

## 2.3 Development of an analytical method for the quantitative determination of glycidamide in model systems and in food

On the basis of a recently published method for the selective quantification of AA involving a derivatisation step with 2-mercaptobenzoic acid [9], a new stable isotope dilution assay for the quantification of GA using [$^{13}C_3$]-glycidamide ([$^{13}C_3$]-GA) as the internal standard was developed. The method should also allow the parallel quantification of AA and GA in food samples.

First, the derivatives were synthesised by reacting GA as well as [$^{13}C_3$]-GA singly with 2-mercaptobenzoic acid to obtain stable derivatives according to the reaction scheme illustrated in Figure 3. Derivatisation of GA resulted in two different reaction products. Their structures were confirmed by NMR and mass spectrometry.

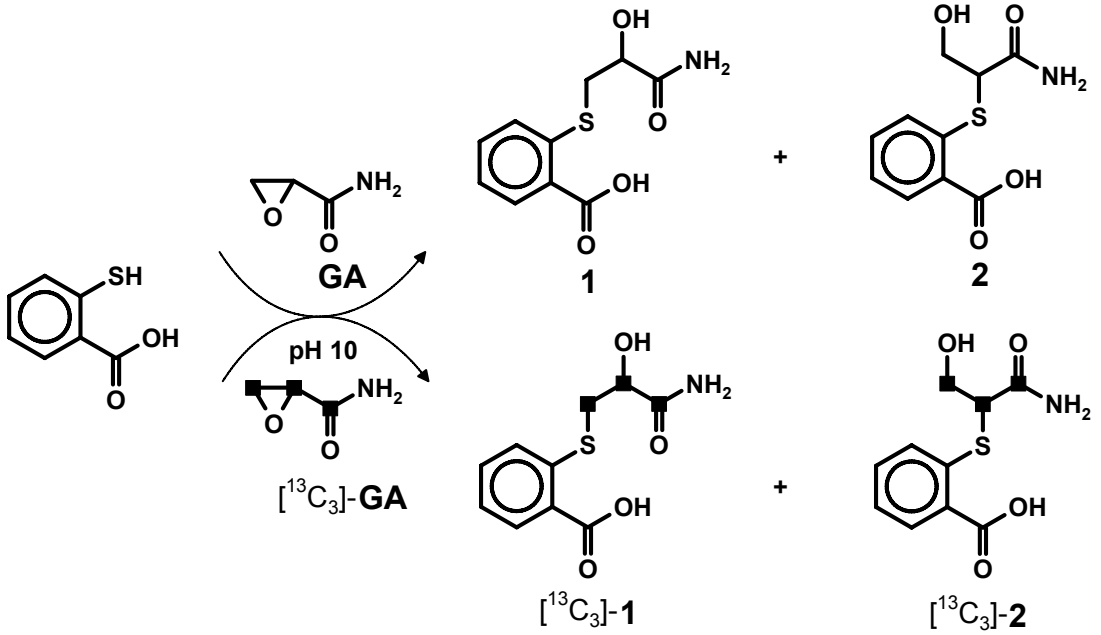


**Figure 3: Conversion of glycidamide (GA) and [$^{13}C_3$]-glycidamide ([$^{13}C_3$]-GA) into the thioethers 1, 2, [$^{13}C_3$]-1, and [$^{13}C_3$]-2, respectively, prior to LC-MS-MS analysis. ■ [$^{13}C$]-label.**

To prevent matrix interferences, and for increased selectivity, tandem mass spectrometry was used for unequivocal identification. The most abundant ion transitions were selected and used for quantification by LC-MS-MS in the SRM mode: *m/z* 242 to *m/z* 72 and *m/z* 242 to *m/z* 153 for 1 and 2 as well as *m/z* 245 to *m/z* 75 and *m/z* 245 to *m/z* 153 for [$^{13}C_3$]-**1** and [$^{13}C_3$]-**2**, respectively. The limit of quantitation (LoQ) for GA was estimated to be 0.001 µg/kg on the basis of a correlation between the intensity of the respective ion transitions and the background noise.

## 2.4 Conversion of acrylamide into glycidamide by fatty acid hydroperoxides in model systems and in food

### Model systems

Hydroperoxides are known reactants forming epoxides by oxidation of a double bond in a given compound. This epoxidation, known as *Prileschajew* reaction, has previously been shown to be involved in the formation of the intensely metallic smelling 4,5-epoxy-(E)-2-decenal from (E,E)-2,4-decadienal in the presence of 13-hydroperoxy-(E,Z)-9,11-octadecadienoate [13]. As indicated in Figure 4, the same reaction might generate GA from AA in the presence of lipid hydroperoxides.

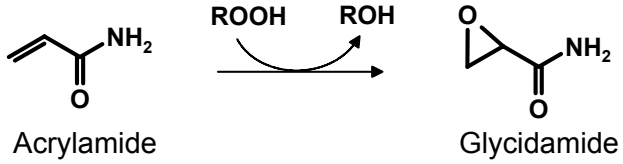


**Figure 4: Possible pathway leading to the generation of glycidamide by epoxidation of acrylamide.**

Linoleic acid hydroperoxides were synthesised by incubation of linoleic acid with soybean lipoxygenase type I. Defined mixtures of AA and linoleic acid hydroperoxides were then reacted at elevated temperatures and the amounts of GA formed were quantified. The data clearly showed the formation of GA by epoxidation of AA. Depending on the reaction temperature (heating time: 45 min), different thermoconversion rates were found (Table 6). Using 120 mg of AA provided about 6 µg of GA at 80 °C (Table 6, Expt. 1) and about 10 µg of GA at 120 °C (Expt. 2), whereas a further increase of the temperature (200 °C; Expt. 3) led to a decrease of GA comparable to the amounts generated at 80 °C. Obviously, the epoxide formed is unstable at higher temperatures. In a next series of experiments, the formation of GA at a fixed temperature (160 °C), but from different amounts of AA, was investigated. The results (Expts. 4-6) clearly showed that GA formation increased with the amounts of AA available in the reaction system. In a control experiment, the hydroperoxides were omitted in the model mixture resulting in no detectable amounts of GA.

**Table 6: Conversion of acrylamide into glycidamide as affected by the reaction temperature and the initial amount of acrylamide**

| Expt. | Reaction conditions[a] | | Amount of Glycidamide (µg)[b] |
|---|---|---|---|
| | Amount of Acrylamide (mg) | Temperature (°C) | |
| 1 | 120 | 80 | 6.1 |
| 2 | 120 | 140 | 9.3 |
| 3 | 120 | 200 | 6.0 |
| 4 | 60 | 160 | 5.6 |
| 5 | 120 | 160 | 12.1 |
| 6 | 600 | 160 | 29.2 |

[a] Reaction was performed in the presence of linoleic acid hydroperoxides in a lipid matrix consisting of saturated triglycerides for 45 min in a closed glass vessel.

[b] Mean values of triplicates. Coefficient of variation was below 5%.

## Food samples

As compared to the recently published method for the determination of AA in food [9], the sensitivity of the quantification of AA after derivatisation with 2-mercaptobenzoic acid was further increased in the present study. By replacing the dichloromethane extraction with a solid phase extraction (Extrelut column), the yield of the derivative was increased by a factor of 2.5. After solvent evaporation, the eluate of the solid phase extraction could directly be used for the determination of AA by means of LC-MS-MS. For GA quantification, a further clean-up step by semi-preparative reversed-phase HPLC was required due to the much lower concentration as compared to AA.

In order to check whether the newly developed method could also be applied to real food samples, the amounts of AA and GA were analysed in commercial potato chips as well as in French fries (Table 7). Besides being identified for the very first time in a food sample, GA showed concentrations of 1.5 μg/kg in chips and 0.3 – 0.6 μg/kg in French fries, depending on the heating time (5 and 8 min, respectively). In potato chips, the amount of GA was 0.5 % of AA, whereas this proportion was only 0.2 % in French fries without showing a clear dependence on the heating time (Table 7).

**Table 7: Concentrations of acrylamide and glycidamide in potato chips and pre-cooked French fries as affected by the heating conditions**

| Expt. | Sample | Acrylamide (μg/kg)[a] | Glycidamide (μg/kg)[a] |
|---|---|---|---|
| 1 | Potato chips[b] | 302.7 | 1.51 |
| 2 | Precooked French fries (light-colored)[c] | n.a.[f] | 0.002 |
| 3 | Precooked French fries (light-colored)[d] | n.a.[f] | 0.21 |
| 4 | Precooked French fries (light-colored)[e] | 200.5 | 0.29 |
| 5 | Precooked French fries (dark-colored)[c] | n.a.[f] | 0.02 |
| 6 | Precooked French fries (dark-colored)[d] | n.a.[f] | 0.41 |
| 7 | Precooked French fries (dark-colored)[e] | 350.1 | 0.63 |

[a] Mean values of triplicates. Coefficient of variation below 5% (acrylamide) and below 10% (glycidamide), respectively.

[b] Commercial sample.

[c] Commercial precooked French fries (50 g) were heated in coconut oil.

[d] Commercial precooked French fries (50 g) were heated in sunflower oil.

[e] Commercial precooked French fries (50 g) were heated in an oven.

[f] Not analysed.

Based on the initial thoughts that hydroperoxides can promote the conversion of AA into GA, French fries were prepared in coconut oil containing mainly saturated fatty acids and in sunflower oil with a high percentage of unsaturated triglycerides. As already shown for the model systems, the results supported the assumption of an influence of hydroperoxides on the formation of GA. While French fries prepared in coconut oil contained GA in amounts of only 0.002 μg/kg (light-colored) or 0.02 μg/kg (dark-colored) (Table7; Expts. 2 and 5), respectively, the same batch fried in sunflower oil revealed concentrations of 0.2 and 0.4

µg/kg (Expts. 3 and 6), respectively. Furthermore, during the frying process in coconut oil, the fat seemed to "protect" AA by avoiding the formation of hydroperoxides and thus the formation of GA. This assumption was corroborated by the data obtained for precooked French fries which were dry-heated in an oven. Under these conditions (Expts. 4 and 7) much higher GA concentrations in comparison to the fries processed in coconut oil were formed. The amounts of GA by heating the fries in air were even higher than in sunflower oil (cf. Expts. 3 and 4 as well as Expts. 6 and 7).

Although only a small number of samples has been analysed so far, GA was unequivocally identified for the first time in food, which is of considerable importance due to the fact that GA was shown to exhibit a much higher toxicity in cell assays than AA [14].

## 2.5 Reactions of acrylamide with amino acids (cysteine) and peptides (glutathione)

At first, adduct formation between AA and cysteine (Cys) was studied. To identify and quantify the acrylamide-cysteine (AA-Cys) adduct, a stable isotope dilution assay using [$^{13}C_3$]-acrylamide-cysteine as the internal standard was developed (Figure 5A). To compensate strong matrix interferences, derivatisation with dansyl chloride according to Figure 5B was used as an optional step. For this purpose unlabelled as well as [$^{13}C_3$]-labelled AA-Cys was synthesised. Initial studies were focussed on the identification and quantification of the adduct in wheat flour as affected by the heating time and the temperature (Table 8). No linear relationship was obtained for the heating time and the concentration of the adduct because its concentration decreased after an initial increase and a maximum at 40 min. It can thus be concluded that the adduct formation is favoured in the initial phase of heating and that an adduct degradation dominates upon longer heating times. The influence of the temperature on the formation of the AA-Cys adduct showed an increase with increasing temperature and, above 160 °C, a plateau with a constant concentration of the adduct.

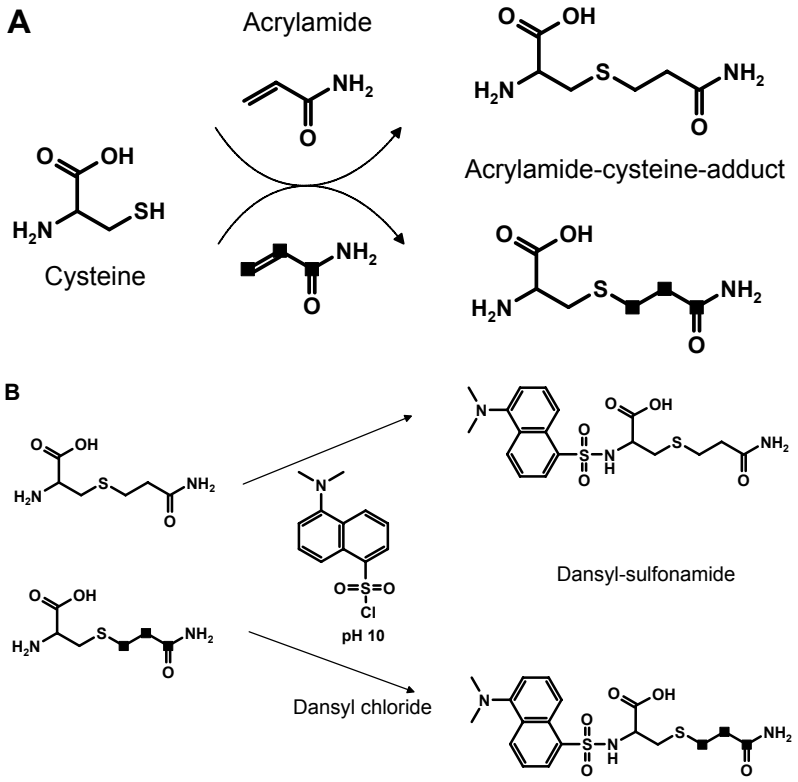


**Figure 5: Formation of the acrylamide-cysteine adduct and the [$^{13}C_3$]-acrylamide-cysteine adduct (A) and subsequent conversion into a stable dansyl sulfonamide (B) prior to LC-MS-MS analysis. ■ [$^{13}C$]-label.**

**Table 8: Concentration of the acrylamide-cysteine adduct in wheat flour as affected by the heating time and the temperature.**

| Time-dependence, constant temperature (170 °C) | | Temperature-dependence, constant heating time (20 min) | |
|---|---|---|---|
| Time (min) | Conc. (µg/kg)[a] | Temperature (°C) | Conc. (µg/kg)[a] |
| 20 | 45.8 | 120 | 24.6 |
| 40 | 76.1 | 160 | 30.3 |
| 60 | 39.5 | 200 | 30.7 |

[a] Mean values of triplicates.

In a next series, the concentration of the AA-Cys adduct of several flours of wheat, spelt, and oats were investigated after heating (20 min, 170 °C). The concentrations ranged from 36 to 277 µg/kg with wheat and spelt showing lower and oats showing higher concentrations (Table 9). In addition to the adduct concentration, the contents of the educts free Cys and AA were determined. In most cases adduct formation was correlated with the AA concentration. In particular oatmeal, which was high in free Asn (Table 5), generated high AA amounts upon heating, and, thus a high concentration of the Cys-AA adduct. As the concentration of free Cys varied less than the content of AA, it can be assumed that the formation of the adduct depends more on the AA and less on the Cys concentration.

**Table 9: Concentrations of free cysteine (Cys) in unheated as well as of acrylamide (AA) and acrylamide-cysteine (AA-Cys) in heated cereal flours.**

| Sample | Conc.[a] in | | |
|---|---|---|---|
| | Unheated flour | Heated flour[b] | |
| | Cys (µg/kg) | AA (µg/kg) | AA-Cys (µg/kg) |
| Wheat 1 | 558 (494 – 622) | 130 (125 - 143) | 36 ( 33 – 39) |
| Wheat 2 | 437 (427 – 446) | 126 (101 - 139) | 40 ( 39 – 41) |
| Wheat 3 | 448 (435 – 460) | 222 (180 - 238) | 70 ( 69 – 70) |
| Wheat 4 | 372 (365 – 378) | 223 (189 - 246) | 76 ( 75 – 76) |
| Spelt 1 | 550 (525 – 575) | 259 (182 - 290) | 44 ( 39 – 45) |
| Spelt 2 | 371 (346 – 395) | 431 (380 - 440) | 65 ( 63 – 67) |
| Oats 1 | 359 (351 – 366) | 663 (594 - 660) | 224 (221 – 229) |
| Oats 2 | 316 (311 – 321) | 708 (650 - 752) | 277 (275 – 284) |

[a] Mean values and range of variation of triplicates.

[b] Flours were heated at 170 °C for 20 min in open glass vessels.

In a last series of experiments several heat-processed foods were analysed for the concentration of the AA-Cys adduct. These foods showed no clear correlation between the formation of the AA-Cys adduct and the concentrations of free AA and Cys. In comparison to the flours, these processed foods had a more complex and also a different composition. It can thus be assumed that, depending on the composition, AA may also

react with other nucleophils such as glutahione or free amino acids. This might affect the formation of the AA-Cys adduct.

Based on the method for the determination of the AA-Cys adduct a stable isotope dilution assay for the acrylamide-glutathione adduct was developed. [$^{13}C_3$]-acrylamide-glutathione was synthesised and used as the internal standard. Initial studies showed that this adduct was also present in different foods.

## 3. Summary

Studies on the role of the *Strecker* reaction highlighted the role of 3-aminopropionamide in the mechanism of acrylamide formation as a potent precursor as well as the key intermediate. In most foods asparagine is the most important precursor of acrylamide, however, especially in fermented or preheated foods 3-aminopropionamide can be formed either as "biogenic" (fermentation) or "thermogenic" (heating) amine, which can easily be converted into acrylamide by heating even if no reducing sugars are present.

S-Deficiency during wheat growth led to a strong increase of free asparagine in the endosperm without affecting the concentration of reducing carbohydrates. Thus heating of S-deficient flours yielded high concentrations of 3-aminopropionamide and acrylamide. However, due to their poor technological properties, S-deficient wheat flours would be excluded from commercial bread processing. Oatmeals have a higher concentration of free asparagine even if the cereal is grown under sufficient S-supply. Heating of oatmeal thus provides more acrylamide as compared to flours from other cereal species.

A method based on [$^{13}C$]-labelled internal standards for the simultaneous quantification of acrylamide and glycidamide in model systems as well as in food was developed. Both in model systems and in fat-containing foods glycidamide can be formed from acrylamide via fatty acid hydroperoxides. Therefore glycidamide formation is favoured during heating in the presence of fats containing a high proportion of unsaturated fatty acids. In this study the carcinogenic glycidamide was identified and quantified in food for the first time.

Acrylamide can react with food constituents containing nucleophilic functional groups such as cysteine and glutathione. These reactions are of special importance as they decrease the concentration of free acrylamide in food. Quantitative data for the acrylamide-cysteine adduct in flours suggest that the acrylamide concentration is more important for the concentration of the formed adduct than the content of free cysteine. In processed foods more constituents, which are able to react with acrylamide, are present and might decrease the acrylamide concentration.

### REFERENCES


[1] Mottram, D.S.; Wedzicha, B.L.; Dodson, A.T. Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature* 2002, *419*, 448-449.

[2] Stadler, R.H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, Ph.A.; Robert, M.-C.; Riediker, S. Food chemistry: Acrylamide from Maillard reaction products. *Nature* 2002, *419*, 449-450.

[3] Stadler, R.H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* 2004, *52*, 5550-5558.

[4] Yaylayan, V. A.; Stadler, R. H. Acrylamide formation in food: a mechanistic perspective. *J. AOAC Int.* 2005, *88*, 262-267.

[5] Zyzak, D.V.; Sanders, R.A.; Stojanovic, M.; Tallmadge, D.H.; Eberhart, B.L.; Ewald, D.K.; Gruber D.C.; Morsch, T.R.; Strothers, M.A.; Rizzi, G.P.; Villagran, M.D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* 2003, *51*, 4782-4787.

[6] Granvogl, M.; Jezussek, M.; Koehler, P.; Schieberle, P. Quantitation of 3-aminopropionamide in potatoes - A minor but potent precursor in acrylamide formation. *J. Agric. Food Chem.* 2004, *52*, 4751-4757.

[7] Muttucumaru, N.; Halford, N.G.; Elmore, J.S.; Dodson, A.T.; Parry, M.; Shewry, P.R.; Mottram, D.S. Formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat. *J. Agric. Food Chem.* 2006, *54*, 8951-8955.

[8] Claus, A.; Schreiter, P.; Weber, A.; Graeff, S.; Herrmann, W.; Claupein, W.; Schieber, A.; Carle, R. Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads. *J. Agric. Food Chem.* 2006, *54*, 8968-8976.

[9] Jezussek, M.; Schieberle, P. A new LC/MS-method for the quantitation of acrylamide based on a stable Isotope dilution assay and derivatization with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J. Agric. Food Chem.* 2003, *51*, 7866-7871.

[10] Calleman, C.J.; Bergmark, E.; Costa, L.G. Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation. *Chem. Res. Toxikol.* 1990, *3*, 406-412.

[11] Randall, P.J.; Spencer, K.; Freney, J.R. Sulfur and nitrogen fertilizer effects on wheat. I. Concentrations of sulfur and nitrogen and the nitrogen to sulfur ratio in grain, in relation to the yield response. *Aust. J. Agric. Res.* 1981, *32*, 203-212.

[12] Granvogl, M.; Bugan, S.; Schieberle, P. Formation of Amines and Aldehydes from Parent Amino Acids during Thermal Processing of Cocoa and Model Systems: New Insights into Pathways of the Strecker Reaction. *J. Agric. Food Chem.* 2006, *54*, 1730-1739.

[13] Gassenmeier, K.; Schieberle, P. Formation of the intense flavor compound trans-4,5-epoxy-(E)-2-decenal in thermally treated fats. *J. Am. Oil Chem. Soc.* 1994, *71*, 1315-1319

[14] Baum, M.; Fauth, E.; Fritzen, S.; Herrmann, A.; Mertes, P.; Merz, K.; Rudolphi, M.; Zankl, H.; Eisenbrand, G. Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. *Mutat. Res.* 2005, *580*, 61-69.

# New solutions for fried potato products with respect to high product quality and reduced acrylamide contents

Deutsches Institut für Lebensmitteltechnik e.V. (DIL), Quakenbrück

Knut Franke, Ulf Strijowski, Martina Kießling

## 1. Introduction

The development of new approaches for the minimization of acrylamide formation during heating of foods was the object of a completed research project [1]. Several solutions for different foods were investigated and validated in laboratory scale. The next step is the creation of the scientific background for the implementation of these approaches into an industrial scale.

One of these tasks is the generation of knowledge for manufacturing par-fried French fries with a lower acrylamide formation potential during final preparation (finishing). Results of the above mentioned previous project demonstrated that a pre-drying of the par-fried French fries and a coating with brine reduced the final acrylamide content [1]. Such a pre-drying and an increase of sodium chloride content could also be established during the manufacturing process of the industrially par-fried French fries. Up to now no data about the integration of such an enhanced pre-drying and brine coating into the production line is available. Also nothing is known about resulting effects on acrylamide reduction and quality issues.

Another task remaining from the previous project was the investigation of opportunities for restructured potato products similar to French fries. With these products an extended intervention into the precursor situation with respect to acrylamide reduction will be gained. This task includes a suitable texture with a crispy crust and a mealy interior, a suitable brown surface after frying and a low fat content. Additionally, the shape of such restructured potato products has to be optimised with respect to avoiding a burst surface and a separation of crust and core. Of course, the potential for influencing precursor contents and the subsequent acrylamide reduction in this type of product is of interest. Especially the application of the enzyme asparaginase for reduction of free asparagine content seems to be a promising way for these products. For example, Pedreschi et al. [2] published reduction rates between 30 and 60 % for French fries incubated with asparaginase.

An unresolved task from the previous project was the acrylamide minimisation during finishing of French fries in household and restaurants. But a considerable part of French fries are prepared at home or in restaurants without any control of the acrylamide contents. So far, recommendations only exist for the preparation of French fries in this field [3]. Therefore, a new approach based on generalized process parameters is necessary to enable a better control of the preparation process in the relevant fryer types.

## 2. Aims

According to the remaining tasks of the previous project the aims with respect to potato products with reduced acrylamide contents can be summarized as follows:

- development of basic data for manufacturing industrially par-fried French fries leading to lower acrylamide formation during finishing by the application of an extended pre-drying of the potato sticks and enrichment of the salt content especially in the outer layers
- development of a formulation and a suitable procedure for manufacturing restructured potato products of French fries type possessing suitable shape and texture with a reduced acrylamide content including opportunities to influencing the precursor contents

– investigations into a better control of frying processes in household and restaurant frying equipment with respect to optimised product quality and reduced acrylamide contents.

## 3. Results

### 3.1 Implementation of the enhanced pre-drying and salt enrichment in an industrial manufacturing process for par-fried French fries

A model process for manufacturing par-fried French fries was established beginning with peeled and graded potatoes and including the processing steps cutting, blanching, pre-drying (traditional or enhanced), par-frying, and freezing. This procedure enables a production of frozen, par-fried French fries in a technical scale at the research institute. The potatoes were supplied by a local potato processor.

The first task which had to be investigated was the required degree of moisture reduction during the enhanced pre-drying of the potato sticks before par-frying. An indication for the required moisture contents of the French fries before final frying (finishing) was extracted from the results of the previous project. Figure 1 shows the generalised moisture courses of potato sticks during processing to French fries including the finishing to a ready-to-eat product in a restaurant fryer. The moisture developments of the traditional process including a short pre-drying only for removing of surface water (solid line), drying of par-fried French fries according to [1] (dotted line) and the new procedure with the enhanced pre-drying based on a prolongation of the traditional pre-drying process after blanching and before par-frying (dashed lines) are compared in this Figure to define the required moisture reduction in the new procedure.

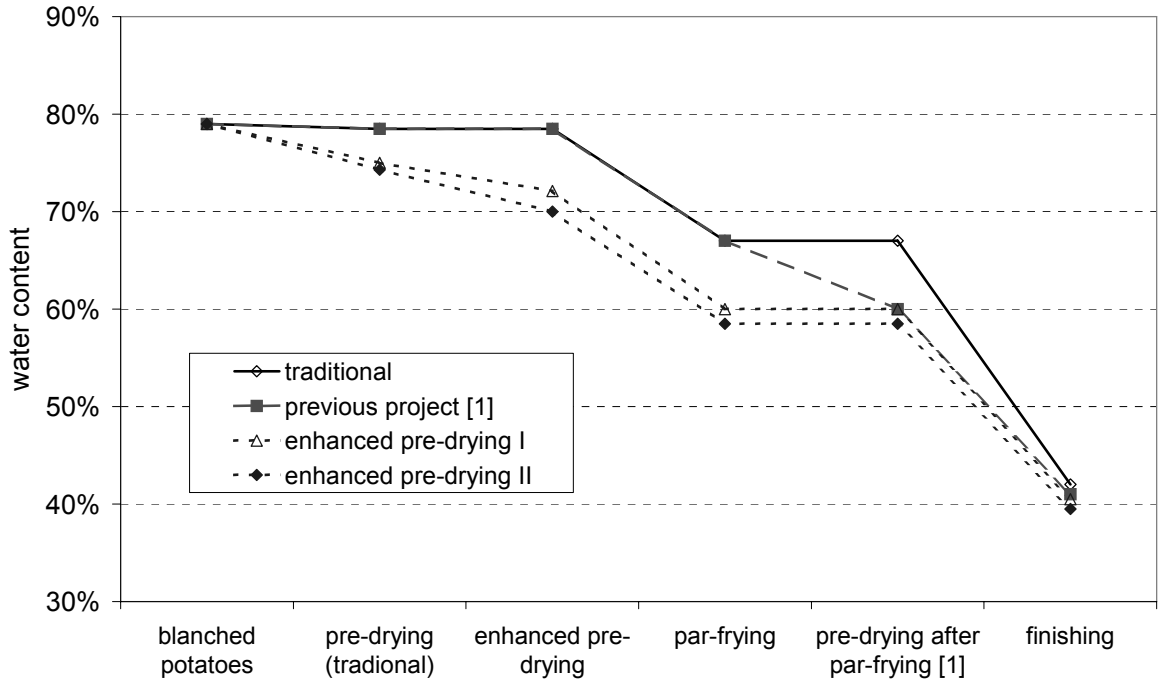


**Figure 1: Schematic moisture courses of potato sticks during processing to ready-to-eat French fries starting with the blanched product according to different manufacturing processes**

As shown in Figure 1, a water content of about 70 to 72 % should be reached during the enhanced pre-drying of the potato sticks in the frame of the new procedure. Potato sticks with such moisture content can

be par-fried similar to the traditional process for reaching a water content of about 60 % before finishing. For these French fries the final frying can be shortened, resulting in lower acrylamide contents [4].

The influence of drying temperature and thickness of the French fries (normal cut or fine cut) on the drying kinetics during pre-drying using a special batch dryer with well defined drying conditions is shown in Figure 2.

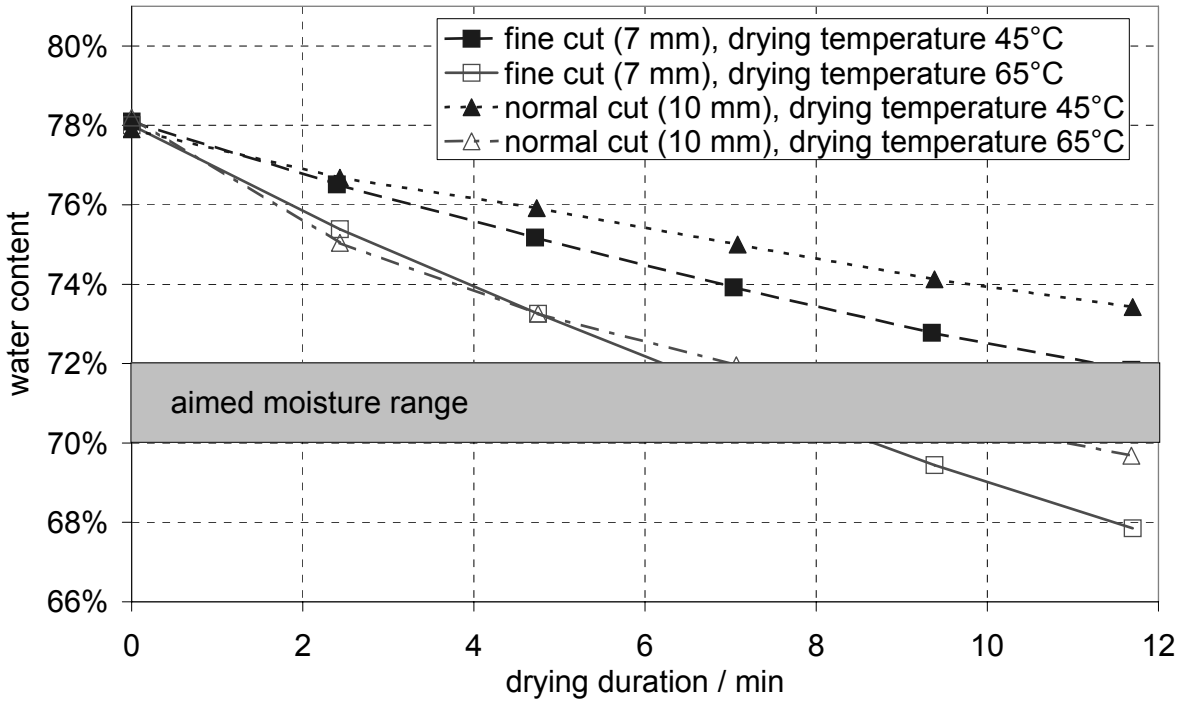


**Figure 2: Drying kinetics of potato sticks of different thickness dried at different air temperatures**

As expected, thickness of the potato sticks (type of cut) as well as air temperature during drying had an effect on moisture reduction rate. However, the influence of drying temperature was much more pronounced than the thickness. Due to the longer transport distances of the water from the core of the thicker potato sticks, these products showed a lower moisture reduction compared to the thinner sticks especially at longer drying times where the surface water had been removed and the diffusion in the stick determined the drying progress. Considering the aimed moisture range between 70 and 72 % for the enhanced pre-drying, a drying time of 6 to 8 min was sufficient to reach this drying state for the higher drying temperature of 65 °C. Therefore, such a drying time was applied during the following investigations for evaluation of the other effects.

In addition to the enhanced pre-drying, a higher salt content in the outer layers of the French fries is suitable to reduce the acrylamide formation during finishing [5]. Therefore, a blanching step in brine was tested to reach the desired higher salt content. For this purpose, potato disks with a thickness of 20 mm were used and the one-dimensional salt diffusion was investigated after blanching at 70 °C for 20 min in brine with a sodium chloride concentration of 1 %. Directly after blanching and also after a pre-drying of the disks for 8 min, the local salt concentrations in the potato disks were determined. Therefore, three thin slices with a thickness of 1 mm were cut from both sides and analysed. Measured salt content in the outer three layers (I to III) and in the inside after blanching and after pre-drying step are shown in Figure 3.

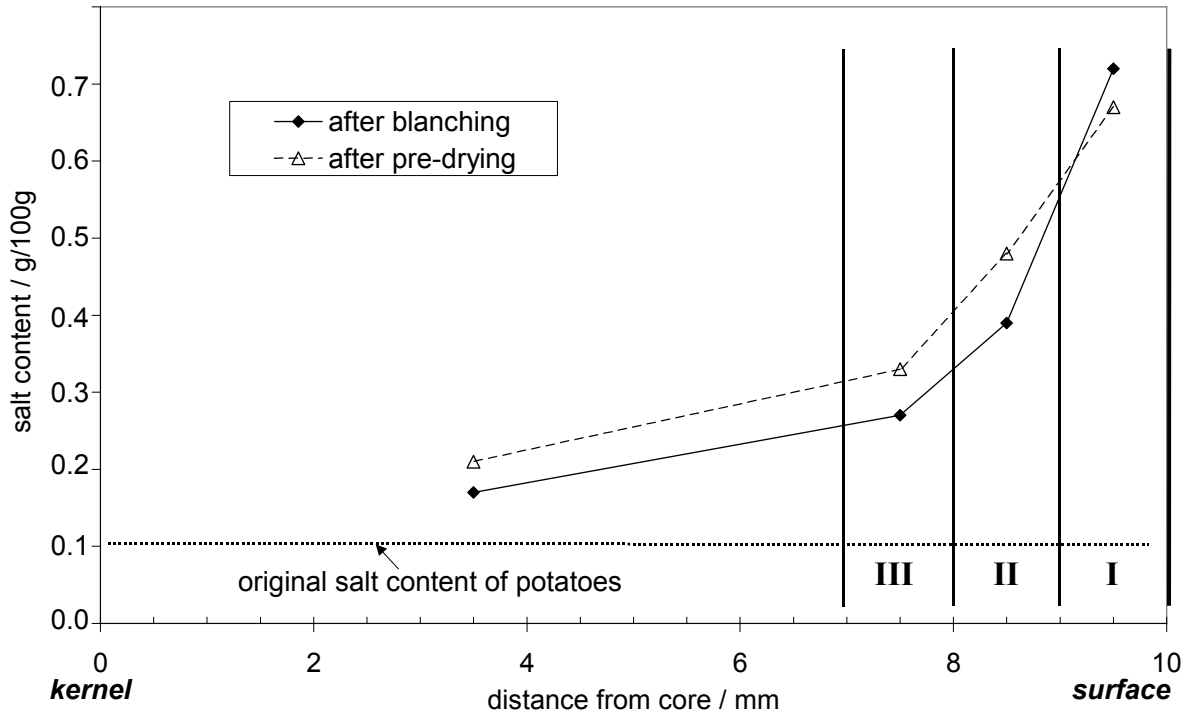


**Figure 3: Salt contents of different layers (I to III) of a potato disk which was blanched in salt solution (1 % w/w) and pre-dried**

The results directly after blanching showed a high salt content in the outer layers with a considerable decrease in core direction (inside). The absolute salt content in the outermost layer (I) was close to the salt content of the blanching solution. This indicates that the blanching step with brine solution is sufficient for a desired salt increase in the outer layers which is relevant for acrylamide reduction during frying [4]. On the other hand, the total increase in salt content was relatively low since the salt concentration in the core increased only slightly compared to the original salt content of the potatoes which is in the range of about 0.1 %. This means that the impact of salt enrichment on sensory properties and health issues [6] seemed to be quite acceptable. This concentration profile was marginally shifted after the pre-drying step. A small decrease could be observed for the outermost layer (I) because the outer salt concentration of the blanching solution was no longer present during pre-drying. The concentrations in the other layers increased slightly due to the inner concentration gradients. However, after this step the concentration in the outer layers also remained on a high level which is necessary for the reduction of acrylamide formation during frying.

The effects of the enhanced pre-drying and the enrichment of salt content in the outer layers of French fries during processing on quality and on the final acrylamide contents after finishing were investigated applying different pre-drying and par-frying conditions. For these purposes French fries (fine cut) with and without an enriched salt content in the outer layers were pre-dried in two different ways. According to the traditional process (also see Figure 1), the first way was a relatively short pre-drying time of 2 min to remove surface water after blanching. In the second setting an enhanced pre-drying time of 6 min enabling a much higher degree of drying (see Figure 2) was performed. Additionally, different par-frying times were tested for an adaptation to the enhanced pre-drying process. These par-fried French fries were subsequently finished in a restaurant fryer with three different frying times to enable a broader range of final water content and degree of browning. The frying time for the French fries with the enhanced pre-drying was adapted to obtain comparable final water contents of the ready-to-eat products. The acrylamide contents of the French fries vs.

the CIE-colour value a* indicating the degree of browning are shown in Figure 4. The higher the values for a* the darker are the French fries and vice versa.

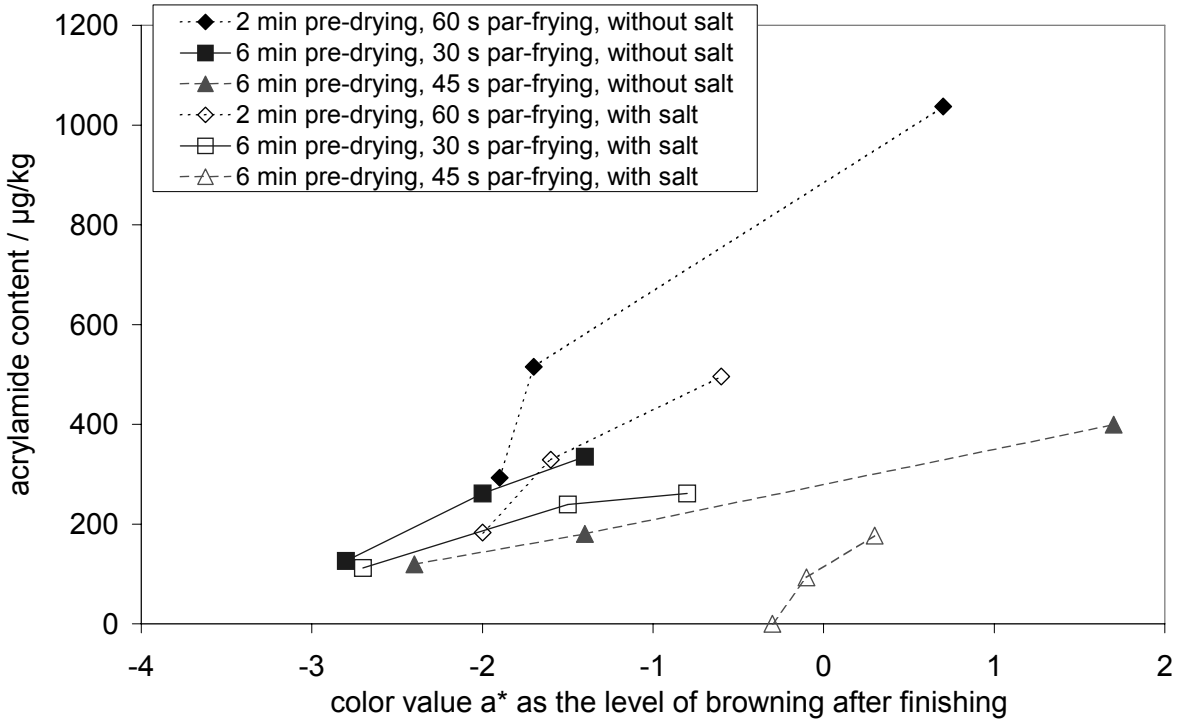


**Figure 4: Acrylamide contents vs. color value a* as browning indicator for ready-to-eat French fries after different preparation procedures for the par-fried products**

For all preparation settings of par-fried French fries an increase of acrylamide contents with increasing browning could be observed due to longer frying times. Additionally, an influence of preparation procedure of par-fried French fries was detectable. By comparing the acrylamide contents for the shorter pre-drying according to the more traditional process (diamonds) a distinct effect of salt enrichment could be observed. For similar degrees of browning, lower acrylamide contents could be reached. An effect of the enhanced pre-drying on the relationship between browning and acrylamide formation was detectable. Especially for the salt enriched French fries the longer pre-drying (empty symbols) resulted in lower acrylamide contents in the final products in particular for higher degrees of browning (a* > -1). Additionally, salt enriched French fries with enhanced pre-drying and a longer par-frying time (45 s instead of 30 s) seemed to be more successful in minimizing the acrylamide contents for a comparable degree of browning of the final product.

### 3.2 Development of restructured potato products of French fries type and possibilities for reducing acrylamide contents in these products

For the development of the restructured potato products being similar to French fries a suitable formulation and manufacturing procedure had to be developed. The main challenges with respect to quality determining parameters were the texture, the fat intake and the acrylamide content. For the texture a crispy crust and a mealy interior are favourable, the fat intake during frying depends on water content because these two parameters are related reciprocally [7] and the acrylamide contents should be minimized for a given degree of browning. A further task which emerged in the pre-tests was the preservation of the shape during processing, especially throughout frying. A considerable increase in thickness was often observed due to the separation of an impermeable crust from the core caused through steam generation in the core. Such an inflated crust tore at the end of frying or collapsed after cooling leading to an undesired product surface.

Therefore, the conservation of thickness of the products during frying was evaluated in addition to the other quality parameters.

The first step was the development of the basic formulation consisting of different dried potato products (flakes and granules) and water. Also a basic manufacturing procedure had to be evolved (Figure 5).

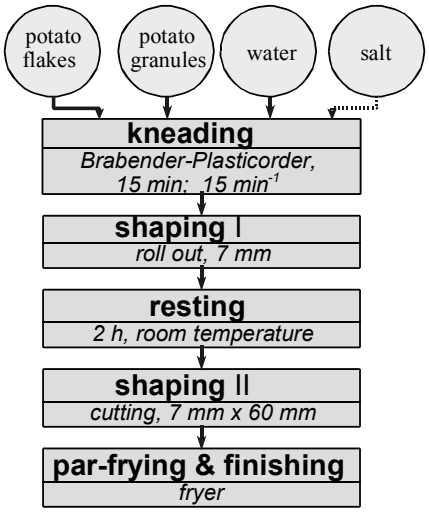


**Figure 5: Schematic flow sheet for the manufacturing procedure of restructured French fries type potato products**

Finally, a formulation consisting of 35 % flakes, 15 % granules and 50 % demineralised water resulted in the best thickness conservation and a texture close to that of the French fries. A further improvement of the constancy of thickness and surface appearance could be reached by splitting the frying process into a par-frying step, a subsequent storage of the par-fried products and a separate finishing step according to the last step in Figure 5. The effects of the separate par-frying step on thickness conservation and on acrylamide content vs. brightness as colour indicator of the fried products (degree of browning) are shown in Figure 6. Higher values for brightness represent lower browning of the product surface and vice versa. A set of different total frying times of the restructured products were used to generate varying degrees of browning.

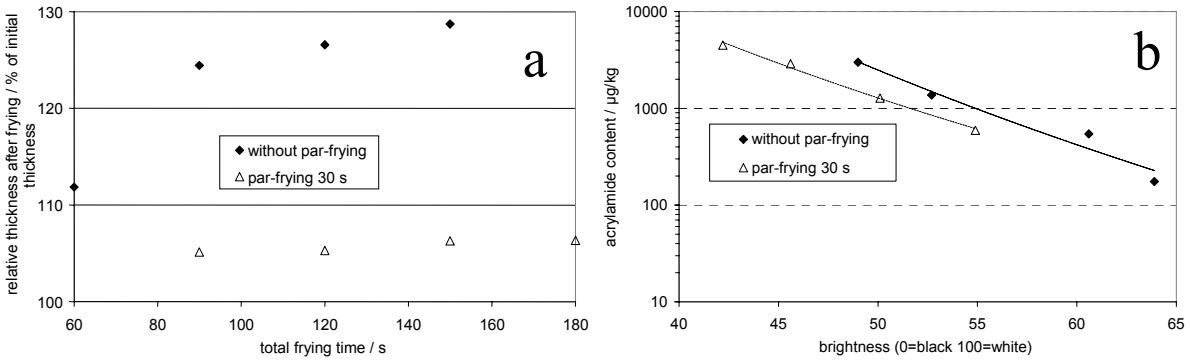


**Figure 6: Effects of total frying time and splitting of frying process on thickness preservation (a) and on relationship between acrylamide content and brightness (b)**

As shown in Figure 6a the splitting of the frying process into a par-frying step of 30 s and a finishing step of various lengths resulted in products with a better preservation of shape compared to the one step frying.

Additionally, such splitting had a positive effect on acrylamide formation (Figure 6b). For the same degree of browning (level of brightness) lower acrylamide contents were measured. As a major drawback, the splitting of the frying process led to an increase in fat uptake during the second frying step resulting in a higher final fat content of these products (results not shown).

Therefore, an additional coating step for the restructured potato products was tested as a possibility to lower the fat uptake for a given water content. Several authors described such a reduction of fat contents by coating with starch or cellulose solutions before frying [8, 9]. The effect of a starch coating of restructured potato products before par-frying on the relationship between fat and water content is shown in Figure 7. The coating solution consisted of demineralised water and potato starch (2.5 %) which had been gelatinised by a suitable temperature pre-treatment.

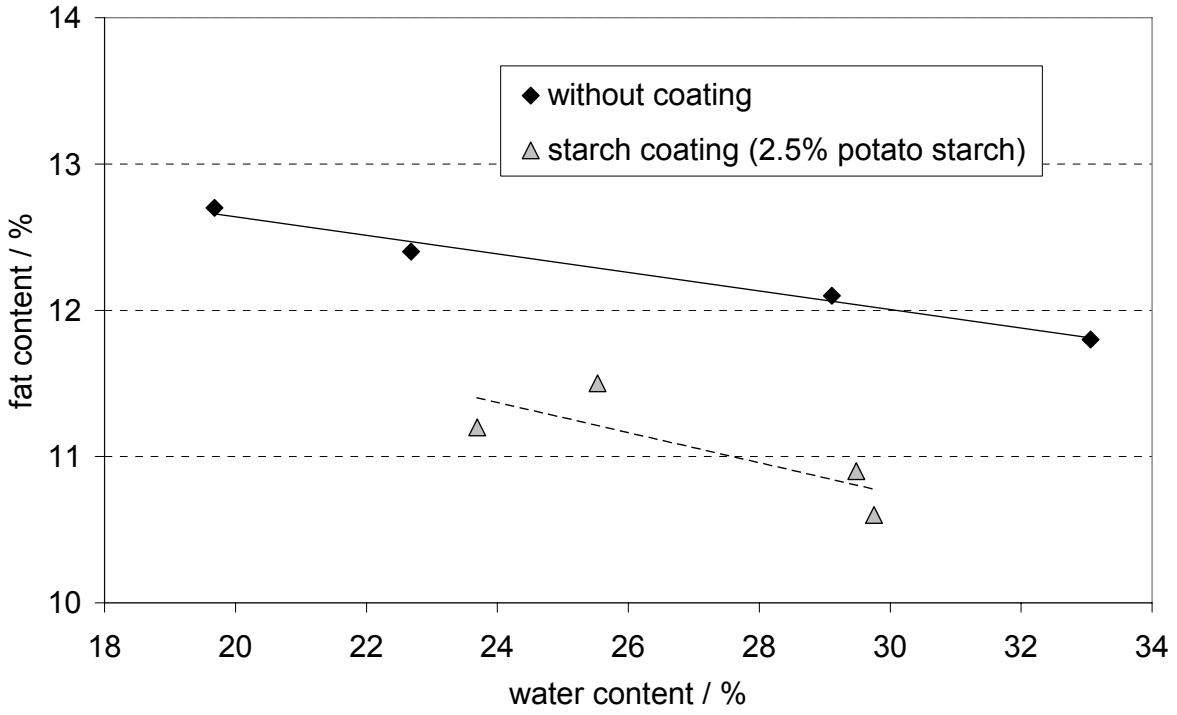


**Figure 7: Fat content vs. water content of restructured potato products without and with a coating in a starch solution and fried for different times**

As could be observed the coating influenced the dependencies between fat and water content and could contribute to a lower fat uptake. A similar effect could be observed for coating with a solution of methylcellulose (results not shown).

A coating with a high viscosity polysaccharide solution also offered the opportunity to influence the salt content in the outer layers in a defined way in order to achieve a reduction of acrylamide contents in such products comparable to the effects in French fries (Chapter 3.1). Effects of salt addition (5 %) to the coating solution on the correlation between acrylamide content and degree of browning expressed as colour value a* are shown in Figure 8.

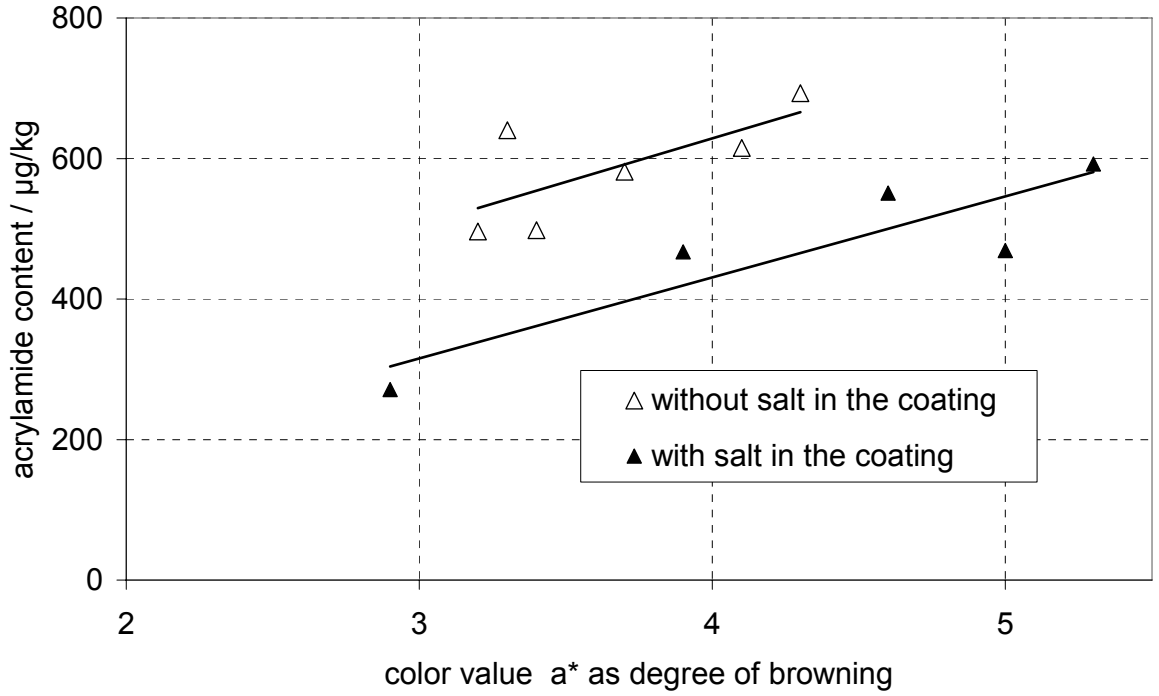


**Figure 8: Acryamide content vs. degree of browning (colour value a*) for coated restructured potato products with and without addition of salt (5 %) in the coating**

Addition of salt led to a significant increase of salt concentration in the outer layers of the restructured product and contributed to a distinct reduction of acrylamide formation during frying.

Another opportunity for acrylamide reduction in these products is the direct impact of precursor contents during preparation process by suitable enzymatic treatment. Therefore, the application of the enzyme asparaginase for hydrolysation of free asparagine into aspartic acid significantly reduces the precursor contents and leads to fried products with lower acrylamide content [10]. The advantages of this enzyme application in restructured potato products of French fries type are the possibility to add it directly to the matrix and to enable a sufficient action time during the preparation procedure. Therefore, the use of a commercial asparaginase was tested by adding different amounts of enzyme directly to the water phase before mixing. After a total dough resting time of 1 hour free asparagine contents were determined and the restructured products were fried according to the standard frying conditions without splitting of frying as described before. The effects on the content of free asparagine and resulting acrylamide contents in the fried potato product are shown in Figure 9.

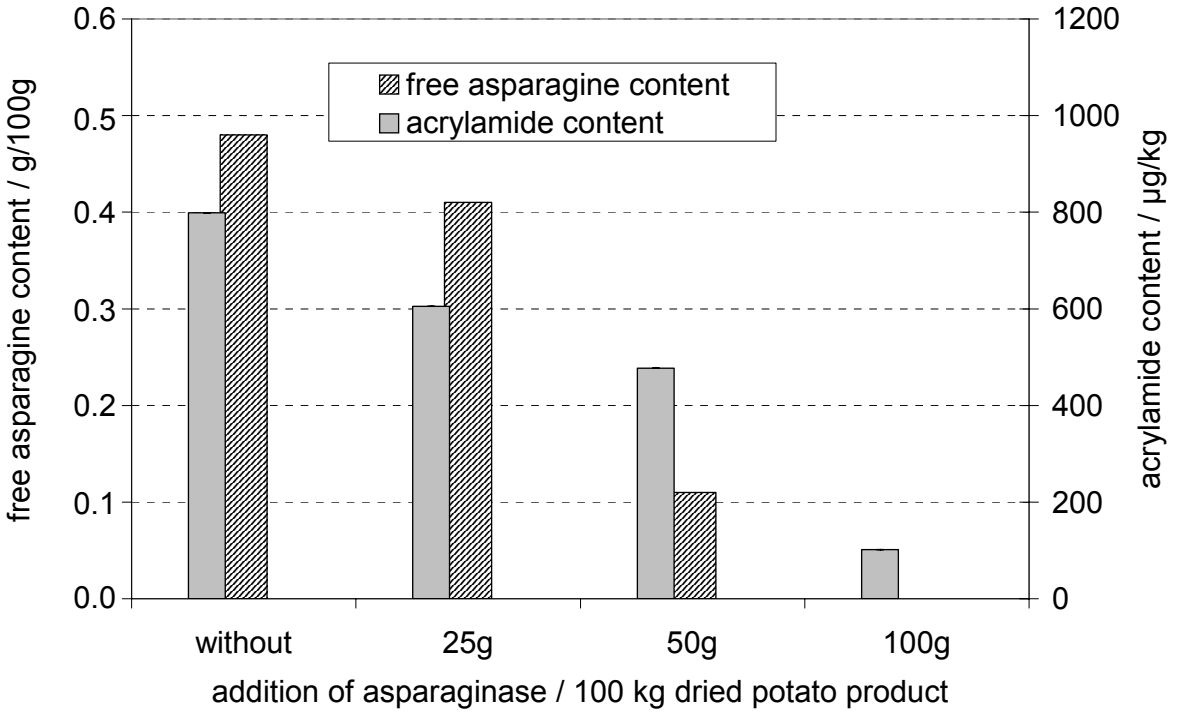


**Figure 9: Acrylamide and free asparagine content after use of different amounts of asparaginase in restructures potato products**

A strong influence of enzyme addition on the contents of free asparagine could be observed. An addition of 100 g enzyme per 100 kg of dried potato products resulted in a free asparagine content below quantification limit of 0.1 g/100 g. As expected, the acrylamide formation could be reduced due to reduction of free asparagine. Therefore, the application of asparaginase is a suitable tool for the specific reduction of precursor contents in such restructured potato products. However, even at the highest enzyme addition resulting in free asparagine content below quantification limit, detectable amounts of acrylamide were determined in the fried products indicating the importance of alternative formation pathways for potatoes with very low asparagine contents [11].

## 3.3 Investigations into a better control of frying processes in households and restaurants

### a) Restaurant fryers

For the investigations into the frying process in restaurant fryers two different fryers were used (Figure 10).


**Figure 10: Photographs of the restaurant fryers used for the investigations**

These fryers (RF I and RF II) with comparable fat capacities (about 11 kg) were equipped with different heating power. RF I had a heating power of 7.2 kW whereas the heating power of RF II was 8.4 kW. Additionally, RF II had a newer control unit with a cooking computer. This control unit enabled an automatic adaptation of the frying time depending on the oil temperature course during the frying process. In the case of a heavy product load leading to a larger drop of oil temperature at the beginning and a slower temperature increase during re-heating, the frying process could automatically be extended. Such a procedure was realised to enable similar frying results independent of the product load in the fryer.

Using these two restaurant fryers, frying tests were carried out using a partially hydrogenated rapeseed oil and the standard restructured potato product according to Chapter 3.2 were carried out. Different initial oil temperatures and a product/oil ratio of 0.1 were applied. Additionally, for RF II the frying process was carried out with and without the automatic adaptation of frying time. The parameters (temperature and time) and the results (mass change during frying, final oil temperature at the end of the frying process, colour value a* and acrylamide content) of the French fries are presented in Table 1.

As shown in column 6, the decrease of mass during frying did not only depend on the frying time but also on the type of fryer and type of control (frying time adaptation). The fryer RF I with the lower heating power led to less water evaporation and therefore less mass reduction. On the other hand, the automatic extension of frying time resulted in a higher mass loss indicating that there still was considerable water evaporation even during the prolonged period of the frying process. However, especially at this stage, the temperature of the outer product layers increased considerably leading to darker products (higher values for a*). This was especially observable for the higher frying temperature settings (Table 1). But this intensified browning also led to very high acrylamide contents in the fried products. A good indicator for the acrylamide contents in the final product was the oil temperature at the end of the frying process ("final oil temperature" in column 5). Especially in the case of RF I with the lower heating power, a direct influence of the oil end temperature was obvious. Lower end temperatures also for higher initial oil temperatures resulted in lower acrylamide contents in the fried product.

**Table 1: Results of frying processes of restructured potato products in different restaurant fryers with and without adaptation of the frying time**

| Fryer | Oil temperature setting / °C | frying time setting / s | real frying time / s | final oil temperature / °C | mass decrease / % | colour value a* | acrylamide content / µg/kg |
|---|---|---|---|---|---|---|---|
| RF I | 160 | 120 | 120 | 140 | 9 | 0.3 | 66 |
| | 160 | 180 | 180 | 144 | 15 | 4.8 | 194 |
| | 170 | 120 | 120 | 147 | 12 | 3.6 | 206 |
| | 170 | 180 | 180 | 148 | 16 | 5.9 | 291 |
| RF II with adaptation | 160 | 120 | 141 | 152 | 12 | 2.5 | 139 |
| | 160 | 180 | 190 | - | 20 | 11.5 | 1388 |
| | 170 | 120 | 138 | 162 | 17 | 7.3 | 333 |
| | 170 | 180 | 200 | 174 | 22 | 12.3 | 1943 |
| RF II without adaptation | 160 | 120 | 120 | 153 | 9 | 1.9 | 122 |
| | 160 | 180 | 180 | 165 | 17 | 9.9 | 855 |
| | 170 | 120 | 120 | 154 | 12 | 2.4 | 177 |
| | 170 | 180 | 180 | 173 | 19 | 11.0 | 1270 |

With respect to acrylamide minimisation in restaurant fryers the level of final oil temperature which is reached at the end of the frying process seems to be the critical parameter in restaurant fryers together with the duration of frying. To enable the desired degree of final water content (texture) but avoiding an intensive browning, a lower final oil temperature should be aimed at by an adapted control program. On the other hand, prolongation of frying process (often at high oil temperatures) at this stage as realised by current cooking computers seemed to give a higher degree of browning and much higher levels of acrylamide contents in the fried product. Therefore, a parallel optimisation of specific heating power, temperature course and frying time is required for each type of restaurant fryer and product type.

### b) Household fryers

Five household fryers (HF I to HF V) with different oil capacities and heating powers were tested (Table 2) with respect to process parameters, e. g. oil temperature settings, and the resulting product quality. Additionally, the specific heating power as the ratio between heating power and oil capacity is presented in Table 2.

**Table 2: Household fryers used for the investigations into frying processes**

| Parameter | HF I | HF II | HF III | HF IV | HF V |
|---|---|---|---|---|---|
| Photograph |  |  |  |  |  |
| Heating power (measured) / kW | 1.6 | 1.7 | 1.8 | 1.9 | 2.1 |
| Oil capacity / l | 1.1 | 3.0 | 2.0 | 3.5 | 3.3 |
| specific heating power / kW/l | 1.3 | 0.6 | 0.9 | 0.6 | 0.7 |

A broad range of fryers with different oil capacities and specific heating power were chosen to permit a comprehensive variation of process conditions and equipment parameters. The first step was the control of the oil temperature in the fryer without product load to generate a basis for more comparable frying processes regarding product quality during the next investigations. For these purposes, the fryers were switched on and were in operation with a closed lid for at least one hour to reach their steady-state with respect to temperature distribution in the oil. Subsequently, the temperature in the middle of the frying basket was recorded for 10 min using a calibrated temperature probe with an accuracy of ± 1 °C. Average temperatures and standard deviation were calculated for each fryer from these data (Table 3).

**Table 3: Average temperatures and standard deviations of oil temperatures in household fryers for different temperature setting points**

| Set point temperature | Measured oil temperatures without product (mean value ± standard deviation) | | | | |
|---|---|---|---|---|---|
| | HF I | HF II | HF III | HF IV | HF V |
| 150 °C | 137.2 ± 3.2 | - | 154.0 ± 3.1 | 146.0 ± 2.6 | - |
| 160 °C | 149.3 ± 3.0 | 139.1 ± 2.8 | - | 152.9 ± 2.9 | 165.9 ± 2.3 |
| 170 °C | 158.8 ± 3.0 | 151.0 ± 2.6 | 169.7 ± 2.7 | 163.4 ± 3.2 | 179.2 ± 2.3 |
| 180 °C | 170.9 ± 3.4 | 164.7 ± 2.3 | - | 173.9 ± 3.1 | 189.6 ± 2.3 |
| 190 °C | 180.6 ± 3.1 | 175.1 ± 2.3 | 187.3 ± 2.4 | 183.7 ± 3.0 | 199.3 ± 2.1 |

- This temperature could not be set in this fryer

As can be seen from the data, the different fryers showed a broad range of measured temperatures for the same setting temperature. Both lower and higher temperatures could be observed. To enable comparable frying processes these deviations in the real oil temperature have to be reduced by a better adjusting of the fryer control units. The solution of this task by the fryer manufacturers is the absolute prerequisite for any other developments with respect to a more standardized frying process resulting in comparable product qualities.

In the next step extensive investigations regarding the influences of process settings on product quality and also on the process itself were carried out using the standard restructured potato product. For these purposes two oil temperatures were used (160 und 175 °C) which were controlled externally to get comparable conditions for all fryers. Additionally, the product/oil ration was varied between 0.1 and 0.25. To obtain a variation in the degrees of browning, three frying times between 2 and 6 min were applied. For the fried product properties like water content, browning and acrylamide content were evaluated. Additionally, parameters for the description of the frying process, e. g. oil temperature course during frying or evaporation of water, were recorded or calculated. Some of these results will be discussed in the next paragraphs.

The influence of fryer and frying time on browning of the fried products shown as brightness is shown in Figure 11. It has to be mentioned that all frying processes shown in Figure 11 were carried out at the same product/oil ratio of 0.1 and initial oil temperature of 160 °C. Therefore, similar quality results should be expected for the same frying time.

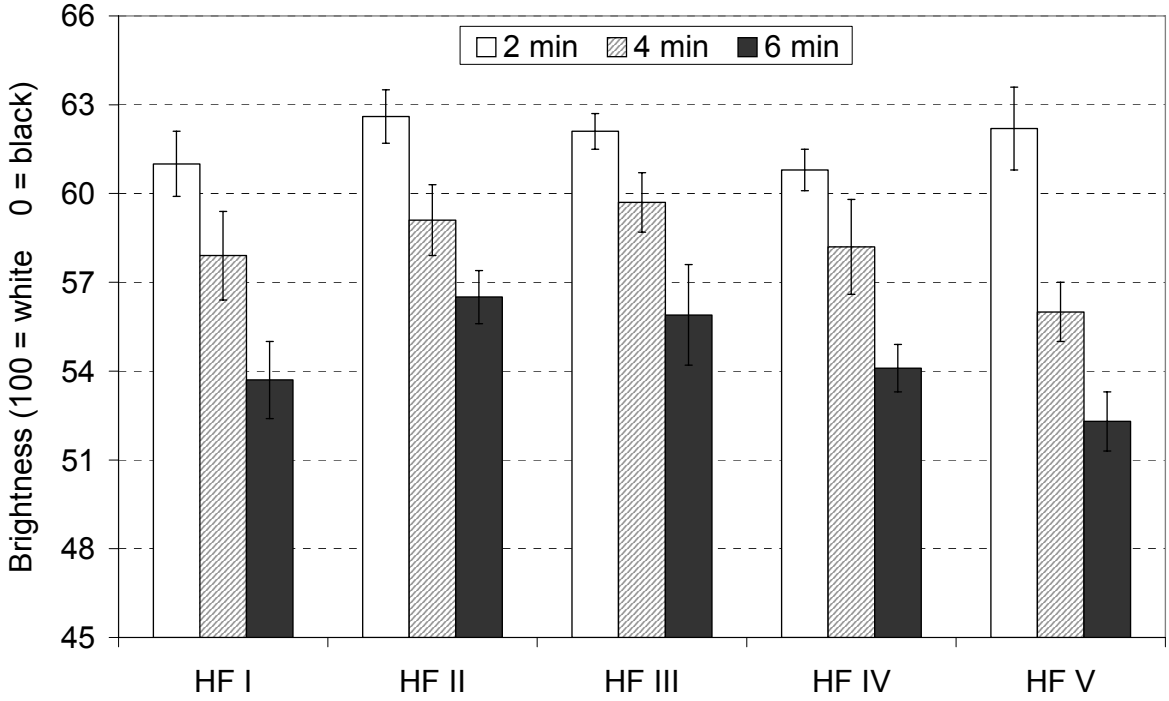


**Figure 11: Brightness of restructured potato products fried for 2 to 6 min in different household fryers (160 °C, product/oil ratio 0.1)**

Although the brightness of the products which were fried for a similar time were in the same range, there were significant differences in the degree of browning between the household fryers for entirely comparable frying conditions, e. g. between the results of HF II and HF IV and the longest frying time of 6 min. Such differences could also be observed for the whole range of frying times. Comparable results were detected for other quality parameters, e. g. acrylamide content.

In the next step it was tested whether these differences are related to fundamental engineering parameters describing the individual frying process, e. g. heat transfer from oil or energy balances, using a suitable mathematical model. For these purposes, statistically significant correlations between settings of frying process, the fryer itself and engineering process parameters were determined using regression analysis.

Considering the total heat energy input into the product during frying, one part of this heat energy is required to increase the product temperature from its initial value to an average temperature of >100 °C at the end of the process. Another part of heat energy is required for evaporation of water from the fried product to form the crust starting when the outer layers of the product reaches 100 °C. The amount of evaporated water and therefore the energy for water evaporation determines the main product quality parameters like final water content or crust thickness.

For the regression analysis, the fryer number ($x_1$) which is sorted according to heating power (Table 2), frying temperature ($x_2$), product/oil ratio ($x_3$) and frying time ($x_4$) were included in the model for calculation of heat energy consumption for water evaporation (y). The resulting regression model with the significant coefficients is presented in equation (1). The coefficient of determination B was 0.89 indicating a good representation of process by the model.

$$y = 140.2 - 18.1 \cdot x_1 - 2941 \cdot x_2 - 29.5 \cdot x_4 + 77.4 \cdot x_1 \cdot x_2 + 8.2 \cdot x_1 \cdot x_4 + 14.2 \cdot x_2 \cdot x_3 + 230.5 \cdot x_2 \cdot x_4 \quad (1)$$

Based on the regression model in (1) an average of water evaporation energy can be calculated for each frying time valid for all fryers. Therefore, such a general parameter characterises a standard frying process

with respect to water evaporation. The next step is the determination of standard frying conditions leading to standardized water evaporation. These conditions are specific for each fryer. As an example the data for the frying time of 4 min are presented in Table 4. The calculated standardized energy consumption for water evaporation was 170 kJ for the frying time of 4 min corresponding to a water evaporation of about 76 g.

**Table 4: Calculated frying conditions for the standard frying process of 4 min leading to similar water evaporation in each household fryer considered**

| Fryer | Standard frying conditions | |
|---|---|---|
| | Product/oil ratio | Oil temperature / °C |
| HF I | 0.22 | 172 |
| HF II | 0.17 | 170 |
| HF III | 0.26 | 173 |
| HF IV | 0.11 | 163 |
| HF V | 0.12 | 174 |

The differences in the settings of the fryers for the calculated standard frying process include the individual parameters of each frying regarding geometry, e. g. location of heating and temperature control probe, heat transfer from heating, temperature distribution in the oil and others. Therefore, such a standard frying process is a tool to get generalised parameters describing the individual features of the fryers in an indirect way.

Applying these conditions in the different household fryers, similar levels of water evaporation can be expected after a frying time of 4 min and therefore also comparable qualities. This was positively tested for the degree of browning after 4 min frying using the settings in Table 4 for the individual fryers (results not shown).

## 4. Summary

A model plant in a technical scale had been established for the investigations regarding the integration of coating and an enhanced pre-drying into the manufacturing process of industrially produced par-fried French fries. It could be demonstrated that the drying of blanched French fries is much more effective than drying of par-fried French fries permitting shorter drying times for the aimed moisture reduction. Furthermore, the successive par-frying process could be shortened due to the enhanced pre-drying. Blanching of the potato sticks in a solution containing about 1 % of sodium chloride permits a defined increase of the salt content in the outer product layers without an extensive raise in the core. This internal salt gradient leads to the required reduction of acrylamide formation in the outer layers, but minimises the impacts on sensory and on possible health issues. Additionally, the potentials for optimizing the par-frying time depending on salt enrichment for a maximized acrylamide reduction could be described.

With respect to the manufacturing of recombined potato products, formulations and procedures were determined and tested to obtain products with a suitable sensory quality (texture, water and fat content, browning) and minimized acrylamide contents. A coating of these products with a polysaccharide solution before frying contributes to lower fat intake during frying. Furthermore, the addition of sodium chloride to the coatings reduces acrylamide contents in the final product. A more distinct reduction of acrylamide

formation for a comparable degree of browning is possible by application of the enzyme asparaginase to these products as could be demonstrated.

With respect to investigations regarding control of frying in households and restaurants to obtain an optimized quality and reduced acrylamide contents as a result of these frying processes, extensive investigations were carried out. Different types of restaurant and household fryers were included and variable settings for frying temperature and time as well as product load were considered. Besides the determination of the product quality, the frying process in the fryers was analysed with respect to engineering process parameters, e. g. energy balances. A standard frying process leading to a defined product quality was established for household fryers based on such engineering process parameters. These results could be validated and further improved by investigations of frying with different potato products. Such a standard frying process represents a tool which permits evaluation of frying processes in different household fryers and the derivation of the relevant control parameters to obtain the desired final quality.

## REFERENCES


[1] Franke, K.; Kießling, M.; Reimerdes, E.H.; Sell, M. Importance of frying fat and frying equipment conception on the acrylamide contents in fried products, In: Development of New Technologies to Minimize Acrylamide in Food, pp. 35-44, Bonn: Research Association of the German Food Industry, German Federation of Food Law and Food Science, 2005.

[2] Pedreschi, F.; Kaack, K.; Granby, K. The effect of asparaginase on acrylamide formation in french fries, Food Chemistry: An International Journal 109 (2) 386-392, 2008.

[3] Maschkowski, G.; Groeneveld, M.; Müller, C. Acrylamid - Wie Sie sich und Ihre Familie schützen können, Bonn: aid Infordienst und BMVEL, 2002.

[4] Reimerdes, E.H.; Franke, K. Engineering and biotechnological aspects for the manufacturing of high quality fried potato products, Biotechnology Journal 1 (4) 413-419, 2006.

[5] Franke, K.; Sell, M.; Reimerdes, E.H. Quality related minimization of acrylamide formation - an integrated approach, In: Friedman, M., Mottram, D. S. (Eds.), Chemistry and Safety of Acrylamide in Food, pp. 357-369, New York: Springer, 2005.

[6] Seal, C. J.; Mul, A. de; Eisenbrand, G.; Haverkort, A. J.; Franke, K.; Lalljie, S. P. D.; Mykkänen, H.; Reimerdes, E.; Scholz, G.; Somoza, V.; Tuijtelaars, S.; Boekel, M. van; Klaveren, J. van; Wilcockson, S.J.; Wilms, L. Risk-benefit considerations of mitigation measures on acrylamide content of foods - A case study on potatoes, cereals and coffee, British Journal of Nutrition 99 (S2) S1-S46, 2008.

[7] Kozempel, M.F.; Tomasula, P.M.; Craig, J.C. Jr Correlation of moisture and oil concentration in French fries, LWT - Food Science and Technology 24 (5) 445-448, 1991.

[8] Mellema, M. Mechanism and reduction of fat uptake in deep-fat fried foods, Trends in Food Science & Technology 14 (9) 364-373, 2003.

[9] Garcia, M.A.; Ferrero, C.; Campana, A.; Bertola, N.; Martino, M.; Zaritzky, N. Methylcellulose coatings applied to reduce oil uptake in fried products, Food Science and Technology International 10 (5) 339-346, 2004.

[10] Pedreschi, F.; Kaack, K.; Granby, K. The effect of asparaginase on acrylamide formation in french fries, Food Chemistry 109 (2) 386-392, 2008.

[11] Yaylayan, V.A.; Stadler, R.H. Acrylamide formation in food: a mechanistic perspective. Journal of AOAC International 88 (1) 262-267, 2005.

# Examination of the process conditions and the thermal processes to reduce acrylamide formation during baking

Institut für Lebensmittel- und Umweltforschung e.V. (ILU), Nuthetal/Bergholz-Rehbrücke

Heinz Kaiser, Annette Lehrack, Maja Eigner, Alexander Voss

## 1. Introduction/Aim

The formation of acrylamide (AA) during baking is a very complex process of the combined action of material and technological conditions. The baking process is at the centre of the project. The project is aimed at developing process conditions during baking at which the acrylamide formation possibly can be suppressed to a large extent. This includes the following objectives:

- Reduction of the AA-content by technological measures during baking according to common or company specific recipes
- Development of technological parameters and conditions during baking for the reduction of AA in the product
- Recommendations for machine building for the implementation of the specific baking conditions in new or adapted baking aggregates.

The results will lead to recommendations for the baking oven branch, a basis for the implementation of new baking processes. The most important products of the basic assortment, i.e. wheat rolls, wheat bread, mixed rye bread, wholemeal bread, and of the biscuit and cracker assortment, i.e. brown gingerbread, were used for the tests. The product groups were selected under the aspect that the acrylamide contamination of bread and rolls is relatively low, but due to the high per-capita consumption of bread and rolls up to 87 kg per consumer [1] per year constitues a risk potential [2]. Brown gingerbread belongs to the product group with the highest contamination [3].

## 2. Solution and methods

### 2.1 Solution for bread and rolls

As a uniform reaction during baking, crumb and crust are developed simultaneously under the influence of the temperature gradient. Inevitably, the peripheral parts of the dough and baked goods which form the crust at the end of baking are subjected most intensively to the effect of heat. This suggests to possibly largely break up the baking process. The development of the product structure, i.e. the development of volume and shape, and the crumb should be uncoupled from the development of the crust, i.e. the crust thickness and browning. This is implemented by different temperature and humidity parameters within a defined baking time.

For the split-up or division of the baking process which possibly has to be performed in two baking aggregates, two very different strategies were developed (Figure 1):

- First strategy (1): Process splitting → (baking) steaming and (post)baking
- Second strategy (2): Phase baking → (pre)baking and (final) baking

An acrylamide-poor baking should be implemented by the variation of the temperature, the time course of heat action and the observance of the baking climate in a clearly different structuring of at least two baking steps.

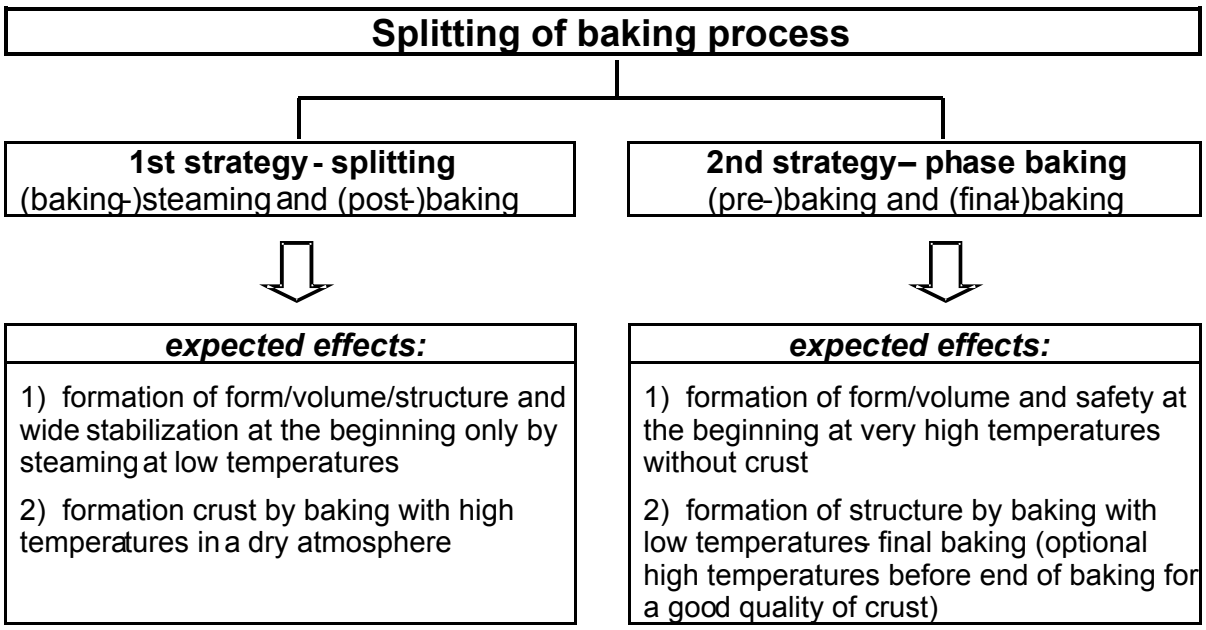


Figure 1: Strategies for the reduction of the formation of acrylamide during baking of bread and rolls

According to the 1st strategy, the process splitting (Figure 2), (post) baking takes place after steaming on the variation of the varieties of heat transfer by different technical baking processes or principles of heat transfer, respectively, in the storey oven, by baking in circulating air (shop oven and/or rack oven) and in the storey oven with selectively transformed infrared radiation - STIR® [4].

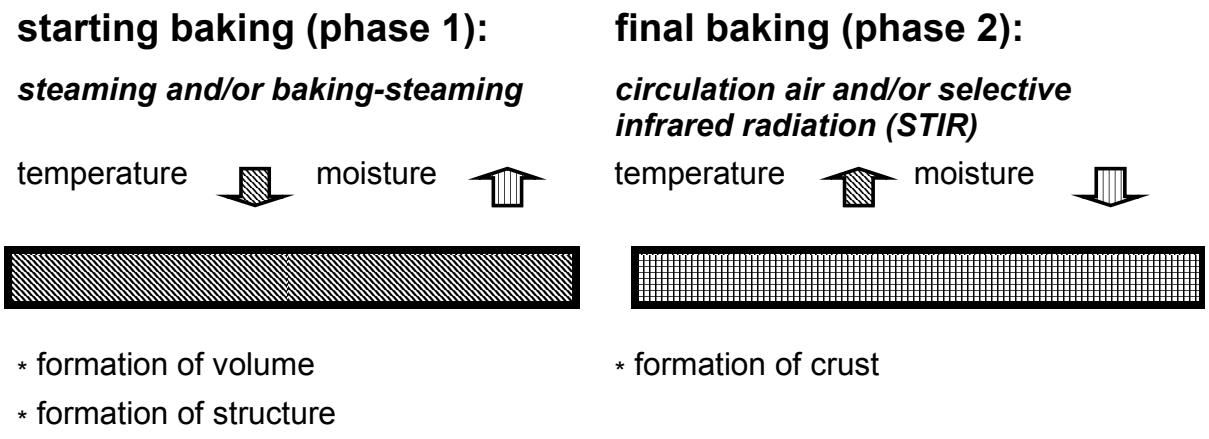


Figure 2: Expected results of the 1st strategy "Steaming/Baking" – splitting of baking

The 2nd strategy, phase baking (Figure 3), de facto is the total reversion of the 1st strategy because baking is started at very high temperatures. The reason for that is the improvement of the baking results, especially the volume of products of break and shred. After the impact of the very high temperatures, baking has to be continued at low temperatures. In the phase of the drying out of the sides of the baked product, only small quantities of acrylamide should be formed. (Final-) baking takes place in the storey oven, with circulating air and in the storey oven with selective infrared radiation.

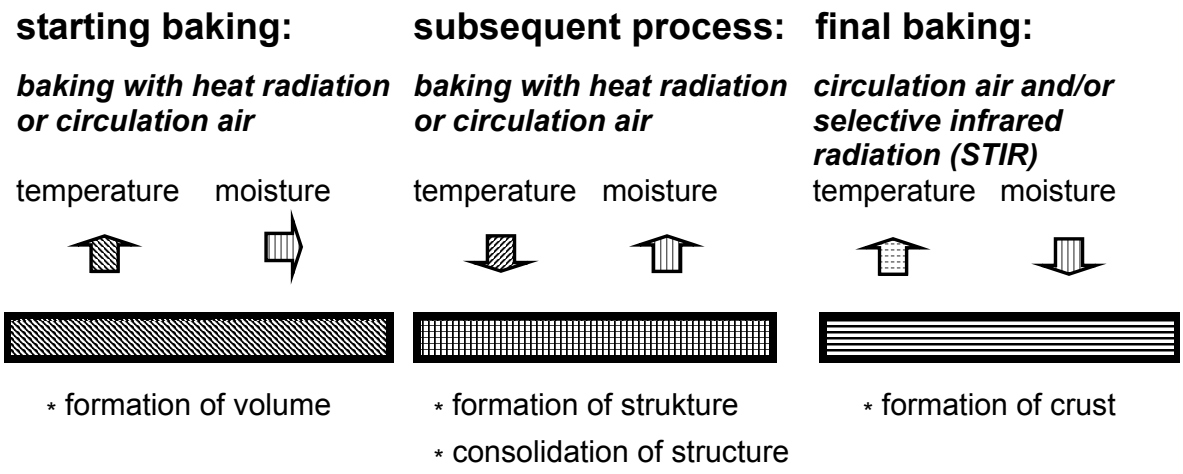


Figure 3: Expected results of the 2nd strategy (Pre) baking/(final) baking – phase baking

Baking temperature and –time are very closely interrelated. A high level of temperature has an effect on the consumption of energy and on the formation of form of the baked goods, especially of rolls [5,6].

### 2.2 Solution for brown gingerbread

Generally, for long-keeping baked goods "dry baking" should be applied in order to achieve the typical product properties. The product moisture of the traditional brown gingerbread is the result of the conditioning after baking. A division of baking into two steps is tested during baking of brown gingerbread. The development of volume and structure at high temperatures, which is necessary because of the leavening with chemical improvers, should be separated from the subsequent stabilization and dehumidification ("Dry baking")(Figure 4).

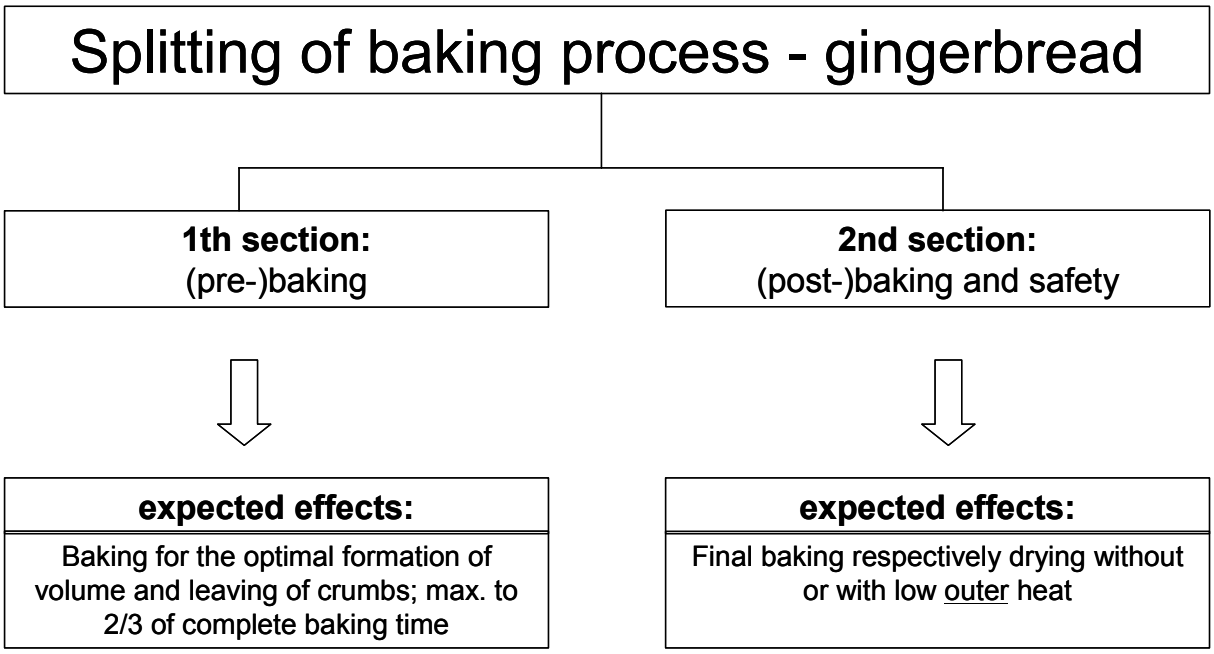


Figure 4: Strategy to reduce acrylamide formation during baking of brown gingerbread

"Dry baking" should take place without exterior heat exposure if possible. Microwave baking and vacuum technique should be applied.

Process parameters for a technical conception of the splitted baking process should be developed in a work parcel on the basis of the findings on the influence parameters and test methods.

### 2.3 Material and methods

The products were produced according to traditional recipes and with a minimum of necessary ingredients. The wheat products were produced according to the straight dough method. Improvers (malt containing wheat improver) were added only to wheat rolls. Rye bread and mixed rye bread were produced according to the sour dough method, the Detmold one-step method on the basis of starter sour and alternatively with dough acidifier. The flours of the types W 550, R 1150 and rye wholemeal and rye meal were taken from two harvest years and correspond to the usual mean standard qualities.

Brown gingerbread was prepared traditionally from a basic dough with 70 % wheat flour and 30 % rye flour, the addition of lactic acid and with a storage time of at least 3 days. **A**mmonium **b**i(-hydrogen)**c**arbonate (ABC rise, E 503i and E 503ii) and potash (potassium carbonate C 501) in a ratio 60:40 were used as raising agents. Additionally, sodium bicarbonate (E 500) and a baking powder (sodium bicarbonate E 500, disodium hydrogendiphosphate E 450i, dicalcium phosphate E 450v) were used [7].

The tests were performed on a small scale [8]. For the realization of the partly extremely different baking conditions, exclusively commercial baking ovens and aggregates including microwave ovens [9] and vacuum chamber [10] were used. Small-scale machines were used for dough making and dough handling.

For the determination of the acrylamide, the outer layer of the loaves and rolls, the crust, was cut off in a thickness of 5 mm and predried at 45 °C. In case of gingerbread the whole product was measured. For the sample treatment an interior recovery standard was added (10 µg acrylamide 2,3,3,-d3). After the suspension in water, dissolution was performed in 1-propanol by shaking and ultrasonic treatment. After centrifugation, the supernatant was rotated and given into acetonitrile. Acrylamide was determined by LC-MS-MS. Good correlation between the acrylamide contents determined by the project partners according to different methods was achieved in the framework of the preliminary project [11, 12]. The detection limit was at 20 µg/kg.

## 3. Results

A reduction of the acrylamide content of the most important bread and rolls varieties on wheat and rye basis can be achieved according to the two strategies with the corresponding baking process. All the acrylamide values are related to the crust layer of 5 mm. A differentiation under the aspect of the sensitivity of the method is not reliable satisfactorily at acrylamide values <30 µg/kg.

### 3.1 Baking according to the splitting method

#### a) Wheat rolls

Wheat rolls were produced as simple round piece as well as a split roll by dough curling. Baking in the storey oven and baking with air circulation in a shop oven /Dibas with the corresponding standard baking programmes served as control. Baking in the storey oven with selective infrared radiation was applied for post-baking after steaming (Figure 5).

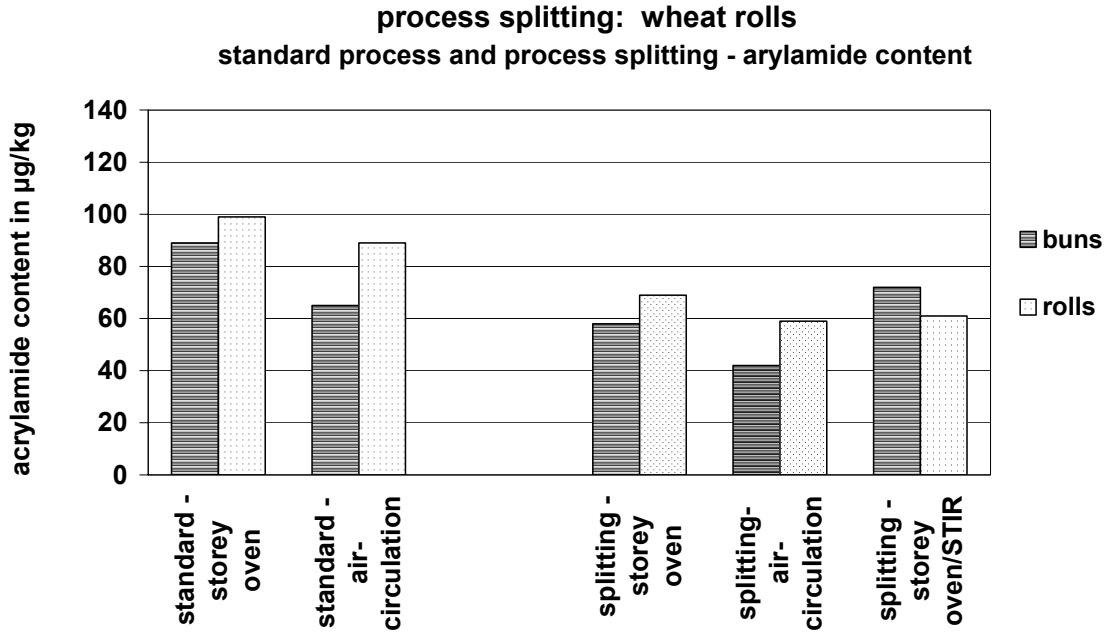


Figure 5: Reduction of the acrylamide content of wheat rolls by baking splitting in various baking ovens (STIR = storey oven with selective infrared radiation)

An average acrylamide reduction between 80 and 65 % of the initial values in wheat rolls is possible using baking splitting. Already in the standard baking process with air circulation lower acrylamide values are achieved which observation is continued in baking splitting. An intensive heat load by selective infrared radiation is detrimental to wheat rolls. This situation is observed in weakened form in wheat bread and in increased form in mixed rye bread.

### b) Wheat bread

Wheat bread was produced predominantly as oven bottom loaf. The results of acrylamide reduction of the three baking methods are compared (Figure 6).

The reduction of acrylamide in wheat bread amounted to 85 to 70 %. The acrylamide content was reduced even below 50 % in Baguette which has a smaller cross-section and a stronger development of break and shred. There are only insignificant differences between the absolute values of the processes of post-baking.

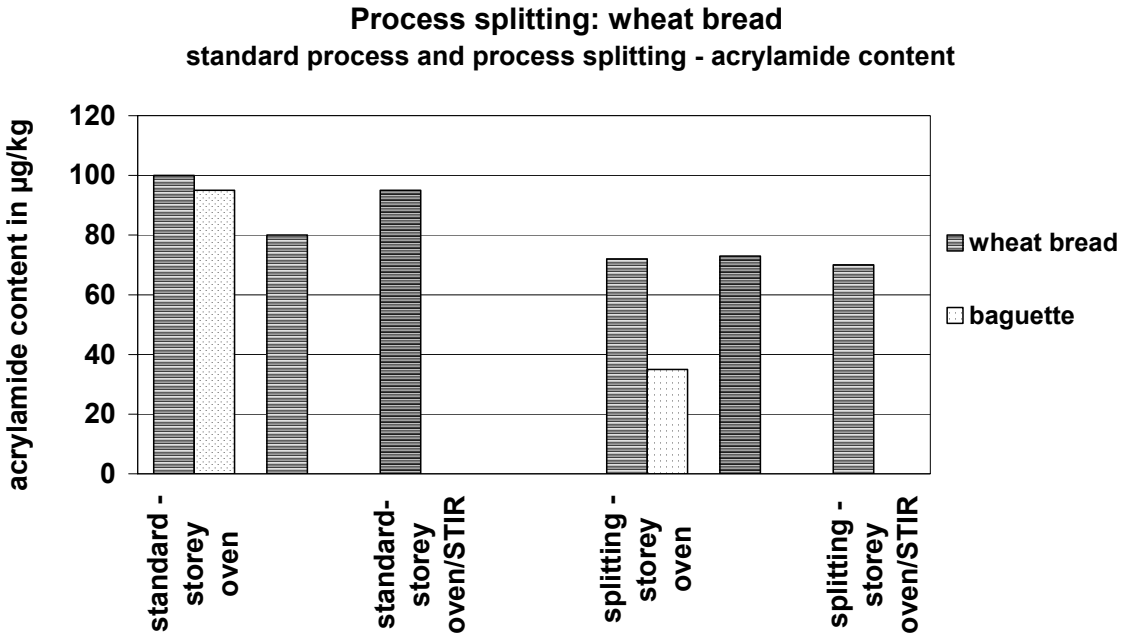


**Figure 6: Reduction of the acrylamide content of wheat bread by baking splitting in different baking ovens (STIR = storey oven with selective infrared radiation)**

### c) Mixed rye bread and wholemeal bread

Mixed rye bread with a rye ratio of 70 to 60 of the total flour is the bread variety most frequently consumed in Germany. Mixed rye bread 70/30 was tested during baking splitting after steaming in the three post-baking processes (Figure 7).

In mixed rye bread an acrylamide reduction to 70 and 80 % is possible. In case of wholemeal bread due to the higher dough moisture and the larger buffer capacity of the minerals (wholemeal doughs and wholemeal bread have a higher degree of acidity at comparable pH-values) changed milieu conditions are given which cause by far lower acrylamide contents. Under these conditions, acrylamide reductions of nearly 50 % were observed. The reaction milieu overlaps the potentially higher asparagine content of wholemeal with regard to the expected acrylamide content.

Further, significant differences were observed between dough making with acidifier and according to the sour method (Detmold one-stage method, fermentation time of the sour 18 hours). These differences were observed also with mixed rye bread and with flours of the two harvest years.

With the method of baking splitting largely equal sensory properties of the products were achieved, especially with regard to crust thickness and browning. In the loaf volume partly minor reductions were observed. Larger deviations in the volume below 85 % as against the standard baking method were observed in wheat rolls with break and shred and in Baguette the cross section of which is similar to that of the rolls, and a similar development of the break and shred.

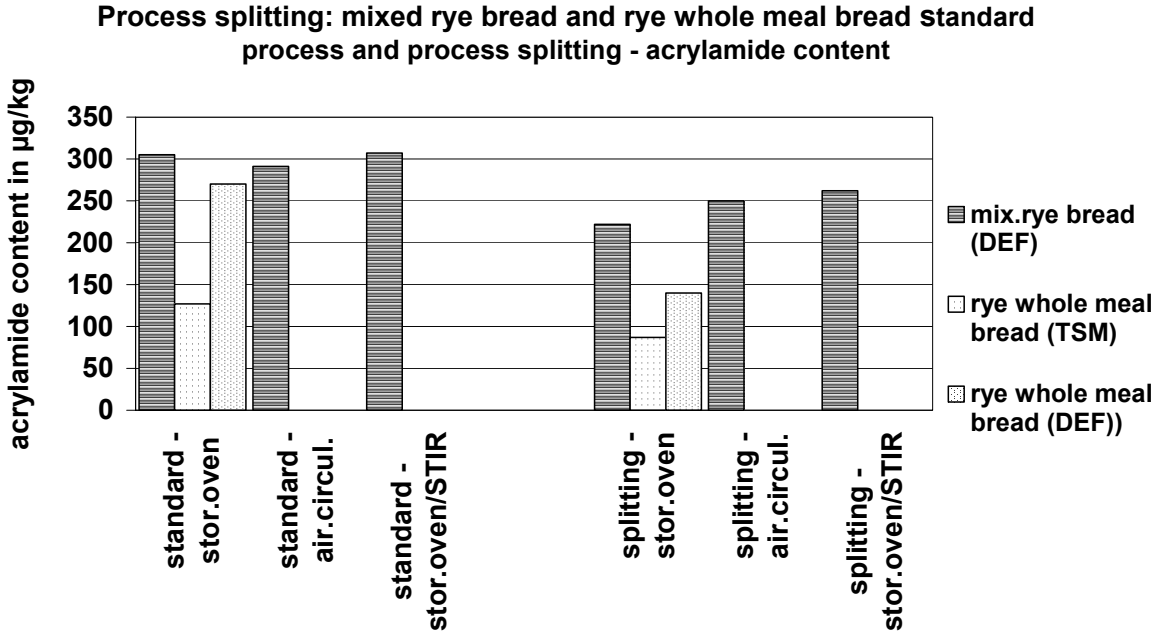


Figure 7: Reduction of the acrylamide content of mixed rye bread and whole meal bread by baking splitting in different baking ovens (STIR = storey oven with selective infrared radiation; air.circul. = air circulation /shop oven or rack oven; TSM = dough acidifier; DEF = Detmold one-stage method)

## 3.2 Baking according to the phase method

The volume reductions and the intention to further optimize the reduction of acrylamide by baking resulted in the solution of baking at very high initial temperatures defined as phase baking. The results of the different product groups are summarised in the following Figures 8 to 10.

### a) Wheat rolls

Compared with wheat rolls as buns have a lower acrylamide load as in the case of baking splitting, the differences being clearer. It should be noted that final baking of the rolls with selective infrared radiation in the low temperature range does not result in increased acrylamide values. On the one side this is due to the short baking time of the rolls. An the other side based on the physical aspects of heat radiation. The width of the wave length distribution of the heat radiation and the concentration of the energy density on preferred wave lengths (depending on the material properties of the radiator) is dependent on the temperature [13]. Only at a higher baking temperature the radiation intensity reaches a maximum in the wave length range which preferably is absorbed by the surface of the baked goods.

The achievable reductions of acrylamide are very differentiated. The reductions for rolls amount to app. 50 % after baking in storey ovens and storey ovens with selective infrared radiation (STIR). In ovens of the storey principle the products had to be re-set. After baking with circulating air an acrylamide reduction only to 95 to 85% was achieved.

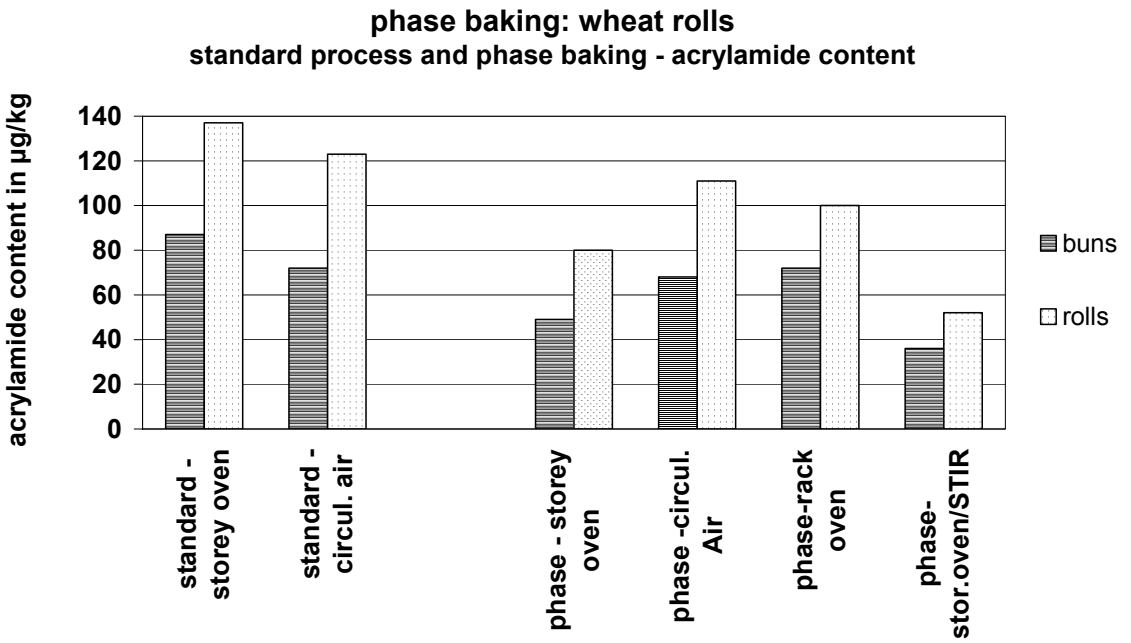


**Figure 8: Reduction of the acrylamide content of wheat rolls by phase splitting in different baking ovens (STIR = storey oven with selective infrared radiation; air.circul. = circulating air/shop oven or rack oven)**

The temperature flexibility in ovens with circulating air allows a temperature change and thus spares the re-set. However, the reduction effect is lower. As ovens with air circulation are the preferred baking aggregate for rolls, a more flexible temperature change should be possible.

### b) Wheat bread

An acrylamide reduction to 95 to 85 % is possible, i.e. a relatively low reduction (Figure 9). The best reduction is achieved in storey ovens. Despite the high temperature difference of 90 °C between pre-baking and post-baking in the rack oven acrylamide cannot be reduced considerably. An improvement is possible if the products are given into an oven of lower temperature for post-baking.

### c) Mixed rye bread and wholemeal bread

For mixed rye bread a reduction of acrylamide of 85 to 75 % is possible. The trend is observed that baking in circulating air and with selective infrared radiation results in smaller acrylamide reductions (Figure 10). There are great differences between baking wholemeal bread as oven bottom loaf (wholemeal) and as tin loaf (coarse wholemeal). Reductions of 85 to 75 % and of 75 to 50 % for tin loaves, respectively, are possible. The same trend is observed for baking in the storey oven, with circulating air, and in the storey oven with selective infrared radiation. The intensive heat radiation is not suited for loaves with a longer baking time than rolls.

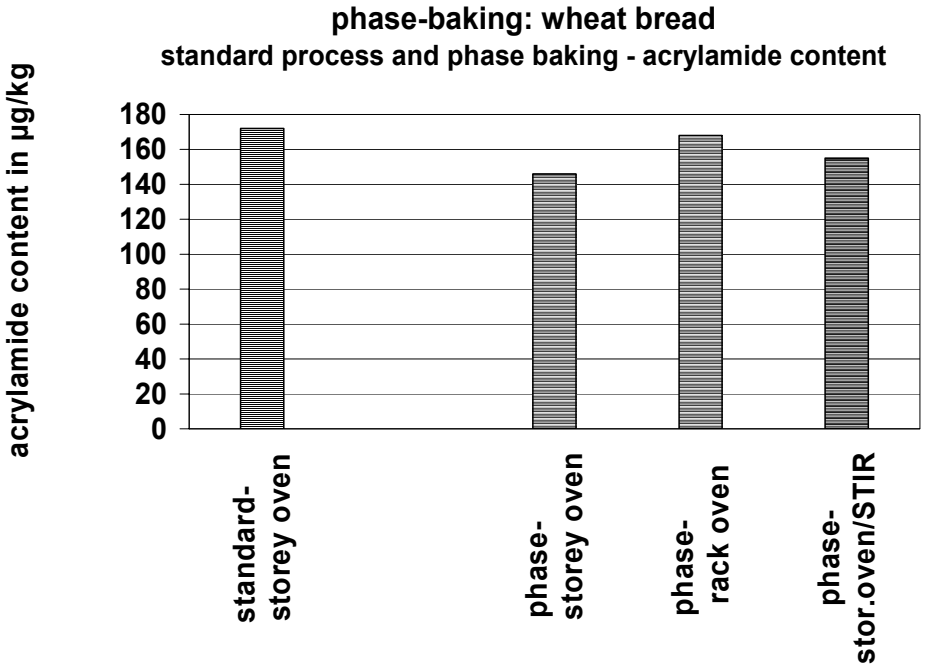


Figure 9: Reduction of the acrylamide content of wheat bread by phase baking in various baking ovens (STIR = storey with selective infrared radiation)

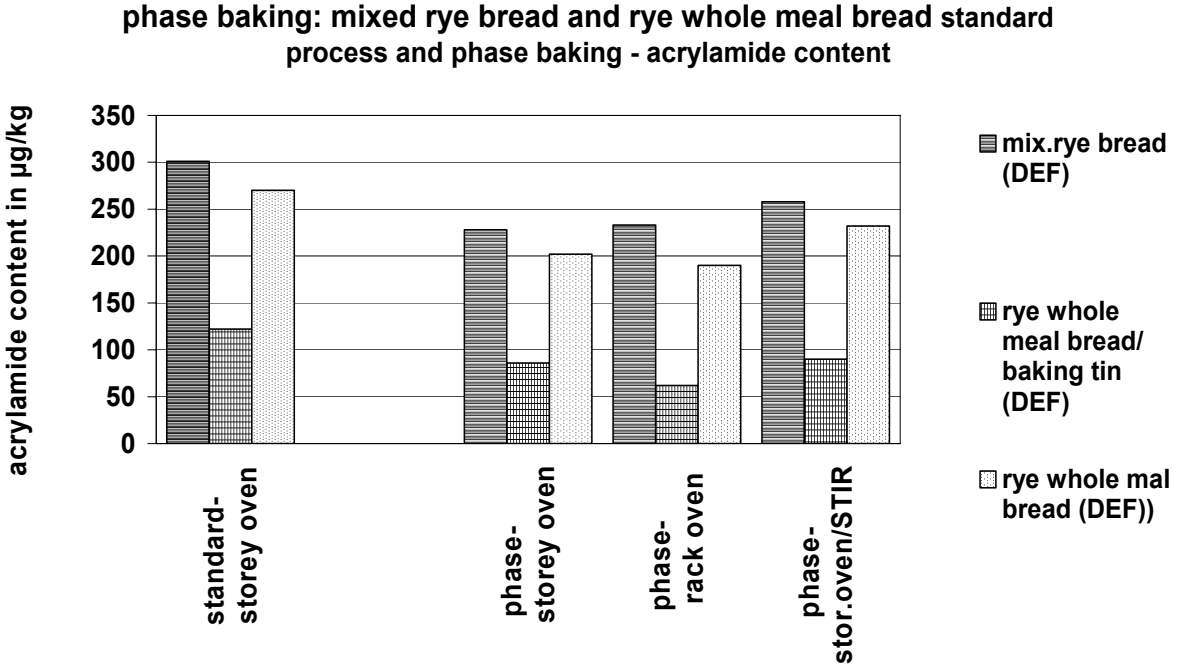


Figure 10: Reduction of the acrylamide content of mixed rye bread and rye whole meal bread by baking splitting in various baking ovens (STIR = storey oven with selective infrared radiation; DEF = Detmold one-step method)

## 3.3 Summary: Reduction of acrylamide in bread and rolls

The initial values of the acrylamide content in the outer layers of the baked products applying the standard baking process, amount to 65 to 100 µg/kg for wheat rolls, to 200 to 300 µg/kg for mixed rye bread, 70/30 depending on the kind of acidification, to 80 to 100 µg/kg for wheat bread (oven bottom loaf), and to 90 to 130 µg/kg for rye whole meal bread. The results reveal that a reduction of the acrylamide content is possible according to the two baking methods, baking splitting and phase baking. Depending on the product variety, differentiated results for the total quality and the possible reduction of the acrylamide content are achieved according to the one or the other baking method. The results are summarized in Table 1.

**Table 1: Total evaluation of the reduction of acrylamide by splitting the baking process for bread and rolls**

| Kind of product | Process splitting | | Phase baking | |
|---|---|---|---|---|
| | Volume adaptation | Acrylamide reduction | Volume adaptation | Acrylamide reduction |
| Wheat rolls, buns | ++(+) | ++(+) | +++ | +++ |
| Wheat rolls, split roll | 0 | +++ | +++ | ++(+) |
| Wheat bread, Baguette | 0 | +++ | n.d. | n.d. |
| Wheat bread, loaf bread | +++ | ++ | ++ | + |
| Mixed rye bread 70/30[(1)] | +++ | +(+) | +++ | +(+) |
| Mixed rye bread 70/30 pan bread[(2)] | n.d. | n.d. | ++…+++ | 0…+++ |
| Rye whole meal, loaf bread[(3)] | ++ | +++ | ++ | ++ |
| Rye meal bread, pan bread | n.d. | n.d. | ++ | ++(+) |
| [(1)]dependent on the use of dough acidifier/sour method<br>[(2)]dependent on the tin material<br>[(3)]dependent on the use of dough acidifier/sour method | | | | |
| Evaluations compared with the standard process | | | | |
| Characteristic | Scale | | | |
| | +++ | ++ | + | 0 |
| Relative volume | | | | |
| Achieved approach to the standard | 100-95 | 95-90 | 90-85 | <85 |
| Relative acrylamide content | | | | |
| Achieved reduction to | <70 | 70-80 | 80-90 | 90-100 |

The tests were performed such as always, the same condition as in common standard baking processes was achieved. The differentiation of the total quality is reflected mainly in the volume, largely ensuring an equal crust thickness and browning. Regarding the achievable product volume, phase baking is of advantage because of the high initial baking temperature. In no case special bread and roll varieties were used. However, the potential of acrylamide reduction, abandoning the same browning and crust thickness, posssibly is not fully exhausted.

The values of the total product were only partly determined because in the interest of a better differentiation the determination of acrylamide is related to the outer side of the product and the minimization potential should be shown. Acrylamide values of the whole product are of importance for the consumer. Selected experimentally determined values for the whole product are given in Table 2.

**Table 2: Comparison of experimentally determined acrylamide contents of the outer side and of the whole product**

| Product variety | Process | AA-content (µg/kg)/Individual tests | |
|---|---|---|---|
| | | Outer side of the product | Total product |
| Wheat rolls, buns and rolls | Standard/storey | 112 | 49 |
| | Splitting/storey | 69 | 16 |
| Wheat bread, loaf | Standard/storey | 100 | 17 |
| | Splitting/storey | 72 | 11 |
| Mixed rye bread (Detmold process) | Standard/storey | 276 | 52 |
| | Splitting/storey | 201 | 38 |
| Rye wholemeal bread, loaf | Standard/storey | 127 | 39 |
| | Splitting/storey | 87 | 22 |

With regard to the technical feasibility, phase baking has lower requirements for the realization of the highly different climate conditions during baking. The decision on one of the baking methods is very complex and requires the consideration of the production conditions and of economic aspects.

### 3.4 Possibilities to reduce acrylamide in brown gingerbread

"Brown gingerbread plain" was tested. It was produced of 90 parts of a mixture of sugar varieties (invert sugar cream and glucose sirup DE° 40) related to 100 parts flour mixture (30 % rye flour type 1150 and 70 % wheat flour type 550) and for leavening a combination of potash and ABC leavener [14]. Gingerbread leavener was not used. The process corresponds to a small-scale production with short storage time of the basic dough. For achieving a good conditioning ability sufficiently high initial temperatures during mixing of the basic dough of app. 45 °C, a sufficient storage time for swelling of the ingredients of at least 3 days and a very dry baking are necessary [15]. Gingerbread as all dry flat baked products is baked without clear differentiation between crumb and crust. Acrylamide is formed in the whole product. Acrylamide was determined in the conditioned product.

#### a) Starting data

During baking of brown gingerbread according to a standard recipe the acrylamide content varies in independent tests by up to ±20 % (Table 3). The variations are resulting from the extremely strong reaction of the system to minor milieu variations during the preparation and baking. The absolute value for acrylamide is above the observed value of the 7$^{th}$ signal value determination [3]. Recipe modifications and material measures were disregarded because the acrylamide reduction by the baking prodcess should be determined.

Table 3: Starting data of the production of brown gingerbread (control tests, two flour batches, n = 13)

| Statistical figure | Storage time | pH Values | | | Size of the product | | Acrylamide |
|---|---|---|---|---|---|---|---|
| | | Storage dough | Basic dough | Baked product | Height | Width | |
| | in days | | | | in mm | in mm | in µg/kg |
| Mean | 7 | 5.19 | 8.03 | 5.98 | 18.6 | 70.9 | 3.839 |
| Highest | 21 | 5.59 | 8.33 | 6.51 | 20.6 | 74.5 | 4.918 |
| Lowest | 3 | 4.65 | 7.53 | 5.48 | 16.1 | 65.2 | 2.876 |
| Variance | | 0.27 | 0.26 | 0.33 | 1.6 | 2.7 | 706 |

### b) Splitting of the baking process and post-baking by microwave energy

After (pre-) baking in the storey oven or alternatively with circulating air in the shop oven Dibas, (post-) baking for the stabilization and drying to the baking moisture is performed in a commercial microwave and a combined circulating air/microwave oven on the basis of the Dibas shop oven (Figures 11 and 12).

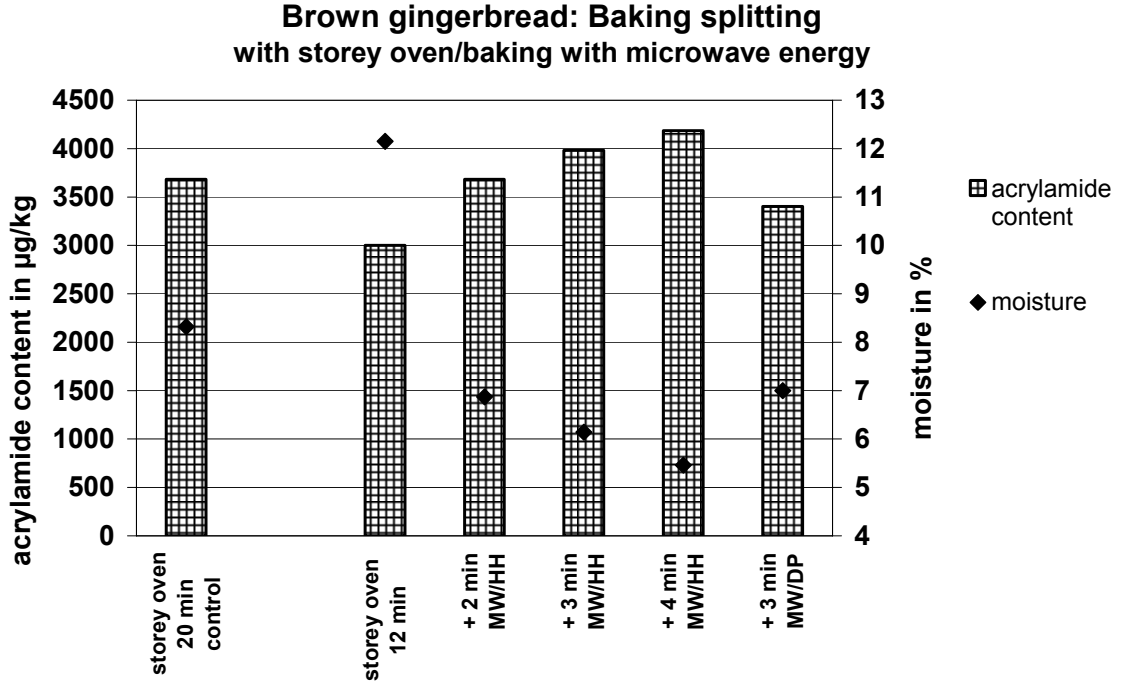


Figure 11: Splitted baking of brown gingerbread: First baking step 12 min in the storey oven and continuation with microwave energy (HH = household/industrial microwave; DP = Dibas power microwave oven) – Acrylamide and moisture values at increasing microwave effect

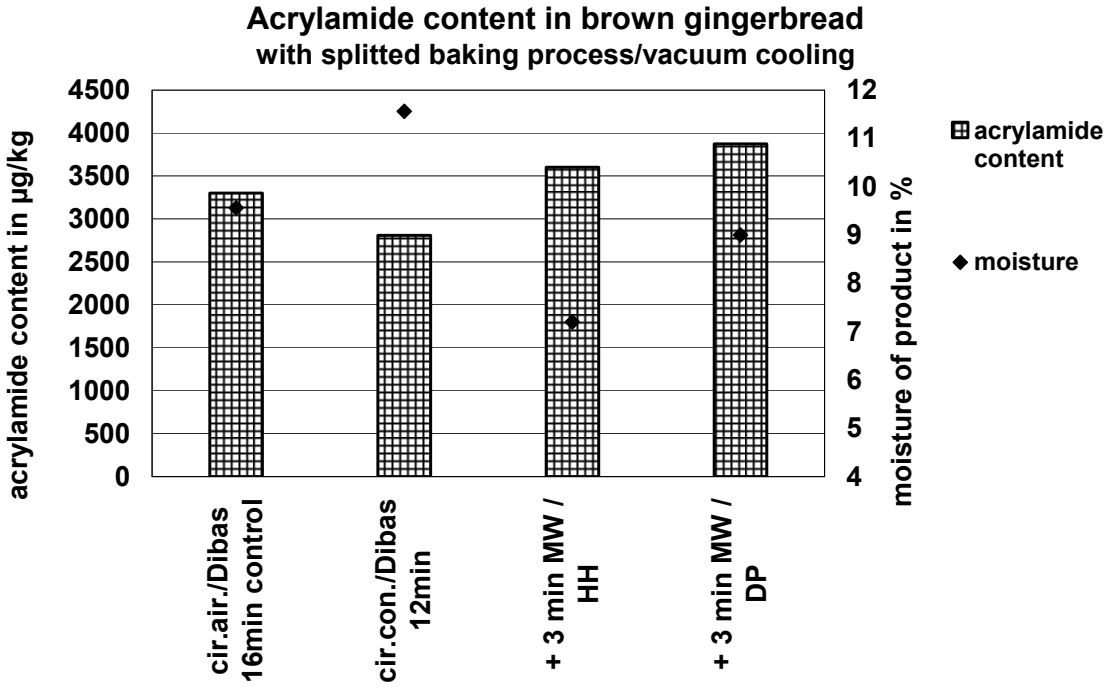


Figure 12: Splitted baking of brown gingerbread: First baking step 12 min in the circulating air oven/Dibas and continuation with microwave energy (HH = household/industrial microwave oven; DP = Dibas power microwave oven) - Acrylamide and moisture values

Post-baking with microwave energy ensures a sufficient dehumidification and stabilization of the products, but at the same time causes a further increase of the acrylamide formation to nearly 140 %. In the two processes of prebaking, in the storey oven and during baking with circulating air in the Dibas, the initial value of acrylamide is too high. Shorter baking times and lower temperatures in the first baking step do not provide the necessary leavening and the typical porosity and generally the stability of the products. The poor and untypical browning involved cannot be corrected by a longer application of microwave energy. At a too short pre-baking time sensory faults in the chewability and solubility will occur. Therefore, the application of microwave energy in the second baking step despite different processes is not suited. The preliminary tests do not confirm these results.

### c) Baking splitting and post-treatment by vacuum

For reasons of work organisation for the baking ovens used, pre-baking is performed in a rack oven. After the first baking phase the loaded rack is given into the vacuum chamber. In the first baking phase of 12 min at slightly reduced baking temperature the higher air circulation and the full load of the rack oven will cause a reduction of the acrylamide content by app. 800 µg/kg as compared with the circulating air/shop oven (Figure 12). As a consequence of the changed pre-baking the browning as of the initial product is not achieved. Vacuum treatment varies in three programmes which differ in the speed of vacuum development (slowly and flat with reduced pumping capacity, quickly and steep with increased pumping capacity, and combined vacuum curve), in the final pressure and the hold-up time (Figure 13).

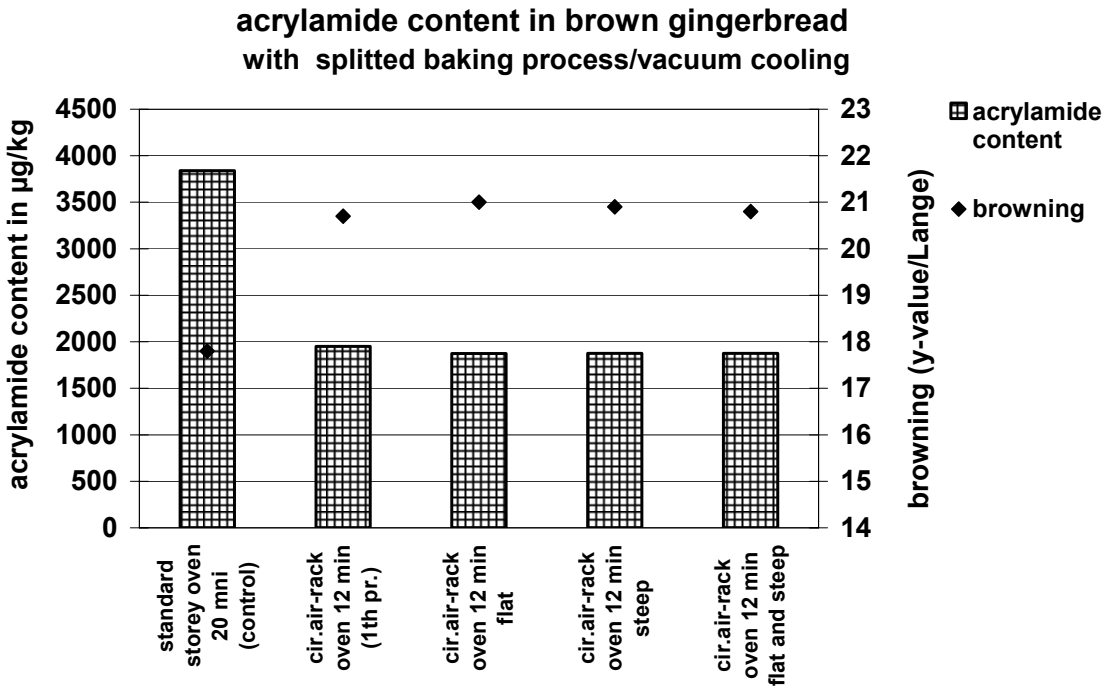


Figure 13: Acrylamide formation and browning of brown gingerbread during splitted and reduced baking with circulating air and subsequent vacuum treatment

The acrylamide values of the three characteristic vacuum programmes do not show any differentiations as against baking in circulating air after a baking time of 12 min. The most favourable dehumidification and stabilization is reached at a flat/steep vacuum development with a long hold-up time. In all the vacuum programmes the volumes are reduced because despite the vacuum the product stabilization in the first baking phase with the aim of less acrylamide is insufficient. As the dehumidification takes place without thermal impact, no further acrylamide is formed, however, a reduction is not possible at these reaction conditions.

Concerning the sensory properties the gingerbread has a good leavening and porosity and product typical properties, but the touch and chewability are rather solid after conditioning despite product moisture contents between 15 and 16 %.

On balance of the tests to reduce the acrylamide content of brown gingerbread by changing the baking process the result was: in each case, the acrylamide load after (pre) baking is too high. The reduction of (pre) baking has an adverse effect on the product stability and causes sensory damages. (Post) baking does not reduce acrylamide.

### d) Influence of conditioning and storage time

During conditioning and storage the acrylamide content of brown gingerbread is not constant. In case of direct conditioning by water coating and subsequent drying a temperature-dependent reduction of the acrylamide content is observed up to a storage temperature of 80 °C (Figure 14). For an actual reduction of acrylamide this effect at a high initial value is rather low.

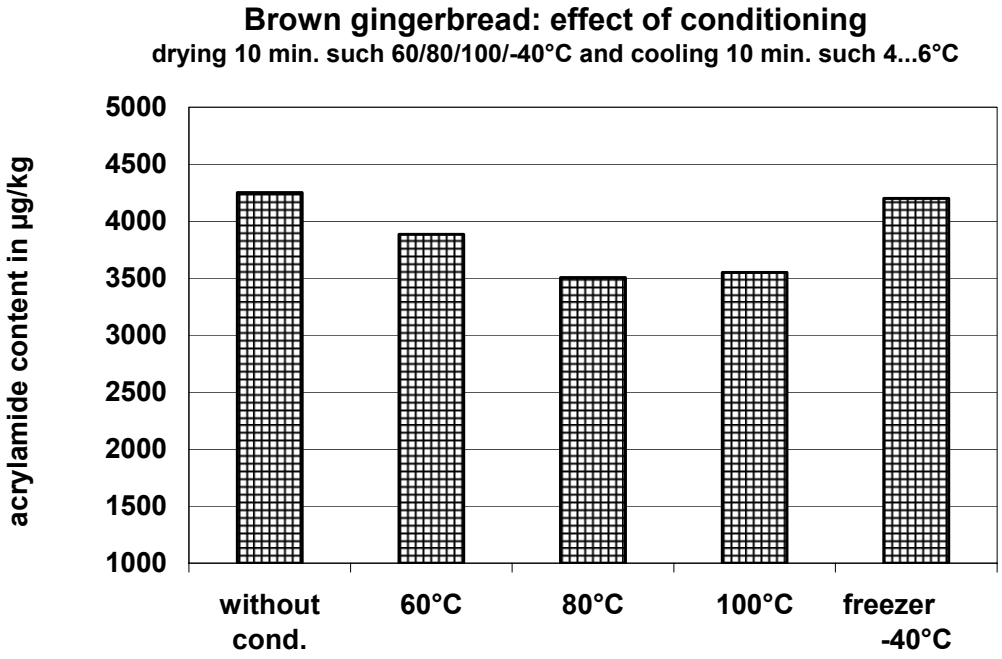


Figure 14: Brown gingerbread: change of acrylamide during direct conditioning with various temperatures and during deep-freezing

Indirect conditioning common in practice, with increasing conditioning time facilitates a reduction of the acrylamide content of >500 µg/kg. At the same time, this reduction is connected with a change of the pH value. At a high initial level this reduction is insufficient (Figure 15).

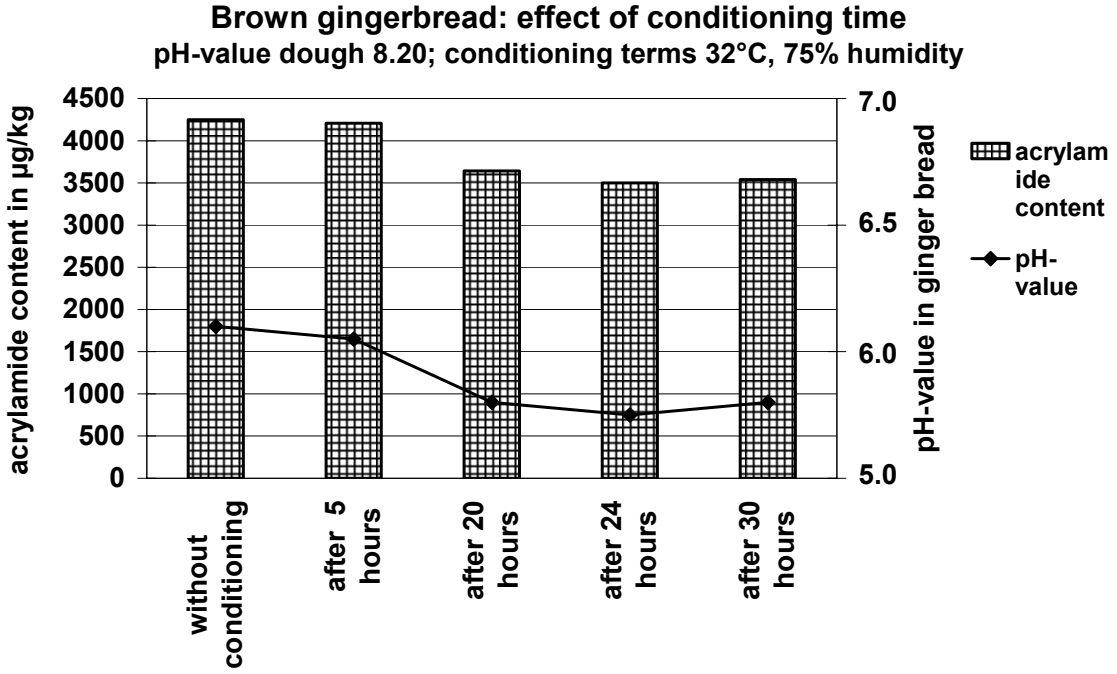


Figure 15: Brown gingerbread: Change of acrylamide at indirect conditioning and change of pH value after various conditioning times

A (hot) storage of the standard gingerbread at 20 °C reduces the acrylamide content up to 1,000 µg/kg and at 37 °C up to 2,000 µg/kg within 12 weeks. A long-time storage at 20 °C of more than 32 weeks reduces the acrylamide content up to 3,500µg/kg. The increase of the acrylamide content at freeze storage is an unexpected phenomenon (Figure 16). Despite the high reduction of acrylamide a long-time storage is neither effective nor economical, can, however, be applied. The increase during freeze storage was confirmed by other teams but cannot be explained.

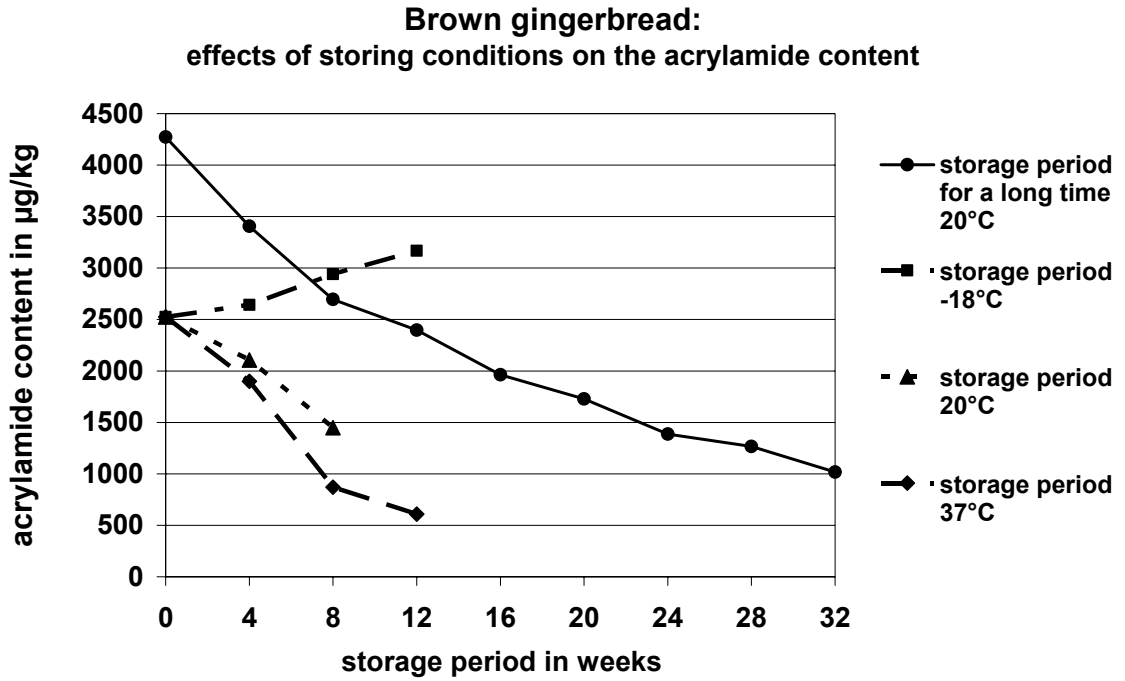


Figure 16: Brown gingerbread: change of acrylamide at various storage conditions

### e) Influence of the replacement/substitution of the leavening agent

A leavening agent which is based on various salts of the ammonium ion $NH_4^+$ (E503i and E503ii) is regarded to be the main cause of the formation of acrylamide in gingerbread and biscuits. The leavening agent is **a**mmonium **h**ydrogen **c**arbonate shortly ABC or AHC leavening or commercial ammonium carbonate as trivial name. The leavening agent can be a mixture of ammonium carbonates and the ammonium compound of carbonic acid and carbamic acid (amino methane acid or monoamide of carbonic acid) [14]. Carbamic acid is very instable, decomposes spontaneously to ammonia and carbonic acid and *de facto* exists only in its salts.

The tests to substitue the ABC leavener by a recommended baking powder were performed without any change of the baking conditions. They are closely connected with the change of the pH value (Figure 17). The substitution of only 50 GT of the ABC leavener is without any effect to the reduction of acrylamide.

The results show that an effective reduction of the content of acrylamide to 10 to 12 % is possible by substitution of the ABC leavener; however, the gingerbread loses its typical characteristics. More favourable sensory results are achieved if instead of baking powder with an acid component only sodium hydrogen carbonate (E 500) is used.

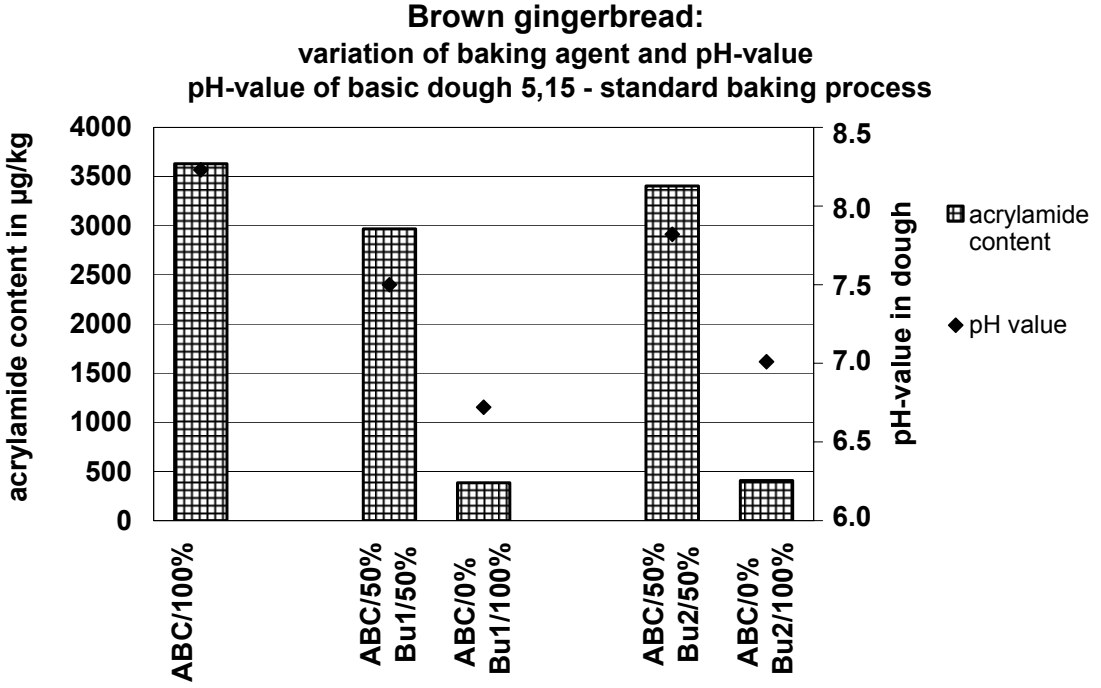


Figure 17: Substitution of ABC leavener by baking powder keeping the part of potash (Bu 1 = baking powder 1 with E 500 and E 450i; Bu 2 = baking powder with E 450i and E 450v [16])

Further tests with the substitution of leaveners and additional influence of lactic acid and sodium hydroxide solution on the pH value confirm the great importance of the pH value. Acrylamide formation also depends considerably on the presence of ABC leaveners and ammonium ions and their carbonates despite the "correction" of the pH value.

The typical character of gingerbread is bound to reducing sugars, especially fructose, and to the alkalizing taste [17]. A certain potential to reduce acrylamide, connected with the influence of the pH value, gains in efficiency only if the system is free from ammonium nitrogen. If the hypothesis of the independence of the formation of acrylamide from ammonium nitrogen is accepted [18], the ABC leavening must bring about a significant reaction supporting effect. This does not only depend on the pH value which is confirmed also in the CIAA research report [19]. The substitution of ABC leavener is of importance not only for gingerbread but also for many other biscuits [20]. A total substitution in these products is deemed infeasible [21].

The formation mechanism after the reaction of ammonia with a carboxylic acid derivative is conceivable but requires for the carboxylic acid derivative a basic body of corresponding structure. The carboxylic acid derivative could originate from lactic acid by cleavage of the hydroxyl group with an additional hydrogen atom in form of a water molecule. Formation mechanisms did not have been subject of the project. Upon the meaning of Amrein [22], the ammonia released from ammonium hydrogen carbonate has a "promoting effect" for the formation of acrylamide from hexoses with asparagine. The cause of the supporting effect of $NH_4^+$-ions and the inhibitary effect of $Ca^{2+}$-ions is not clear [23].

Tests with wholemeale rye bread with gingerbread-typical ingredients as model system confirm the role of the pH value but also the promoting effect of the ammonium carbonates on the formation of acrylamide. For the reduction of the formation of acrylamide the two methods, i.e. substitution of leaveners and a moderate influence on the pH value, are feasible and applicable with insignificant adaptation of the recipe.

### f) Influence of the enzymatic degradation of asparagine

The formation of acrylamide in brown gingerbread also can be influenced by the use of enzymes for the degradation of asparagine as accepted precursor [24, 25,26].

The addition of the enzyme to the basic dough in the dosage given by the producer results in a reduction of asparagine up to 5 % after a storage time of three days (Table 4). The pH value of the basic dough is below the optimal range of effectiveness of the enzymes. The optimal storage temperatures of <60 °C can be reached in the storage dough only for a short time or not at all. The extension of the storage time is possible to a larger scope. Differences can occur between the commercial samples. The degradation of asparagine in gingerbread results in a significant reduction of the acrylamide up to <10 % of the initial value (Figure 18). Keeping the ABC leavener and potash, app. 250 µg/kg remain in the product. Thus the reduction potential is not fully exhausted and can be completed by substitution of leavener and optimization of the recipe.

**Table 4: Asparagine/aspartic acid in initial flour and asparagine degradation in the basic dough**

| Raw material/Intermediate product | Asparagine<br>mg/kg | Aspartic acid<br>mg/kg |
|---|---|---|
| Flour mixture 70 % rye 1150/30 % wheat W 550 | 210 | 168 |
| Gingerbread basic dough without Enzyme | 67 | 87 |
| Use of enzyme A<br>Use of enzyme B<br>Use of enzyme C | 30<br>4<br>4 | 101<br>123<br>125 |

The pH value conditions are rather unfavourable for the use of the at present allowed and available enzymes in the main dough. The optimal temperature range is exceeded during the start of baking. The addition of enzymes to the main dough causes a slight reduction of acrylamide, anyhow reaches app. 1/5 of the initial value.

The enzyme additions in the basic dough cause only marginal sensory changes. Small differences are observed in the texture and chewability which for all the enzymes were softer and had a slightly reduced solubility. In case of addition to the main dough there were no untypical changes and also the trend of a slightly reduced solubility. The use of enzymes in the basic dough is more practicable. This has to be preferrred because of the higher potential of acrylamide reduction.

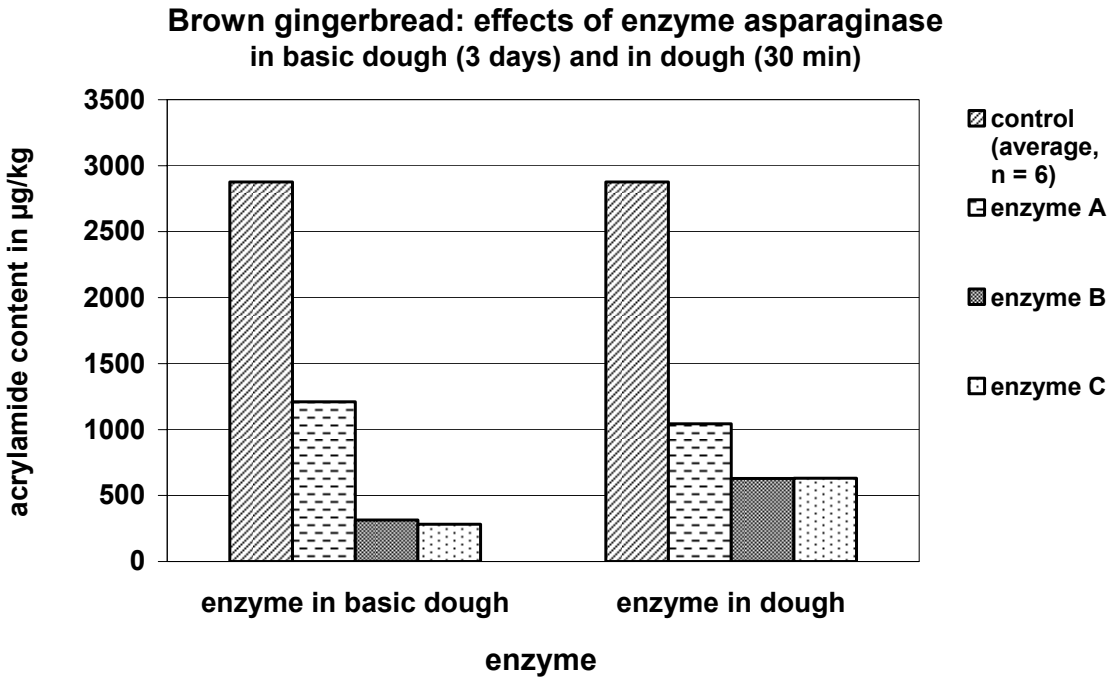


Figure 18: Formation of acrylamide in brown gingerbread using commercial asparaginases in the basic dough and main dough

The results in Chapter e) show that the pH value is a major factor. From ABC-leavening is a specific effect. But the trials of degrading enzymes confirm the recognized effect of the Precursor Asparagins compared to other material factors.

## 3.5 Summary – Reduction of acrylamide in brown gingerbread

The formation of acrylamide in brown gingerbread with ABC leavening and potash cannot be reduced efficiently only by changing the baking process. The high temperatures which are necessary for a rapid gas development and leavening at the beginning of baking, result in a relatively high formation of acrylamide in the first half of baking. The subsequent stabilization and dehumidification can be performed successfully at completely changed process conditions, but does not result in a change of the acrylamide level observed. Beside an exact adaptation of the baking conditions in the interest of a good leavening, the content of acrylamide in gingerbread can be reduced exclusively by material measures – addition of asparaginases and substitution of leavening agents, respectively. In case of substitution of the leavening agent, sensory changes of the character of gingerbread are unavoidable.

## 3.6 Baking parameters for the technical realization

The process technical parameters resulting from the baking results of the solution strategies for the process splitting (combined steaming and (post) baking) are summarized in Table 5 for wheat products and in Table 6 for rye products.

Table 5: Baking parameters - strategy 1 ("process splitting"): wheat bread and -rolls

| 1st step: (baking-)steaming | 2nd step: (post-)baking |
|---|---|
| **baking equipment** (for laboratory tests)<br>combisteamer<br>3 storey<br>standard baking tray 400 x 600 mm | **baking equipment** (for laboratory tests)<br>storey oven<br>rack oven (rotat.)<br>circulation air/shop oven<br>storey oven with STIR |
| Baking parameters (generally) wheat baked goods | |
| temperature:<br>100°C starting/from 100°C to 150°C increasing<br>dewpoint: app. 100°C decreasing<br><br>contingent of baking time[1)] 70/100 | temperature:<br>220 to180°C wheat rolls; 200 bis180°C wheat bread<br>steaming….damper opened<br>dewpoint at the end of baking < 80°C<br>contingent of baking time[1)] 30/100 |

[1)] Total baking time elongated, normal baking time 18 min wheat rolls, 25 min wheat bread

Table 6: Baking parameters - strategy 1 ("process splitting"): rye- and mixed rye bread

| 1st step: (baking-)steaming | 2nd step (post-)baking |
|---|---|
| **baking equipment** (for laboratory tests)<br>combisteamer<br>3 storey<br>standard baking tray 400 x 600 mm | **baking equipment** (for laboratory tests)<br>storey oven<br>rack oven (rotat.)<br>storey oven with STIR |
| Baking parameters (generally) rye baked goods | |
| temperature:<br>120°C /170°C to 150°C increasing<br>dewpoint : app. 90°C decreasing<br><br>contingent of baking time[1)] 60/100 | temperature:<br>240°C to 230°C to 210°C decreasing<br>no steaming … damper opened<br>dewpoint at the end of baking < 80°C<br>contingent of baking time[1)] 40/100 |

[1)] Total baking time elongated, normal baking time 40 min

The process technical parameters resulting from the baking results of the solution strategies for the phase baking are summarized in Table 7 for wheat products and Table 8 for rye products.

The most important development potential for baking ovens which facilitate low-acrylamide baking is the realization of baking conditions which more clearly must differ on from the other. The technically practicable handling requires the integration of the differentiated functions into a compact baking oven. For

discontinuous baking ovens in which the product handling is based on the rack principle, the development potential is focussed on the integration of very different process conditions with regard to steaming and baking. For continuous processes problems of making proof the steaming and baking zones in the tunnel oven or in case of connecting two aggregates energy saving seal from environment.

**Table 7: Baking parameters - strategy 2 ("Phase baking"): wheat bread and -rolls**

| 1st step: (pre-)baking | 2nd step: (final-)baking |
|---|---|
| **baking equipment** (for laboratory tests)<br>storey oven<br>circulating air (fixed)/Dibas [shop oven]<br>changing of oven optional | **baking equipment** (for laboratory tests)<br>storey oven<br>circulating air (fixed)/Dibas [shop oven]<br>rack oven (rotat.)<br>storey oven with STIR |
| Baking parameters (generally) wheat baked goods | |
| temperature:<br>270°C storey oven / 250 to 230°C circulating air<br>steaming before starting, damper closed<br>dewpoint < 85°C<br><br>contingent of baking time[1)] 40/100 | temperature:<br>180°C storey oven with STIR / 160 to 175°C circulating air<br>no steaming ... damper closed<br>dewpoint increasing to 90°C<br><br>contingent of baking time[1)] 60/100 |

[1)] Total baking time app. 2 min elongated

**Table 8: Baking parameters - strategy 2 ("Phase baking"): rye- and mixed rye bread**

| 1st step: (pre-)baking | 2nd + 3rd step: (final-)baking |
|---|---|
| **baking equipment** (for laboratory tests)<br>storey oven<br>changing of oven optional | **baking equipment** (for laboratory tests)<br>storey oven<br>rack oven (rotat.)<br>storey oven with STIR |
| Baking parameters (generally) rye baked goods | |
| 1st step: temperature high:<br>300°C<br>steaming before starting<br>2 min damper closed/5 min damper open/ damper closed<br>dewpoint < 85°C<br>contingent of baking time[1)] 20/100 | 2nd step: temperature low:<br>180°C to 170°C storey oven<br>no steaming ... damper closed<br>changing of oven optional |
| | 3rd step: temperature (optional) high:<br>210°C to 220°C storey oven/rack oven/storey oven with STIR<br>no steaming ... damper closed<br>contingent of baking time[1)] 60…70/100 (2.),10…20/100 (3.) |

[1)] Total baking time elongated

For decisions in mechanical engineering a variety of direct or indirect economic factors play an important role. The cost factor remained ignored.

## 4. Summary

### a) Bread and rolls

The separation of the baking process into various phases with low initial temperature by baking/steaming and baking for crust development (baking splitting: strategy 1) as well as alternatively with significantly increased initial temperature and final baking at low temperature and corresponding humidity of the baking chamber (phase baking: strategy 2) has proven that a reduction of acrylamide in the main bread and small varieties of wheat and rye is possible.

For wheat rolls splitting of the baking process results in a mean reduction of acrylamide to 66 to 70 %, and phase baking in a reduction to 56 to 65 %. For mixed wheat bread the minimization averages up to 78 % (baking splitting) and 91 % (phase baking). When both methods are applied, mixed rye bread reaches a level of app. 80 % of the initial value of acrylamide. For wholemeal rye bread baking splitting results in a reduction of app. 61 % and phase baking in a reduction of app. 77 %. For rye products there are differences between the sour dough method and dough acidifiers. The contamination of tinned loaves is lower than that of oven bottom loaves.

### b) Brown gingerbread

It is not possible to reduce the formation of acrylamide in gingerbread efficiently with ABC leavening and potash by changing the baking process alone. The acrylamide content of gingerbread can be reduced by an exact adaptation of the baking conditions for a good leavening as well as by the use of material factors, i.e. asparaginases and substitution of leavening agents. Sensory changes of the character of gingerbread are unavoidable.

The content of acrylamide can be influenced by conditioning and storage. The variation of the conditioning can result in a reduction of up to 500 µg/kg acrylamide.

The use of enzymes for the degradation of asparagine as accepted precursor is a further solution to influence the formation of acrylamide in brown gingerbread. The addition to the basic dough results in a significant reduction of acrylamide in gingerbread to app. 10 % or 250 µg/kg, keeping the ABC leavening and potash. The addition of enzymes to the basic dough is technologically more feasible. Further reduction potential is possible by the substitution of leavening agents and infuencing the pH value.

## REFERENCES


[1] GMF Vereinigung Getreide-, Markt- und Ernährungsforschung 2007 (Internet: gmf-info 2000-2008)

[2] Umweltbundesamt: Stellungnahme der Kommission Human-Biomonitoring: Acrylamid und Human-Biomonitoring. - Bekanntmachung des Umweltbundesamtes, in: Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz 1/2008; S. 98 ff

[3] 7. Berechnung der Acrylamidsignalwerte. - Rundschreiben BLL-025-2008; Bund für Lebensmittelrecht und Lebensmittelkunde 2008

[4] John, P: Neue Zeiten - Die neue S & Q-Technologie verkürzt die heute bekannten Backzeiten drastisch. - Back-Journal - Neue Technik (2003)9, S. 24

[5] Kriems, P. u. Ch. Hermann: Niedrigtemperaturbacken von Roggenmischbrot. - Getreide, Mehl, Brot 51(1997), S. 287

[6] Kaiser, H: Einfluss der Backbedingungen und des Wassergehaltes auf das Backen von Rühr- und Sandmassen. - Getreide Mehl und Brot 56(2002)1, S. 44 - 51

[7] Sicherheitsdatenblatt 31 (Handelsname N 54-82 [Dinatriumdihydrogenpyrophosphat] v. 04.07.2007; Handelsname C 52-80 [Dicalciumphosphat] v. 14.08.2007). - Chemische Fabrik Budenheim

[8] Seitter, M., M. ,Kuhn, H.-G. Ludewig, J.-M. Brümmer: Handwerkliche Herstellung von Braunen Lebkuchen. - Arbeitsgemeinschaft Getreideforschung e.V., 8. Tagung für Konditorei-Technologie Detmold 2002

[9] ABZ: Mikrowellentechnik intergriert im Heißluftofen - Prototyp "Dibas-Power" von Wiesheu. - ABZ 63(2008)2, S. 9

[10] Ziegler, Th.: Prozessoptimierung durch Simulation - Vakuumkühlung mathematisch modelliert. - Brot und Backwaren 54(2005)10, S. 42 ff

[11] AiF-FV ZGB 108, in: Development of New Technologies to Minimize Acrylamide in Food. - BLL Bonn 2005

[12] Springer, M. , Th. Fischer, A. Lehrack u. W. Freund: Acrylamidbildung in Backwaren. - Getreide, Mehl und Brot 57(2003), S. 274-278

[13] v. Böckh, P.: Wärmeübertragung - Grundlagen und Praxis. - Springer Verlag Berlin, Heidelberg, New York 2004

[14] Richtlinien für Backtriebmittel, Backpulver, Hirschhornsalz und Pottasche / Gutachten v. 14.03.1962. - Schriftenreihe des BLL Heft Nr. 41, Behr's Verlag Hamburg, Berlin, Düsseldorf 1962

[15] Ludewig, H.-G.: Grundzüge der Herstellung von Feinen Backwaren, in: Handbuch Backwaren, Kap. 3.6.2, 5. Aktualisierung 1998

[16] Phosphate zur Reduzierung von Acrylamidgehalten. - Chemische Fabrik Budenheim 2005

[17] Hanneforth, U.: Herstellung von Feinen Backwaren - Feinteige ohne Hefe, in: Seibel (Hrsg.) Feien Backwaren. - Behr's Verlag Hamburg, 2. Auflage 2001

[18] Amrein, Th.M.: Mitigation of acrylamide in food: Research at ETH Zürich. - "Workshop on Acrylamide" on 16/17 March 2006 in Brussels

[19] Anklam E. and Th. Wenzl: Acrylamide: Update on Selected Rersearch Activities Conducted by the Europran Food and Drink Inustry - A Survey of Two Years of Reseaarch; J. of AOAC International Vol. 88, No. 1. 2005, S. 234 ff

[20] Grothe, K.-H., G. Unbehend u. N. U. Haase: Einfluss von Backtriebmitteln auf die Acrylamidgehalte von Braunen Lebkuchen und Mürbkeksen. - Getreidetechnologie 59(2005)3, S. 163 ff

[21] Grob, K.: Reduction of Exposure to Acrylamide: Achivements, Potential of Optimization, and Problems Encountered from the Perspectives of a Swiss Enforcement Laboratory. - J. of AOAC International Vol. 88, No. 1. 2005, S. 253 ff

[22] Amrein, Th.M., B. Schönbächler, F. Escher and R. Amado: Acrylamide in Gingerbread: Critical FActors für Formation and Possible Ways for Reduction. - J. Agric. Food Chem. 2004, 52, 4282 - 4288

[23] CIAA: The CIAA Acrylamide "Toolbx", Annex 1 to FCP/229/06E, 29.09.2006

[24] Clauß, A., A. Schieber und R. Carle: Acrylamid reduzieren. - Brot & Backwaren 56 (2007) 2, S. 32 ff

[25] Kretschmer, P., A. Lehrack, A. Habel, M. Springer und U. Tietz: Acrylamidbildung bei der Backwaren-herstellung - ein Sachstandsbericht. - Tagung Deutsche Gesellschaft für Qualitätsforschung, 22.03. 2004

[26] Lindhauer, M.G., N.U. Haase, G. Unbehend: Acrylamid in Backwaren. - Arbeitsgemeinschaft Getreideforschung e.V., 9. Tagung für Konditorei-Technologie Detmold 2003

# Impact of food matrices on bioavailability and biological effects of acrylamide in rats

Technische Universität Kaiserslautern, Fachbereich Chemie, Fachrichtung Lebensmittelchemie und Toxikologie

Matthias Baum, Franz Ingo Berger, Julia Feld, Natalie Gerhardt, Gerhard Eisenbrand

## 1. Introduction

Acrylamide (AA) is considered to be carcinogenic as demonstrated in several trials with rats after oral application [1, 2]. Crucial factors affecting the biological activity of AA are its bioconversion into glycidamide (GA) and its bioavailability. The ultimate genotoxic metabolite GA is generated metabolically from AA by cytochrome P450 2E1 (CYP2E1) mediated epoxidation (Figure 1). GA is mutagenic in the Ames test [3] and in the HPRT test with V79 cells [4]. Several in vitro studies have demonstrated GA to induce DNA damage [4-6] while AA has been found inactive. In rats and mice, AA administration resulted in DNA adducts, mainly resulting from binding of GA to N7 of guanine [7-10]. AA and GA are detoxified by conjugation with glutathione [11]. In vivo, glutathione adducts follow the degradation pathway to mercapturic acids to be excreted via the urine. Binding to hemoglobin (Hb) and other proteins in blood also affects biological activity of AA and GA.

Up to now, only few data are available to assess wether food matrices influence the bioavailability and biological activity of AA. Previous animal trials concerning bioavailability and biological effects of AA were almost exclusively conducted with oral application of AA via drinking water [7, 12, 13]. Studies in mice and rats reported higher bioavailability of AA after aqueous gavage than via basal diet fortified with AA [8, 9]. In contrast, recent studies in mice found no differences in bioavailability of dietary AA taken up via different diets [14], in comparison to subcutanous injection [15].

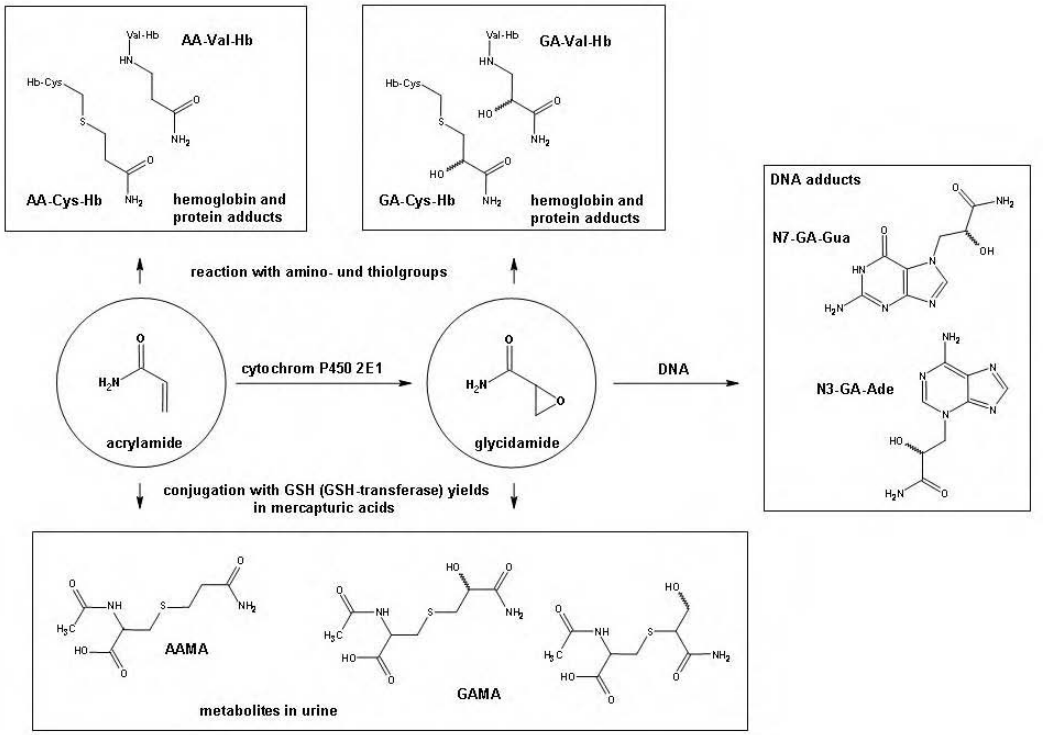


Figure 1: Metabolism of acrylamide [7,11,12,16,17]

## 2. Aim

The study investigated whether the food matrix of French fries and bread crust affect bioavailability and biological activity of AA in comparison to its uptake via drinking water. Mercapturic acids (LC-MS/MS) in urine as well as hemoglobin (Hb) adducts of AA and its genotoxic metabolite glycidamide (GA) (GC-MS) were determined as biomarkers for internal exposure, bioactivation and detoxification. Genotoxicity in leukocytes and hepatocytes was determined by alkaline single cell gel electrophoresis (Comet Assay). Foods were administered over 1-9 days to male Sprague-Dawley rats (daily uptake of 50 µg/kg bw over bread crust; 100 µg AA/kg bw over French fries). Urine and feces were collected for 24 h after the last feeding. Blood and liver samples were collected 24 h after the last AA administration (Figure 2).

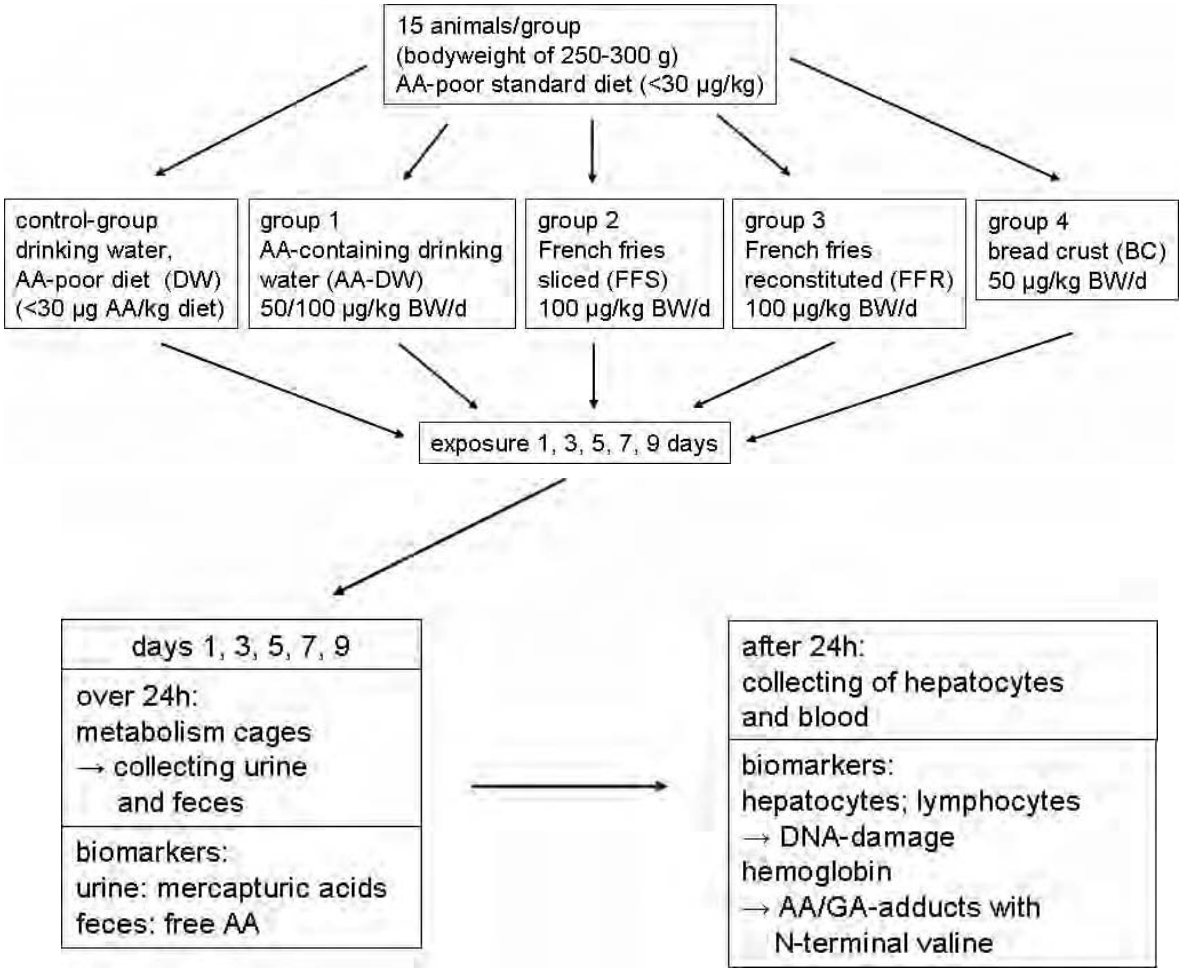


Figure 2: Concept of the animal trial

## 3. Results

### 3.1 AA- and GA-hemoglobin adducts in blood

Hemoglobin is an abundant protein in blood that provides information about exposures to reactive chemicals or electrophilic metabolites through the formation of covalent adducts [17]. Acrylamide and Glycidamide are reactive towards nucleophilic regions in peptides and proteins. Particularly the hemoglobin

adducts of AA and GA are used as convenient biomarkers for external AA and GA exposure. For analysis of the adducts by gaschromatography/mass spectrometry, pentafluorophenyl thiohydantoin (PFPTH) derivatives of valine adducts were generated by treatment of hemoglobin with pentafluorophenyl isothiocyanate [18, 19]. In the case of glycidamide, PFPTH derivatives were further modified by acetonisation to improve GC/MS quantification [20]. We found levels of AA-val-adducts significantly increased depending on the time of treatment and the cumulative dose (Figure 3). The extent of AA-val-adduct formation in FFS- and FFR- groups corresponds to administration of AA over drinking water. Significantly lower AA-valin-adduct contents were observed in the BC-group. In contrast to AA-val-Hb-adducts, GA-val-Hb-adduct levels did not show significant modulation throughout the experiments.

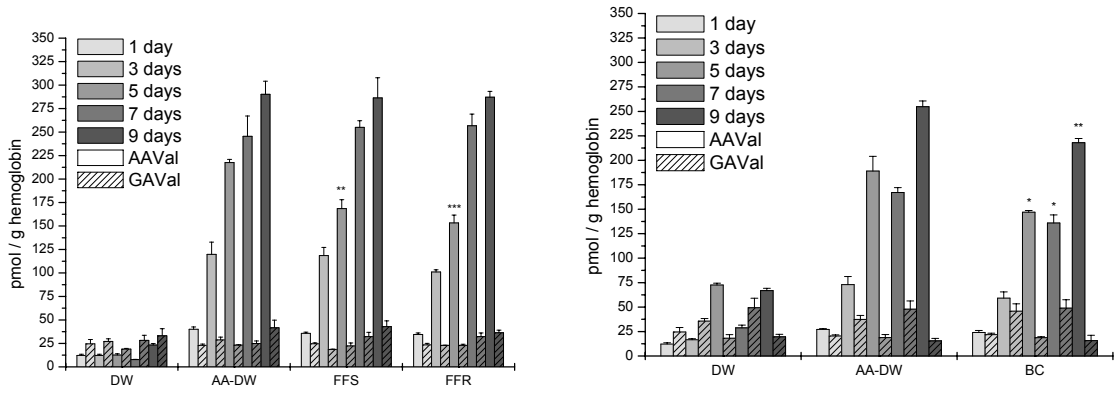


**Figure 3: Formation of acrylamide- and glycidamide-valine-adducts in hemoglobin of rats 24h after the administration of AA over 1, 3, 5, 7 and 9 days via French fries (100 µg/kg BW/d; FFS: sliced from potatoes; FFR: made of potato products) and breadcrust (50 µg AA/kg BW/d) respectively drinking water (AA-DW) in contrast to negative control (DW);**

**mean ± SD, 3 animals per group**

***: significantly different from AA-DW (*p<0,05; **p<0,01; ***p<0,001)**

## 3.2 Mercapturic acids of AA and GA in urine

AA and GA are detoxified by conjugation to glutathione. These adducts are further converted to the respective mercapturic acid metabolites N-Acetyl-S-(2-carbamoylethyl)cysteine (AAMA) and N-Acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine (GAMA). Both metabolites can be isolated from urine by solid phase extraction to be determined by ESI-tandem mass spectrometry in negative ionisation mode (HPLC-MS/MS) and internal calibration by $^{13}C$-labelled standards.

After all time periods significant increases of the mercapturic acids from acrylamide (AAMA) and glycidamide (GAMA) were observed in the AA treatment groups (Figure 4). Each treatment interval resulted in very similar amounts of AAMA and GAMA throughout the repeated AA applications, independent of sequence and number of repeated AA-doses. There was no memory or cumulative effect detectable. Within the food groups, only the BC group response was indicative for some reduction in bioavailability, whereas the French fries groups showed responses very similar to AA ingestion by drinking water. Overall, within equal time periods of treatment, the AAMA and GAMA contents indicated higher variability in comparison to hemoglobin adducts. Taken all treatment groups together, 50 % on average of the ingested daily dose was recovered as mercapturic acids in urine and thus detoxified.

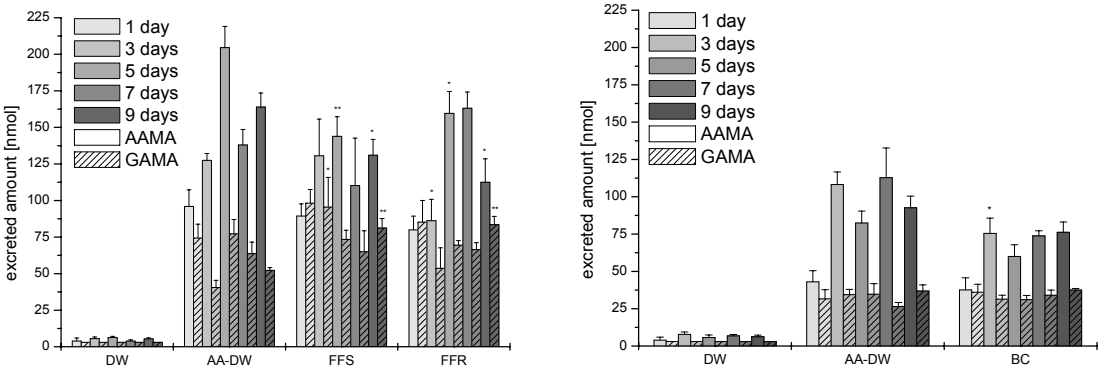


**Figure 4: Formation of mercapturic acids after the administration of AA over 1, 3, 5, 7 und 9 days via French fries (100 µg/kg BW/d; FFS: sliced from potatoes; FFR: made of potato products) and breadcrust (50 µg/kg BW/d) respectively drinking water (AA-DW) in contrast to negative control (DW)**
**AAMA: acrylamide mercapturic acid; GAMA: glycidamide mercapturic acid;**
**mean ± SD, 3 animals per group *: significantly different from AA-DW (*p<0,05; **p<0,01)**

### 3.3 DNA damage in leukocytes and hepatocytes

DNA damage in hepatocytes and leukocytes was monitored by alkaline single cell gel electrophoresis (comet assay). Liver and blood samples were collected 24 hours after the last administration of AA via bread crust (50 µg/kg bw/d), French fries (100 µg/kg bw/d) and drinking water (50 or 100 µg/kg bw/d). Liver cells were isolated from rat liver tissue [21]. Briefly, tissue was minced in 1 ml of ice-cold Hanks buffered salt solution (HBSS). Aliquots of the hepatocyte suspension were used for alkaline single cell gel electrophoresis and for determination of viability by trypan blue exclusion assay. For comet assay, aliquots of blood and liver cells in low melting agarose were put onto slides (Figure 5). After lysis, cells were treated with the DNA repair enzyme formamidopyrimidine DNA glycosylase (FPG) for 30 min. FPG recognizes certain DNA damage events such as oxidized and ring-opened purines as well as apurinic and apyrimidinic sites, rendering them detectable as DNA strand breaks. Likewise, DNA lesions resulting from adducts of GA with DNA nucleotides, like N7-GA-guanine adducts (N7-(2-carbamoyl-2-hydroxyethyl)-guanine) can be detected by the comet assay with FPG. After DNA unwinding and electrophoresis, DNA was stained with ethidium bromide and viewed by computer based microscopy. DNA migration was expressed as change of tail intensity (TI) versus negative control (DW).

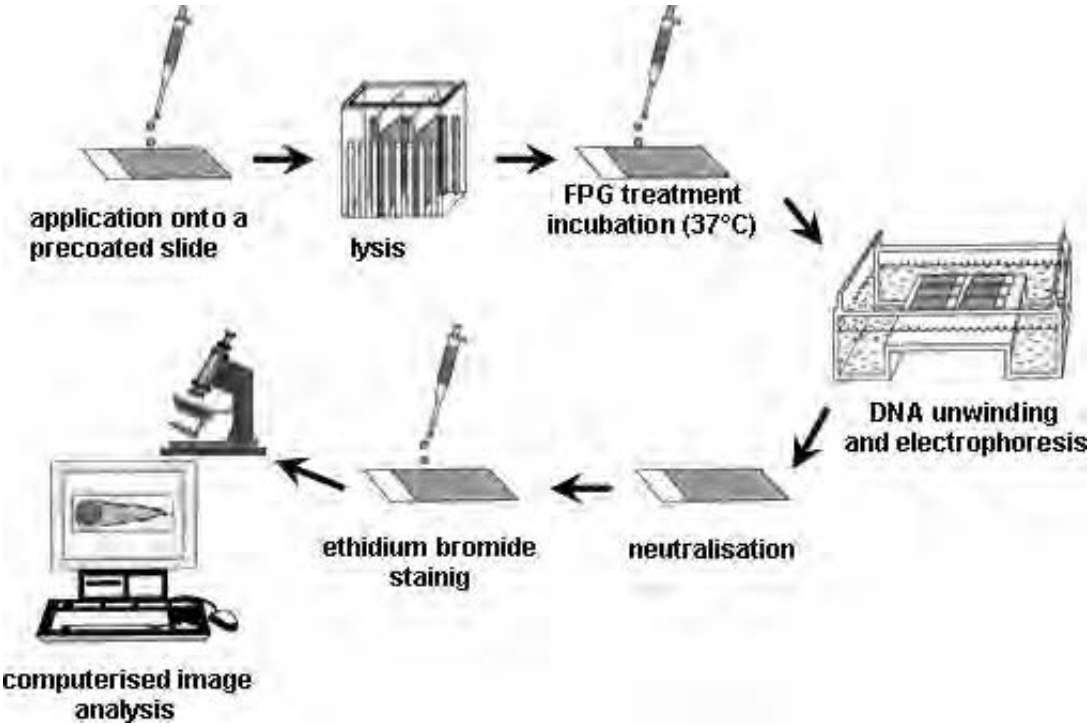


Figure 5: The comet assay

Under all conditions tested, no significant induction of DNA damage was detected (Figure 6). After administration of 100 µg AA/kg bw/d in French fries over 1 to 9 days TI values of exposed groups differed from DW about ± 5 %. The TI values of animals fed with 50 µg AA/kg bw/d by intake of bread crust or drinking water for 1 to 9 days remained within about ± 2 % versus TI values of DW.

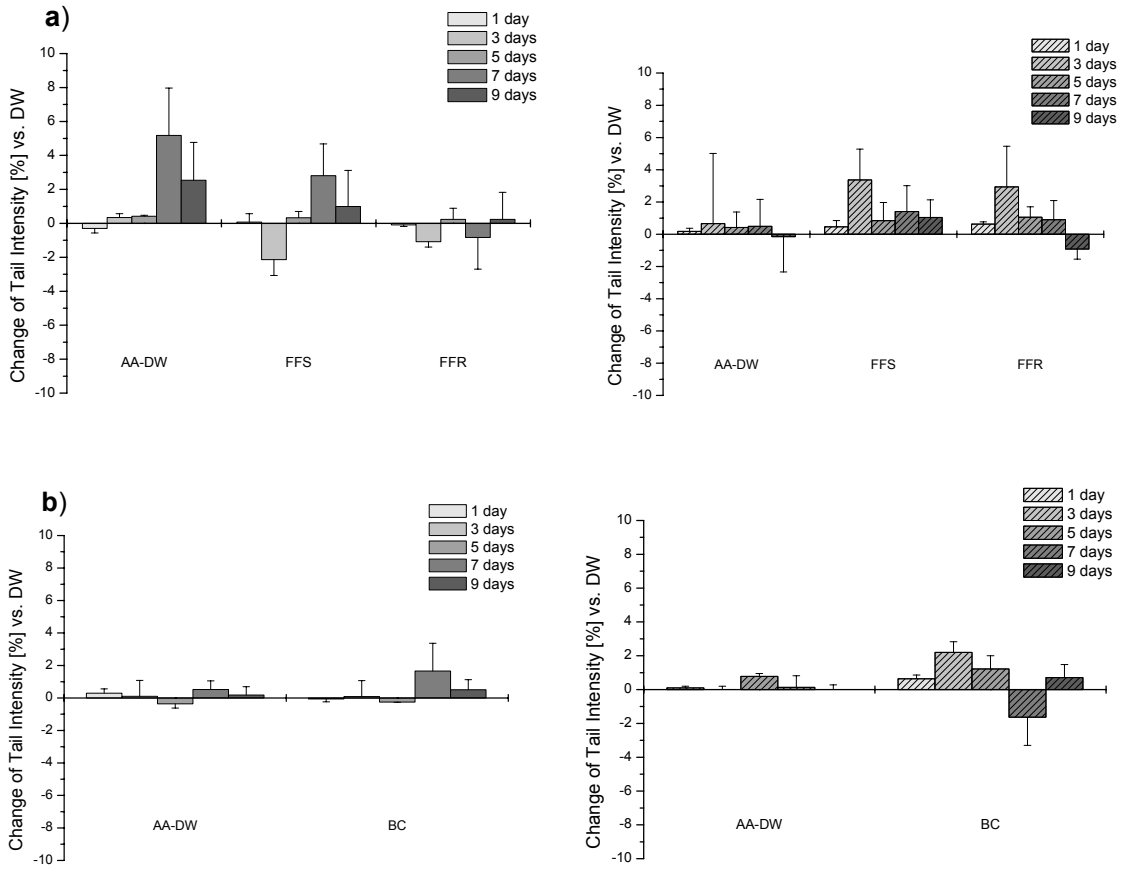


**Figure 6: Analysis of DNA damage in hepatocytes and leukocytes of rats 1, 3, 5, 7, and 9 days administration of 100 µg AA/kg bw/d via French fries (FFS: sliced from potatoes; FFR: made of potato products) and drinking water (AA-DW) (a) respectively 50 µg/kg bw/d via bread crust and drinking water (AA-DW) (b) in contrast to negative control (DW);**

**(open bars: hepatocytes, striped bars: leukocytes; mean ± SD, 3 animals per group)**

## 3.4 AA and GA in feces

The determination of AA and GA was performed using LC-MS/MS with positive electrospray ionisation ($ESI^+$) in multiple reaction monitoring mode (MRM). The samples were extracted with water. After purification and concentration by solid phase extraction the analytes were quantified using internal calibration by $^{13}C$-labelled AA and GA.

Figure 7 shows the excretion of AA as percentage of the daily dose after administration of AA via French fries or breadcrust as compared to its intake in drinking water. The samples were collected over 24 h after the last daily dose and the total amount of feces had a range between 4 g and 18 g. Considerable variability in fecal AA excretion within individual animals of the respective treatment groups concerning excretion of AA over feces was observed. In 37 % of the samples the AA contents were below the limit of detection. No significant differences between the treated groups and in comparison to the negative controls were observed. AA was found in feces in the range from 0.4 % to 8.2 % (calculated as percentage of dose).

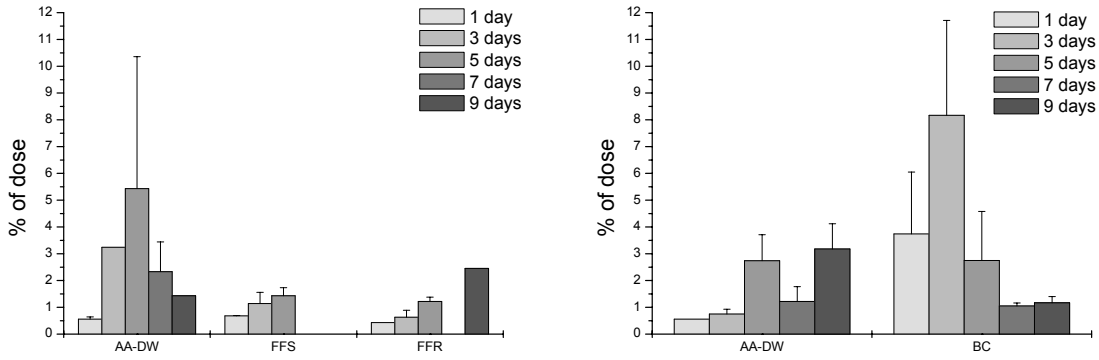


**Figure 7: AA contents in feces of rats as percentage of dose after administration of AA over 1, 3, 5, 7 und 9 days via French fries (100 µg/kg BW/d; FFS: sliced from potatoes; FFR: made of potato products) and bread-crust (50 µg/kg BW/d) respectively drinking water (AA-DW);**

**mean ± SD, 3 animals per group *: significantly different from AA-DW (*p<0,05; **p<0,01)**

## 4. Summary

Taken together, the data give no evidence for a significant influence of the food matrix on the bioavailability and biological activity of AA.

After administration of 50 and 100 µg AA/kg bw/d in food and drinking water, 40-60 % of the administered AA dose are found excreted in the urine within 24 h in the form of mercapturic acids (AAMA and GAMA). At each repeated dosage time point, levels of AAMA and GAMA increased significantly after administration of AA in drinking water or food without any evidence for cumulation. In contrast, levels of AA-val-Hb-adducts steadily cumulated, reflecting the repeated administration of AA. Whereas GAMA excretion in the urine in terms of dose/time response clearly followed those of AAMA excretion, Hb adduct formation was found strikingly different. Under no circumstances there was a significant deviation of GA-val-Hb-adducts from negative control background levels detectable. Likewise, with the comet assay no significant variation in DNA damage response was detectable in blood and liver cells at any timepoint and dose.

### REFERENCES

[1] Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW (1986): Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol. Appl. Pharmacol. 85 (2), 154-168

[2] Friedman MA, Dulak LH, Stedham MA (1995): A lifetime oncogenicity study in rats with acrylamide. Fundam. Appl. Toxicol. 27 (1), 95-105

[3] Hashimoto K, Tanii H (1995): Mutagenicity of acrylamide and its analogues in Salmonella typhimurium. Mutat. Res. 158, 129-133

[4] Baum M, Hoffmann M, Loeppky R N, Michael S, Thielen S and Eisenbrand G (2005): Kinetic of induction and disappearance of DNA-strandbreaks and mutations of different alkylating agents: 3-nitrosooxazolidine-2-

one (NOZ-2), 3-nitrosooxazolidin-5-one (NOZ-5) and glycidamide using comet-assay and hPRT-gene-mutation-assay in V79-cells. Fifth Annual AARC International Conference, Nov 12-15, 2006

[5] Puppel N, Tjaden Z, Fueller F, Marko D (2005): DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. Mutat. Res. 580, 71-80

[6] Thielen S, Baum M, Hoffmann M, Loeppky RN, Eisenbrand G (2006): Genotoxicity of glycidamide in comparison to (+/-)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide and alpha-acetoxy-N-nitroso-diethanolamine in human blood and in mammalian V79-cells. Mol. Nutr. Food Res. 50 (4-5): 430-6

[7] Gamboa da Costa G, Churchwell MI, Hamilton P, von Tungeln LDS Beland FA, Marques MM, Doerge DR (2003): DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. Chem. Res. Toxicol. 16, 1328-1337

[8] Doerge DR, Young JF, McDaniel LP, Twaddle NC, Churchwell MI (2005): Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. Toxicol. Appl. Pharmacol. 202 (3): 258-67

[9] Doerge DR, Young JF, McDaniel LP, Twaddle NC, Churchwell MI (2005): Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicol. Appl. Pharmacol. 208 (3): 199-209

[10] Manière I, Godard T, Doerge DR, Churchwell MI, Guffroy M, Laurentie M, Poul JM (2005): DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide. Mutat. Res. 580 (1-2): 119-29

[11] Sumner SC, Stedman DB, Clarke DO, Welsch F, Fennell TR (1992): Characterization of urinary metabolites from [1,2,methoxy-13C]-2-methoxyethanol in mice using 13C nuclear magnetic resonance spectroscopy. Chem. Res. Toxicol. 5: 553–560.

[12] Calleman CJ, Bergmark E, Costa LG (1990): Acrylamide is metabolized to glycidamide in the rat; evidence from hemoglobin adduct formation. Chem. Res. Toxicol. 3, 406-412

[13] Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI (1999): Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem. Res. Toxicol. 12: 1110-1116.

[14] Vikström AC, Eriksson S, Paulsson B, Karlsson P, Athanassiadis I, Törnqvist M (2008): Internal doses of acrylamide and glycidamide in mice fed diets with low acrylamide contents. Mol. Nutr. Food Res. 52

[15] Bjellaas T, Ølstørn HB, Becher G, Alexander J, Knutsen SH, Paulsen JE (2007): Urinary metabolites as biomarkers of acrylamide exposure in mice following dietary crisp bread administration or subcutaneous injection. Toxicol. Sci. 100 (2): 374-80

[16] Bergmark E, Calleman CJ, He F, Costa LG (1993): Determination of haemoglobin adducts in human occupationally exposed to acrylamide. Toxicol. Appl. Pharmacol. 120, 45-54

[17] Törnqvist M, Fred C, Haglund J, Helleberg H, Paulsson B, Rydberg P (2002): Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. J Chromatogr B Analyt Technol Biomed Life Sci. 778 (1-2): 279-308

[18] Bergmark E. (1997): Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. Chem Res Toxicol. 1997 Jan;10(1):78-84

[19] Schettgen T, Broding HC, Angerer J, Drexler H. (2002): Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicol Lett. 2002 Aug 5;134(1-3):65-70

[20] Paulsson B, Athanassiadis I, Rydberg P, Törnqvist M. (2003): Hemoglobin adducts from glycidamide: acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. Rapid Commun Mass Spectrom. 17(16):1859-65

[21] Kamp HG, Eisenbrand G, Janzowski C, Kiossev J, Latendresse JR, Schlatter J, Turesky RJ (2005): Ochratoxin A induces oxidative DNA damage in liver and kidney after oral dosing to rats. Mol. Nutr. Food Res. 49 (12): 1160-7

# 1. Approaches for industrial implementation of project results

The results of both cooperative research projects with respect to reducing acrylamide in heated cereal and potato products can be summarised to the following proposals. The approaches can be separated according to the relevant food categories and are related to the general strategies listed below:

- 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 manufacturing enabling a more controlled heating process.

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

**Raw material and recipe:**

- Optimum use of resources with respect to minimising content of reducing sugars in potato tubers, e. g. variety
- Continuous water supply supported by irrigation techniques to minimize drought stress during farming; optimised nutrient supply in spring with a decreased availability before lifting
- Minimisation of mechanical impact during lifting and transport during harvest and storage
- Optimum regulation of temperature, humidity and carbondioxide to prevent sugar enrichment.

**Pre-treatment of raw material of intermediates:**

- Application of asparaginase in restructured potato product to reduces free asparagine content

**Process:**

- Removal of solids within blanching medium to prevent enrichments and reduced efficiency of blanching
- Optimisation of temperature profiles and temperature gradients; 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
- enhanced pre-drying of French fries during manufacturing to process to enable shorter frying times for the ready-to-eat products
- Increase of salt content in the outer product layers of par-fried French fries (instead of salting after frying).

**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 and adapted temperature program during frying
- Opto-electronic sorting for removal of dark coloured products
- Education campaigns for consumers to change the overall thermal load of potato food preparation.

Also, opportunities for **baked and extruded cereal products** which were successfully tested in laboratory and pilot scale to reduce acrylamide formation can be described in a similar way.

**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 asparagine 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
- Separation of the baking process for bread and rolls into various phases with
  - low initial temperature by baking/steaming and baking for crust development (baking splitting – strategy 1)
  - significantly increased initial temperature and final baking at lower temperature and corresponding humidity of the baking chamber (phase baking – strategy 2)
- A slight decrease of acrylamide content in brown gingerbread is possible by suitable conditioning and storage
- Substitution of leavening agents and influencing the pH value in brown gingerbread may be measures for acrylamide reduction but have a significant impact on sensory quality
- Use of enzymes for the degradation of asparagine reduces the formation of acrylamide in brown ginger bread

**Equipment and measurement techniques:**

- Possibilities for control and influence product moisture in the extruder
- Development of short time roasting processes for cornflakes
- Control of crust temperature and moisture as well as control of oven climate is recommended during baking
- Realisation of baking conditions which more clearly differ one from the other.

However, it should be pointed out that all these opportunities although positively tested in labsoratory of pilot scale have to be adapted to industrial 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

According to the unsolved tasks remaining from the results of the previous project (AiF 108 ZBG), the technological investigations in this project were focused on development of the scientific background for implementation of the minimising approaches for heated potato and cereal products tested in laboratory scale. A main criterion of these investigations was the maintaining of the quality, e. g. browning, which has been usual and expected for these products because this issue is highly important for acceptance of technological modifications by the market. The accompanying chemical and toxicological research should contribute to a better understanding of the processes during heating as well as to an extension of available data for evaluation of health relevance of acrylamide formed in foods during heating. The results for the sub-projects can be summarised as follows:

A model plant in a technical scale had been established for the investigations regarding the integration of coating and an enhanced pre-drying into the manufacturing process of industrially produced French fries. It could be demonstrated that the drying of blanched French fries is much more effective than drying of par-fried French fries and the successive par-frying process could be shortened due to the enhanced pre-drying. Blanching of the potato sticks in brine enables a defined increase of the salt content in the outer product layers without an extensive raise in the core.

With respect to the manufacturing of recombined potato products, formulations and procedures were developed to obtain suitable sensory quality (texture, water and fat content, browning) with minimised acrylamide contents. Additionally, a specific intervention into the precursor situation in these products by application of the enzyme asparaginase could be shown to enable a distinct reduction of acrylamide content for a comparable degree of browning.

In the frame of investigations regarding control of frying in households and restaurants to obtain an optimised quality and reduced acrylamide contents, a standard frying process leading to a defined product quality was established for very different equipments based on engineering process parameters, e. g. water evaporation. Such a standard frying process represents a tool which enables evaluation of frying processes in different household fryers and the derivation of the relevant control parameters to obtain the desired final quality.

The baking splitting process (lower initial temperature by baking/steaming and final baking for crust development) and the phase baking process (higher initial temperature and final baking at lower temperature) developed in the project are two baking strategies for bread and roll-type products enabling a reduction of acrylamide contents between 55 und 90 % compared to the standard baking process. These strategies have to be adapted individually according to the type of baking good and quality requirements. Therefore, the opportunities to realise baking conditions which differ in a more distinct way compared to those in traditional equipment are one of the main development potentials of new baking ovens. With respect to brown gingerbread which is one of the cereal products with the highest acrylamide contents, an effective minimizing is only possible by interventions on formulation, e. g. exchange of baking agent and application of asparaginases, which also results in unavoidable sensory impacts, whereas only changing of baking processes had not been successful for this product.

The stable intermediate amine 3-aminopropionamid could be quantified as a potent precursor for acrylamide formation not only in model systems but also in foods. An influence of sulfur supplement during growth of cereal plants, e. g. wheat, on the content of free asparagine and also on acrylamide formation potential could be identified. Additionally, the conditions for the minimisation of this influence were described. For the first time, the carcinogenic substance glycidamide could be determined directly in the food matrix using a newly developed stable isotope dilution assay. A formation pathway in the presence of

linolenic acid hydroperoxides was also identified. Additionally, the presence of acrylamide adducts with nucleophilic ingredients, e. g. cysteine, was revealed in foods.

The results of the toxicological investigations in rats revealed that the bioavailability of acrylamide formed in foods (potato, gingerbread) does not significantly differ from that of intake from drinking water for the administration of 50 and 100 µg acrylamide / kg bw/d. Furthermore, in rats it was found that about 40 to 60 % of the acrylamide dose arising from oral intake was excreted through the urine as mercapturic acids within the first 24 h. Under no circumstances was there a significant deviation of glycidamide-hemoglobin adducts from negative control background levels detectable.

All in all, it has been proven that the approaches for the minimisation of acrylamide formation in heated potato and cereal products developed and tested in laboratory scale are generally realisable in pilot scale. However, it has to be mentioned that the results of the technological research represents only one element which is required to establish save processes to manufacture high quality products in industrial scale. Due to the high complexity of the acrylamide problem in heated foods, an individual adaptation of each process for each product is still required after finishing the project and distribution of results. In this pre-competitive project the general scientific backgrounds were developed enabling a faster and more directed adaptation of the relevant processes by the industry.

# Zusammenfassung

Entsprechend den noch zu lösenden Aufgaben, die sich aus den Ergebnissen des Vorläuferprojektes (AiF 108 ZBG) herauskristallisierten, konzentrierten sich die technologischen Untersuchungen im Rahmen dieses Projektes auf die Erarbeitung der Grundlagen für die Umsetzung der im Labormaßstab erarbeiteten Minimierungsansätze bezüglich Acrylamid für erhitzte Kartoffel- und Getreideprodukte. Ein wesentliches Kriterium dabei war die Erhaltung der für diese Produkte gewohnten Qualität, z. B. Bräunung, was nach wie vor von entscheidender Bedeutung für die Akzeptanz technologischer Veränderungen durch den Markt ist. Die begleitenden chemischen und toxikologischen Forschungen sollten zum Verständnis der Vorgänge beim Erhitzen beziehungsweise zur Erweiterung der Daten für die gesundheitliche Bewertung des im Lebensmittel gebildeten Acrylamids beitragen. Für die einzelnen Teilprojekte können die wesentlichen Ergebnisse wie folgt zusammengefasst werden:

Mit dem 3-Aminopropionamid konnte ein stabiles Intermediat als potenter Precursor von Acrylamid nicht nur in Modellsystemen, sondern auch in Lebensmitteln quantifiziert werden. Für Getreide, z. B. Weizen, wurde ein Einfluss der Schwefelversorgung während des Wachstums der Pflanzen auf den Gehalt an freiem Asparagin und damit auf das Acrylamidbildungspotential gefunden und Bedingungen für die Minimierung dieses Einflussfaktors herausgearbeitet. Erstmalig konnte auch mittels einer neu entwickelten Stabilisotopenverdünnungsanalyse die Substanz Glycidamid direkt im Lebensmittel bestimmt sowie ein Bildungsweg in Gegenwart von Linolsäurehydroperoxide aufgezeigt werden. Zudem wurden erstmalig Reaktionsprodukte des Acrylamids mit nukleophilen Inhaltsstoffen, z. B. Cystein, im Lebensmittel nachgewiesen.

Hinsichtlich der Integration des Coatings und der verstärkten Vortrocknung in den Herstellungsprozess industriell vorfrittierter Pommes frites wurde eine Modellanlage im kleintechnischen Maßstab aufgebaut und für unterschiedliche Untersuchungen genutzt. Mit dieser Anlage konnte u. a. gezeigt werden, dass sich die blanchierten Pommes frites wesentlich effektiver als bereits vorfrittierte Pommes frites (Vorläuferprojekt) vortrocknen lassen und dass sich die Vorfrittierzeit entsprechend verkürzen lässt. Das Blanchieren der Kartoffelstäbchen in einer Salzlösung ermöglicht eine gezielte Erhöhung der Salzgehalte in den äußeren Schichten, ohne dass der Gehalt im Inneren deutlich ansteigt.

Bezüglich der Herstellung rekombinierter Kartoffelerzeugnisse vom Typ Pommes frites wurden Rezeptur und Verfahren für eine entsprechende sensorische Qualität (Textur, Wasser- und Fettgehalt, Bräunung) bei minimiertem Acrylamidgehalt ermittelt. Ebenfalls wurde gezeigt, dass der gezielte Eingriff in die Precursorsituation über den Einsatz von Asparaginase der Acrylamidgehalt bei vergleichbarer Bräunung in diesen Produkten deutlich reduziert werden kann.

Bei deren Untersuchungen zur Friteusensteuerung in Gastronomie und Haushalt mit dem Ziel der optimalen Qualität bei reduzierten Acrylamidgehalten von Pommes frites wurde für unterschiedlichste Geräte ein Standardfrittierprozess auf der Basis verfahrenstechnischer Parameter erarbeitet, der zu einer definierten Produktqualität führt. Damit steht ein Tool zur Verfügung, auf dessen Grundlage es möglich ist, das Frittieren in unterschiedlichen Haushaltsfriteusen zu bewerten und die entsprechenden Steuerungs-parameter abzuleiten.

Mit dem Backsplitting (niedrige Starttemperatur durch Back/Dämpfen und Backen zur Krustenentwicklung) sowie dem Phasenbacken (hohe Starttemperatur und Fertigbacken bei niedriger Temperatur) wurden für Brot und Kleingebäcke zwei Strategien erarbeitet, mit denen eine Reduzierung der Acrylamidgehalte auf 56 bis 91% im Vergleich zum Standardbacken möglich ist, wobei je nach Gebäckart und Qualitätsanforderung die geeignete Strategie individuell angepasst werden muss. Entsprechend wird ein wichtiges Entwicklungspotenzial von Backöfen in der Möglichkeit der Realisierung von Backbedingungen, die sich stärker als bisher voneinander unterscheiden, gesehen. Bei braunen Lebkuchen ist eine Minimierung nur

durch stoffliche Eingriffe, z. B. Triebmittelaustausch, Einsatz von Asparaginasen, mit unvermeidlichen sensorischen Veränderungen effektiv möglich, während die alleinige Veränderung des Backprozesses bei diesem Produkt nicht zum Erfolg führt.

Die toxikologischen Untersuchungen zeigten, dass sich in Ratten die Bioverfügbarkeit des Acrylamids aus dem Lebensmittel (Kartoffel, Lebkuchen) bei täglichen Zugaben von 50 bzw. 100 µg Acrylamid/kg Körpergewicht und Tag nicht signifikant von der aus Trinkwasser unterscheidet. Zudem wurde nachgewiesen, dass von der Ratte ca. 40 - 60% der oral aufgenommenen Acrylamiddosis innerhalb von 24 h über den Urin als Mercaptursäuren ausgeschieden werden. Es konnte über den Zugabezeitraum von 9 Tagen keine Zunahme an Hämoglobinaddukten des Metabolits Gylcidamid in Blut gegenüber dem Vergleich ohne Acrylamidgabe gefunden werden.

Insgesamt wird deutlich, dass die im Labor erarbeiteten und getesteten Ansätze zur Minimierung der Acrylamidbildung bei erhitzten Kartoffel- und Getreideerzeugnissen prinzipiell auch im kleintechnischen Maßstab realisierbar sind. Allerdings ist auch anzumerken, dass diese Ergebnisse der technologischen Forschung nur ein Baustein sind, um im industriellen Maßstab qualitativ hochwertige Produkte mit den jeweils vom Hersteller gewünschten Eigenschaften sicher herstellen zu können. Das heißt, auch nach dem Vorhaben besteht, bedingt durch die nach wie vor vorhandene Komplexität der Acrylamidproblematik, die Notwendigkeit der individuellen Anpassung jedes Prozesses für die einzelnen Produkte. In diesem vorwettbewerblichen Projekt konnten jedoch verallgemeinerbare Grundlagen geschaffen werden, die diese Anpassung schneller und zielgerichteter ermöglichen.

## Performing Research Institutes

Deutsches Institut für Lebensmitteltechnik e. V (DIL)
Prof.-v.-Klitzung-Straße 7, 49610 Quakenbrück
Institute management: Dr.-Ing. V. Heinz
Phone: 05431/183-0, Fax: 05431/183114, e-mail: info@dil-ev.de
Projectleader: Dr. K. Franke

Institut für Lebensmittel- und Umweltforschung e. V. (ILU)
Arthur-Scheunert-Allee 40-41, 14558 Nuthetal
Institute management: Dipl.-Ing. P. Kretschmer
Phone: 033200/89-179, Fax: 033200/8922-0, e-mail: igv-manage@igv-gmbh.de
Projectleader: Dr. H. Kaiser, A. Lehrack

Technische Universität Kaiserslautern, Fachbereich Chemie
Fachrichtung Lebensmittelchemie/Umwelttoxikologie
Erwin-Schroedinger-Straße Gebäude 52/322, 67663 Kaiserslautern
Phone: 0631/2052973 Fax: 0631/ 2053085, e-mail: hemm@rhrk.uni-kl.de
Institute management: Prof. Dr. G. Eisenbrand
Projectleader: Dr. M. Baum

Deutsche Forschungsanstalt für Lebensmittelchemie (DFA)
Lichtenbergstraße 4, 85748 Garching
Phone: 089/28914170, Fax: 089/28914183 e-mail: lebensmittelchemie@lrz.tum.de
Institute management: Prof. Dr. Dr. P. Schieberle
Projectleader: Prof. Dr. P. Köhler/ Dr. M. Granvogl

## Coordinating Organisations

Forschungskreis der Ernährungsindustrie e. V. (FEI)
Godesberger Allee 142-148, 53175 Bonn
Tel: 0228/37 20 31, Fax: 0228/37 61 50, e-mail: FEI@fei-bonn.de
Coordinator: Dr. Volker Häusser

Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL)
Godesberger Allee 142-148, 53175 Bonn
Tel: 0228/819930, Fax: 0228/81993200, e-mail: bll@bll.de
Coordinator: Dr. Julia Gelbert

# Publications


[1] Berger, F.; Feld, J.; Baum, M.; Eisenbrand, G. (2008) Acrylamide: Impact of food-matrices on bioavailability and biological effects in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 377, Supplement 1

[2] Franke, K.; Strijowski, U.; Reimerdes, E.H. (2008) Kinetics of acrylamide formation in potato powder, Journal of Food Engineering 90, 135-140

[3] Granvogl, M.; Koehler, P.; Latzer, L.; Schieberle, P. (2008) Development of a stable isotope dilution assay for the quantitation of glycidamide and its application to foods and model systems. J. Agric. Food Chem. 56, (in press).

[4] Granvogl, M.; Koehler, P.; Latzer, L.; Schieberle, P. (2008) Glycidamid - Ein neues Karzinogen in Lebensmitteln. In: Deutsche Forschungsanstalt für Lebensmittelchemie (DFA), Bericht 2008 (DFA, Hrsg.), (in press)

[5] Granvogl, M.; Latzer, L.; Koehler, P.; Schieberle, P. (2007) Interactions of acrylamide with other food constituents. Abstracts of Papers, 234$^{th}$ ACS National Meeting, Boston, MA, USA

[6] Granvogl, M.; Schieberle, P. (2006) Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. J. Agric. Food Chem. 54, 5933-5938

[7] Granvogl, M.; Schieberle, P. (2007) Procedure for minimization of acrylamide formation in heating up a food. Patentschrift: DE 102006007798, A1 20071108

[8] Granvogl, M.; Schieberle, P. (2007) Quantification of 3-aminopropionamide in cocoa, coffee and cereal products. Eur. Food Res. Technol. *225*, 857-863

[9] Granvogl, M.; Wieser, H.; Koehler, P.; von Tucher, S.; Schieberle, P. (2007) Influence of sulfur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. J. Agric. Food Chem. 55, 4271-4277

[10] Koehler, P.; Granvogl, M.; Wieser, H.; von Tucher, S.; Schieberle, P. (2007) Acrylamide formation in cereal flour as affected by sulfur fertilization. Getreidetechnologie 61, 223-227

[11] Köhler, P.; Wieser, H.; Schieberle, P. (2007) Acrylamid-Bildungspotenzial von Weizenmehl in Abhängigkeit von der Schwefeldüngung. In: Deutsche Forschungsanstalt für Lebensmittelchemie (DFA), Report 2007, pp. 20-23

# Acknowledgements

This research project was supported by the Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e. V., Bonn). We also like to thank the German Federation of Food Law and Food Science (Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL)) and the involved industry for its support.

AiF-Project No.: 209 ZBG.

Dieses Vorhaben wurde im Programm zur "Förderung der Industriellen Gemeinschaftsforschung (IGF)" vom Bundesministerium für Wirtschaft und Technologie via AiF über den Forschungskreis der Ernährungs–industrie e. V. (FEI) gefördert. Unser Dank für die Förderung gilt darüber hinaus stellvertretend für zahlreiche beteiligte Verbände und Unternehmen der Lebensmittelwirtschaft dem Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL).

Projekt AiF 209 ZBG

BLL

Bund für Lebensmittelrecht
und Lebensmittelkunde e. V.

Postfach 20 02 12
53132 Bonn
Godesberger Allee 142-148
53175 Bonn

Hauptstadtbüro Berlin
Claire-Waldoff-Straße 7
10117 Berlin

Büro Brüssel
43, Avenue des Arts
1040 Brüssel, Belgien

Für alle Standorte:
Tel. +49 228 81993-0
Fax +49 228 81993-200
bll@bll.de · www.bll.de