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Formation of acrylamide in potato products and its dietary exposure

Thesis submitted in fulfilment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences: Chemistry

Vorming van acrylamide in aardappelproducten
en blootstelling eraan via de voeding

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List of abbreviations

% (v/v)	percentage volume over volume
% (w/v)	percentage weight over volume
% (w/w)	percentage weight over weight
ala	alanine
ALARA	as low as reasonably achievable
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
asn	asparagine
a_w	water activity
BELAC	Belgian organization for accreditation
BMD	benchmark dose
BMDL	benchmark dose limit
bp	boiling point
bw	body weight
CD	(European) Commission decision
CI	confidence interval
CIAA	Confédération des Industries Agro-alimentaires de l'UE (Confederation of the Food and Drink Industries of the EU)
CRM	certified reference materials
DAD	diode array detector
DAG	diacylglycerol
DM	dry matter
DMPS	dimethyl polysiloxane
DNA	deoxyribonucleic acid
EC	European Commission
ECD	electron capture detector
EFSA	European Food Safety Authority
EH	epoxide hydrolase
ESI+	positive electrospray ionization
EU	European Union
FAO	Food and Agriculture Organization
FAPAS	Food Analysis Performance Assessment Scheme
FDA	Food and Drug Administration
FID	flame ionization detector
GAB	Guggenheim-Anderson-de Boer
GC	gas chromatography
GLY	glycerol
gly	glycine

GSH	glutathione
Hb	hemoglobin
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
IRMM	Institute for Reference Materials and Measurements
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
m/z	mass to charge ratio
MAG	monoacylglycerol
MOE	margin of exposure
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
ND	not determined
NOEL	no observable effect level
P	percentile
P	probability
P (%)	mean relative percentage deviation modulus
P 50	50^{th} percentile (= median)
PAV	*p*-anisidine value
PBS	phosphate-buffered saline
phe	phenylalanine
POV	peroxide value
PTR	proton transfer reaction
QDA	quantitative descriptive analysis
RSD	relative standard deviation
SCF	Scientific Committee on Food
SPE	solid-phase extraction
SPME	solid-phase microextraction
TAG	triacylglycerol
UPLC	ultra performance liquid chromatography
US	United States
UV	ultraviolet
var.	variety, cultivar
WHO	World Health Organization

Introduction

The potato (*Solanum tuberosum* L.) is one of the world's major staple food crops. In 2004, 330 million tons were produced worldwide, of which Belgium cultivated about 1% (FAO, 2006). This tuber is moreover an important element in the daily diet for millions of people. On the other hand, deep-fat frying is one of the oldest and most popular cooking methods, among other things due to its cooking speed and the development of desired sensorial properties. The occurring Maillard reaction creates unique flavours, colours and texture in the processed foodstuffs which improves their overall palatability (Pedreschi et al., 2005b). There is however a growing consumer demand for safe, healthy, nutritious and convenient food which is a key driver for improvements and new developments in food processing.

The last decennia, increasing scientific knowledge and better detection techniques have revealed new possible human health risks. Acrylamide was considered as a probable human carcinogen upon exposure via industrial applications and cigarette smoke (IARC, 1994). The exposure could be monitored measuring acrylamide-hemoglobin adducts in the blood (Bergmark, 1997). Yet, the presence of acrylamide in fried and heated foodstuffs was only detected a few years ago. During the construction of a railway tunnel, large quantities of acrylamide leaked into the environment and contaminated some workmen. Considerable amounts of acrylamide adducts were found in the blood of these workers, but also in an unexposed non-smoking control group. This has led to the discovery of acrylamide being present in several fried and baked foodstuffs in 2002 (Tareke et al., 2002; Rosén and Hellenäs, 2002).

This presence of acrylamide in heat-treated carbohydrate-rich foodstuffs sparked international research by local authorities, food companies and universities, focusing on analysis, occurrence and formation in food as well as on toxicological aspects. The desired Maillard reaction was pointed out to be the key for acrylamide formation. The challenge consisted thus out of lowering the formation of acrylamide, while safeguarding product quality for the consumer. Since French fries and crisps belong to the food category with a high acrylamide contamination level (Friedman, 2003) and as these products are moreover frequently consumed (Miranda and Aguilera, 2006), it is clear that these foodstuffs significantly contribute to the daily acrylamide exposure. Therefore, this work specifically focused on the formation of acrylamide in these foodstuffs and on the factors influencing its dietary exposure.

Summary

The **first chapter** presents a literature overview of several aspects dealing with the presence and quantification of acrylamide in foodstuffs. The consumer risks, associated with the intake of this probable human carcinogen are discussed, followed by a review of the proposed formation pathways. Free asparagine and reducing sugars are considered as the most important acrylamide precursors upon heating starch-rich foodstuffs above 120°C. Fried potato products are particularly prone to acrylamide formation due to the abundance of free asparagine, which is present in considerable excess compared to the amount of reducing sugars in the tuber. The latter are consequently the rate limiting factor and of crucial importance to reduce acrylamide formation in potato products. Several agricultural factors, such as cultivar type, fertilization, climate, harvest time and storage conditions may considerably affect the reducing sugar content in the tuber and subsequently the formation of acrylamide.

In the **second chapter**, the reliability of the applied research methodology was assessed. Therefore, the acrylamide analysis was validated in-house for linearity, specificity, limit of detection and quantification, repeatability and recovery. The quality of the analysis was furthermore assured using control samples during each sample run, together with a frequent participation in proficiency tests.
Besides, a new heating methodology, based on a closed tubular reactor, was evaluated for its repeatability. It was shown that the potato powder mixtures, heated in this reactor, should be very homogeneous in order to obtain repeatable results. French fries and potato crisps could also be prepared in a repeatable manner, applying a standardized sample pre-treatment and temperature-controlled frying. Accordingly, it was demonstrated that the entire research methodology should be strictly controlled, starting from the raw material preparation through well-controlled heating or deep-frying methodologies and ending with an accurate quantification of acrylamide in the heated samples.

As a first application of these optimized heating methodologies, the influence on acrylamide formation of the deep-frying oil type was investigated in **chapter 3**. Homogeneous potato powder mixtures containing different oil types were heated in the tubular reactor. By use of this experimental set-up, it became possible to study acrylamide formation in the different mixtures, eliminating some physical and chemical variables during the frying process, such as

heat flux and water evaporation from and oil ingress into the food. The results obtained from the experiments with the reactor were compared with standardized French fry preparation tests. In both cases, no significant difference in acrylamide formation could be found between the various heating oils applied. Consequently, the type of the deep-frying oils did not seem to affect the acrylamide formation in potatoes during frying. Surprisingly however, when artificial mixtures did not contain vegetable oil, significantly lower acrylamide contents were detected compared to oil-containing mixtures, suggesting that the oil content might influence the heat transfer and subsequent acrylamide formation.

To further clarify the role of the deep-frying medium, **chapter 4** focused on the influence of oil oxidation and hydrolysis on the formation of this probable human carcinogen, by means of the same heating methodologies as in the previous chapter. Using the tubular reactor, possible changes in the heat transfer properties of the oil upon degradation were again excluded since direct contact between the food and the heat medium was eliminated. The results obtained from these experiments were once more compared with standardized French fry preparation trials. Using both heating methodologies, acrylamide formation was proven to be independent upon the oil oxidation and hydrolysis status in the experimental conditions used. More specifically, from the experimental results no evidence could be found that, due to oxidative or hydrolytic oil degradation, heat transfer properties of the oil were changed to such an extent that acrylamide formation during French fry preparation would be significantly influenced. Finally, it could be concluded that the investigated oil degradation products, such as glycerol, mono- and diacylglycerols, acrylic acid and several aldehydes did not significantly influence acrylamide formation, especially in the presence of reducing sugars.

Besides oil as a heating medium, water is another important constituent in fried foodstuffs. Based on sorption isotherms of the potato powder, the influence of initial water activity (a_w) and moisture content on both Maillard browning and acrylamide formation was determined in **chapter 5** by heating potato powder mixtures, containing 21% oil, in the tubular reactor. Maillard browning, as determined spectrophotometrically, showed an optimum at intermediate water activities. The yields of acrylamide, expressed relatively to the molar amount of asparagine, remained constant below 0.8 a_w and below moisture contents of about 20% (on dry matter basis). For the more severe heat treatments, an increased acrylamide yield was however observed at higher moisture contents, with an optimum at water contents of about 100% (on dry matter basis). However, this increase and optimum was not observed at

less severe heat treatments. At moisture contents above 100%, a significant decrease in acrylamide yields was assessed, although the water activity increased only marginally in this area of the sorption isotherms. It was thus observed that the acrylamide yield was rather dependent upon moisture content than upon water activity in the high-moisture potato powder model system. Similar to chapter 3, acrylamide formation was lowered upon decreasing the oil content of the potato powder mixtures from 21 to 0%, coinciding with a shift in optimal acrylamide yield to lower moisture contents.

Since it is known that Maillard browning and acrylamide formation are mainly dependent upon the reducing sugar content of the raw material, **chapter 6** focused on the impact of the glucose/fructose ratio on the relationship between acrylamide formation and surface colour of French fries. An exponential correlation was found between both factors. As a consequence, small differences in product colour could result in more pronounced differences in acrylamide contamination. This relationship however appeared to be dependent on the glucose/fructose ratio of the raw material. An excess of fructose compared to glucose stimulated acrylamide formation to a higher extent than Maillard browning. The opposite effect was established with an excess of glucose. In addition, a linear relationship was found between the absorbance of aqueous French fry extracts and acrylamide content, which moreover appeared to be less affected by the addition of extra reducing sugars prior to frying. Yet, to predict acrylamide formation, quantitative and qualitative sugar analysis remains an important instrument and is complementary to surface colour measurement.

Surface colour is however not the only parameter for the final quality of fried products. Other factors such as texture and taste also play a major role. **Chapter 7** further investigated these important quality aspects. In a first step, the impact on acrylamide formation of several additives was investigated as well as the mechanisms behind it. In the potato powder model system, sodium acid pyrophosphate, citric, acetic and L-lactic acid significantly reduced the final acrylamide content, by lowering the pH. Free glycine and L-lysine also lowered acrylamide, while keeping the pH at its original level. Calcium ions induced a supplementary acrylamide reduction, not attributed to a lower pH. These results were confirmed upon addition of the components to blanching water of potato crisps. In contrast to the model system, also NaCl appeared to lower acrylamide formation, in parallel with a reduced oil uptake, suggesting that textural and compositional product changes may also influence acrylamide formation. By means of sensory analyses of these crisps, a successful combination

was demonstrated between acrylamide mitigating treatments and crisps of acceptable or even superior product quality, compared to control crisps blanched in water. Yet, the applied components and concentration levels should be well chosen in order not to generate product-foreign flavours or undesired product colour.

After the identification of several factors influencing or mitigating acrylamide formation in potato products, **chapter 8** investigated the dietary intake of this probable human carcinogen. A food and drink intake survey was carried out among university students and staff members. Consumption data were collected on days when the participants took a hot lunch in a Belgian university canteen. The dietary acrylamide exposure was calculated through a probabilistic approach and revealed a median intake of 0.40 μg.kg $bw^{-1}.day^{-1}$, which is in accordance with previous exposure calculations. Biscuits (35%), French fries (30%), bread (24%) and chocolate (11%) were identified to be the main sources of dietary acrylamide. Foodstuffs consumed in between the three main meals of the day (so called snack type foods) contributed the most to the intake (42%). The exposure was lower in an intervention group which received free portions of fruit and vegetables, indicating that a nutritionally balanced diet may contribute to a decreased acrylamide intake. French fries had a significant impact on the acrylamide intake, due to the frequent consumption in the canteen. This demonstrates the important responsibility of caterers and canteen kitchens in the mitigation of acrylamide exposure through reduction of acrylamide in their prepared products, in particular in French fries, which are regularly consumed in Belgium.

The knowledge about acrylamide formation and intake, acquired in previous chapters, finally led to the formulation of the **general conclusions** and important **recommendations** to government, consumers and industry. Also some **perspectives** for future research were announced, to stimulate further investigations in our ambition to obtain safer, healthier and tastier foodstuffs.

Samenvatting

Het **eerste hoofdstuk** geeft een literatuuroverzicht van de risico's voor de consument, veroorzaakt door de blootstelling aan acrylamide. Daarnaast komen verschillende aspecten aan bod inzake de aanwezigheid, vorming en bepaling van deze vermoedelijk kankerverwekkende stof in levensmiddelen. Vrij asparagine en reducerende suikers worden beschouwd als de belangrijkste acrylamideprecursoren bij verhitting van zetmeelrijke levensmiddelen boven 120°C. Gefrituurde aardappelproducten zijn bijzonder gevoelig voor acrylamidevorming doordat ze zeer rijk zijn aan vrij asparagine. In de aardappel is dit asparagine in ruime overmaat aanwezig ten opzichte van reducerende suikers. Deze suikers vormen dan ook de snelheidsbepalende stap en zijn van cruciaal belang om de vorming van acrylamide te reduceren in gefrituurde aardappelproducten. Verschillende landbouwkundige factoren, waaronder de variëteit, bemesting, klimaat, oogsttijd en bewaaromstandigheden kunnen het reducerend suikergehalte in de aardappel, en bijgevolg de acrylamidevorming, sterk beïnvloeden. Aangezien gefrituurde aardappelproducten frequent geconsumeerd worden, dragen deze aanzienlijk bij tot blootstelling aan acrylamide via de voeding. Precies daarom concentreert deze studie zich op deze levensmiddelen.

In het **tweede hoofdstuk** wordt de betrouwbaarheid van de gebruikte onderzoeksmethodologie beoordeeld. Hiervoor werd de acrylamide-analyse gevalideerd op basis van lineariteit, specificiteit, detectie- en kwantificatielimiet, herhaalbaarheid, reproduceerbaarheid en terugvinding. De kwaliteit van de analyse werd verder gewaarborgd door een regelmatige deelname aan interlaboratorium testen en door het gebruik van controlestalen bij iedere analysereeks.
Daarnaast werd de herhaalbaarheid geëvalueerd van een nieuwe verhittingsmethode, gebaseerd op een gesloten buisreactor. Om herhaalbare resultaten te verkrijgen was de homogeniteit van de mengsels van aardappelpoeder, verhit in de reactor, van cruciaal belang. Door een gestandaardiseerde staalvoorbereiding en temperatuurscontrole tijdens het frituren werden daarnaast eveneens frieten en chips op een herhaalbare manier klaargemaakt. Op deze manier werd aangetoond dat de volledige onderzoeksmethodologie strikt moest opgevolgd worden, startende bij de voorbehandeling van de grondstof, via gecontroleerde verhittingsmethoden en eindigend bij een accurate bepaling van acrylamide in de verhitte stalen.

Bij wijze van eerste toepassing van deze geoptimaliseerde verhittingsmethoden werd de invloed van het type frituurolie op de vorming van acrylamide bestudeerd, zoals besproken in **hoofdstuk 3**. Homogene mengsels van aardappelpoeder, gemengd met diverse types frituurolie, werden verhit in de buisreactor. Deze proefopzet liet toe om acrylamidevorming te bestuderen, waarbij een aantal fysische en chemische variabelen tijdens het frituurproces geëlimineerd werden, zoals de hitte-overdracht, verdamping van water en olie absorptie. De resultaten, bekomen met dit modelsysteem, werden in een volgende fase vergeleken met gestandaardiseerde bakexperimenten van frieten. In beide gevallen kon geen significant verschil in acrylamidevorming worden aangetoond tussen de diverse frituuroliën. Bijgevolg werd besloten dat het type frituurolie geen invloed bleek te hebben op de vorming van acrylamide tijdens het frituurproces. Significant lagere acrylamidegehalten werden echter teruggevonden in aardappelmengsels, waarin geen olie werd gemengd, ten opzichte van oliebevattende mengsels, beiden verhit in de buisreactor. Dit zou erop kunnen wijzen dat het oliegehalte een invloed kan hebben op de hitte-overdracht en vervolgens ook op de vorming van acrylamide.

Om de rol van het frituurmedium verder te onderzoeken, spitst **hoofdstuk 4** zich toe op de invloed van olie-oxidatie en -hydrolyse op de vorming van acrylamide. Hierbij werden dezelfde verhittingsmethoden toegepast als in het vorige hoofdstuk. Aangezien de buisreactor een direct contact tussen levensmiddel en verhittingsmedium verhinderde, konden mogelijke veranderingen in hitte-overdracht, veroorzaakt door de degraderende olie, opnieuw worden geëlimineerd. De resultaten van deze experimenten werden andermaal vergeleken met gestandaardiseerde baktesten van frieten. De vorming van acrylamide bleek bij beide verhittingsmethoden niet af te hangen van olie-oxidatie of -hydrolyse, bij de toegepaste experimentele condities. Er kon meerbepaald niet worden bewezen dat de hitte-overdracht eigenschappen van frituuroliën zodanig veranderden, onder invloed van oxidatie of hydrolyse, dat acrylamidevorming tijdens het bakken van frieten significant werd beïnvloed. Tot slot kon er worden aangetoond dat diverse oliedegradatieproducten, zoals glycerol, mono- en diacylglycerolen, acrylzuur en diverse aldehyden geen significante invloed hadden op de vorming van acrylamide, en zeker niet in de aanwezigheid van reducerende suikers.

Naast frituurolie is water eveneens een belangrijke component in gefrituurde levensmiddelen. Op basis van de sorptie-isothermen van het aardappelpoeder, werd in **hoofdstuk 5** de invloed van de initiële wateractiviteit (a_w) en het watergehalte onderzocht op zowel de Maillard

bruinkleuring als de vorming van acrylamide. Hiervoor zijn mengsels van aardappelpoeder, die 21% olie bevatten, verhit in de buisreactor. De Maillard reactie werd spectrofotometrisch opgevolgd en vertoonde een optimum bij intermediaire a_w. Het rendement van de acrylamidevorming, relatief uitgedrukt ten opzichte van de molaire hoeveelheid asparagine aanwezig in ieder mengsel, bleef constant beneden een a_w van 0.8 en een vochtgehalte lager dan 20% (op droge stof basis). In de mengsels die een meer intense hittebehandeling hadden ondergaan werd een stijgend rendement in acrylamidevorming vastgesteld bij hogere vochtgehaltes, met een optimum bij een vochtgehalte van 100% (op droge stof basis). Deze stijging en dit optimum werden echter niet vastgesteld bij minder intense hittebehandelingen. Bij vochtgehaltes boven 100% werd een significante daling in het acrylamiderendement opgemeten, hoewel de wateractiviteit slechts zeer beperkt veranderde in dit gebied van de sorptie-isothermen. Het acrylamiderendement bleek dus eerder afhankelijk van de hoeveelheid water dan van de wateractiviteit bij deze mengsels met een hoog vochtgehalte. Net zoals in hoofdstuk 3 werd vastgesteld dat de acrylamidevorming verlaagde wanneer het oliegehalte van de aardappelmengsels verminderd werd van 21 naar 0%. Dit ging gepaard met een verschuiving van het optimale acrylamiderendement naar lagere vochtgehaltes.

Aangezien geweten is dat de Maillard reactie en acrylamidevorming voornamelijk afhankelijk zijn van de hoeveelheid reducerende suikers in de aardappel, spitst **hoofdstuk 6** zich toe op de invloed van de glucose/fructose verhouding op de correlatie tussen de acrylamidevorming en oppervlaktekleur van frieten. Een exponentieel verband werd aangetoond tussen de kleur en acrylamidevorming. Bijgevolg konden kleine kleurverschillen aanleiding geven tot meer uitgesproken verschillen in acrylamidecontaminatie. Daarnaast bleek dat dit verband echter afhankelijk was van de glucose/fructose verhouding in het onverhitte startmateriaal. Een overmaat fructose ten opzichte van glucose stimuleerde de vorming van acrylamide meer dan de Maillard bruinkleuring. Het tegenovergestelde effect werd vastgesteld bij een overmaat aan glucose. Daarnaast werd er een lineair verband aangetoond tussen de absorbantie van waterige frietextracten en het acrylamidegehalte. Dit verband bleek bovendien minder gevoelig aan toevoeging van extra reducerende suikers vóór het frituren. Om acrylamidevorming te voorspellen blijft de suikeranalyse echter een belangrijk instrument, complementair aan de meting van de oppervlaktekleur.

Kleur is echter niet de enige parameter welke de finale kwaliteit van gefrituurde aardappelproducten bepaalt. Andere factoren zoals textuur en smaak spelen eveneens een grote rol. **Hoofdstuk 7** gaat verder in op deze belangrijke kwaliteitsaspecten. In een eerste stap werd de impact van diverse additieven op de vorming van acrylamide onderzocht, evenals het werkingsmechanisme ervan. In het modelsysteem, gebaseerd op het aardappelpoeder verhit in de buisreactor, verminderde het finaal acrylamidegehalte significant door het toevoegen van natrium pyrofosfaat, citroenzuur en L-lactaat, en dit door een verlaging van de pH. Vrij glycine en L-lysine verlaagden eveneens het acrylamidegehalte, zonder de oorspronkelijke pH van het modelsysteem te beïnvloeden. Calcium ionen induceerden een extra verlaging, welke niet kon toegeschreven worden aan een verlaging van de pH. Deze resultaten werden bevestigd wanneer deze componenten toegevoegd werden aan het blancheerwater van aardappelchips. In tegenstelling tot het modelsysteem, verlaagde NaCl eveneens het finale acrylamidegehalte. In parallel veroorzaakte NaCl ook een verminderde opname van olie, wat erop zou kunnen wijzen dat veranderingen in de textuur en de samenstelling eveneens de acrylamidevorming kunnen beïnvloeden. Aan de hand van sensorische analysen op deze chips, werd een succesvolle combinatie aangetoond tussen enerzijds behandelingen die acrylamidevorming verminderen en anderzijds chips van aanvaardbare of zelfs superieure kwaliteit, in vergelijking met referentiechips geblancheerd in water. De componenten en concentratieniveaus moeten echter oordeelkundig worden toegepast om geen ongewenste productsmaak of kleur te genereren.

Na de identificatie van diverse factoren die de vorming van acrylamide beïnvloeden of verhinderen, wordt in **hoofdstuk 8** de blootstelling aan acrylamide via de voeding bestudeerd. Hiervoor werd de inname van voeding en drank opgevolgd onder een populatie van universiteitspersoneel en studenten. Deze consumptiedata werden verzameld op dagen wanneer de deelnemers een warm middagmaal consumeerden in een Belgische universiteitskantine. De blootstelling aan acrylamide via de voeding werd berekend aan de hand van een probabilistische benadering. Een mediane acrylamide-inname van 0.40 µg.kg lichaamsgewicht^{-1}.dag^{-1} werd berekend, welke in overeenstemming is met eerdere studies. Koeken (35%), frieten (30%), brood (24%) en chocolade (11%) werden geïdentificeerd als de voornaamste bronnen van acrylamide-inname via de voeding. Levensmiddelen geconsumeerd tussen de drie maaltijden van de dag (de zogenaamde tussendoortjes) droegen het meest bij tot de inname (42%). De blootstelling was lager in een interventiegroep die porties groenten en

fruit gratis toebedeeld kreeg. Dit wijst erop dat een nutritioneel gebalanceerde voeding zou kunnen bijdragen tot een verlaagde acrylamide-inname. Door hun frequente consumptie in de kantine hadden frieten een significante impact op de acrylamide-inname. Dit toont aan dat cateringbedrijven en grootkeukens een belangrijke verantwoordelijkheid hebben inzake de verlaging van de acrylamideblootstelling via een vermindering van acrylamide in hun bereide levensmiddelen, meer specifiek in frieten welke regelmatig geconsumeerd worden in België.

De kennis betreffende de vorming en inname van acrylamide, gebundeld in voorgaande hoofdstukken, leidde finaal tot de formulering van de **algemene conclusies** en belangrijke **aanbevelingen** voor de overheid, consument en industrie. Daarnaast werden een aantal **perspectieven** meegegeven voor toekomstig onderzoek, om zo onze ambities die bijdragen tot het verkrijgen van veilige, gezonde en smakelijke voeding in de toekomst verder waar te kunnen maken.

Chapter 1

Chemistry and safety of acrylamide in foodstuffs

Chapter 1 Chemistry and safety of acrylamide in foodstuffs

1.1 Abstract

This chapter gives an overview of several aspects dealing with the presence, formation and quantification of acrylamide in foodstuffs and the consumers risks associated with it. The first part focuses on the industrial use of acrylamide, as well as on its occurrence in different foodstuffs. Subsequently, the available acrylamide analysis techniques are summarized, followed by a discussion of the toxicological aspects associated with acrylamide intake. For this, the metabolic pathways of acrylamide in animals and humans are described and the most important human safety risks are assessed and characterized. Furthermore, the various acrylamide formation pathways in foodstuffs are discussed, together with the key precursors. Finally, the agricultural factors affecting the formation of acrylamide in potato products are summarized. This chapter serves as a basis for the research presented in the following chapters of this work.

1.2 Use and presence of acrylamide

1.2.1 Chemical properties

Acrylamide (2-propenamide) is an odourless, white solid, with a molecular mass of 71.08 $g.mole^{-1}$ (Figure 1.1). It is industrially produced from the hydration of acrylonitrile. Acrylamide readily dissolves in water and polar solvents such as acetone, methanol and ethanol. It is however not soluble in non-polar solvents. Acrylamide has a melting point of 84.5°C (Friedman, 2003). It contains an α,β-unsaturated amide system which reacts with nucleophilic compounds via a Michael addition (JIFSAN/NCFST, 2002). Monomeric acrylamide readily participates in radical-initiated polymerization reactions, whose products form the basis of most of its industrial applications (Friedman, 2003).

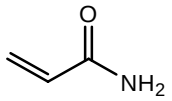


Figure 1.1. Chemical structure of acrylamide

1.2.2 Industrial use and occurrence

The primary use of polyacrylamide is to strengthen paper. In wastewater treatment, polyacrylamide is used as flocculant to improve the process of sludge thickening and dewatering. The polymer has also found applications as a soil conditioner (Friedman, 2003; Törnqvist, 2005). Moreover, polyacrylamide is used in several biomedical and research applications, e.g. for the separation of proteins by electrophoresis (Smith and Oehme, 1991). It is also applied in formulations of several types of personal care and grooming products, such as lotions, cosmetics, deodorants, soaps and shampoos. During manufacture of polyacrylamide, a small amount of acrylamide monomer may however be present in the final product because of incomplete polymerization (Shipp et al., 2006). Low amounts of acrylamide might also migrate from food packaging material into the packed foodstuff (Tritscher, 2004). The specific migration limit for acrylamide from materials which come into contact with foodstuffs was defined to be not detectable, with a limit of detection (LOD) of 10 $\mu g.kg^{-1}$ (EEC, 1992). For drinking water, a maximum guideline value of 0.5 $\mu g.L^{-1}$ was proposed (WHO, 1993). Besides its industrial applications, acrylamide is also present in tobacco smoke, in amounts of 1-2 µg *per* cigarette (Bergmark et al., 1993; Bergmark, 1997; Smith et al., 2000; Friedman, 2003; Urban et al., 2006).

1.2.3 Presence of acrylamide in foodstuffs

Besides its industrial use, acrylamide is also present in a wide range of starch-rich foodstuffs. Acrylamide is typically found in products which are fried, baked or roasted at temperatures above approximately 120°C. Raw or boiled foodstuffs usually do not contain acrylamide (Tareke et al., 2002). Yet, fried meat contains only low amounts of acrylamide, suggesting that carbohydrate-rich foodstuffs provide the precursors of acrylamide, rather than protein-rich foods. Extensive amounts of contamination data were collected in the last years by the European authorities, the Confederation of the Food and Drink Industries in the EU (CIAA) and the US Food and Drug Administration (FDA). These databases (Lineback et al., 2005; US FDA/CFSAN, 2006; IRMM, 2006) are regularly updated. The most important matrices included in both databases are potato crisps, French fries, crisp bread, breakfast cereals, bakery products such as bread and biscuits and coffee (Stadler and Scholz, 2004; Dybing et al., 2005). Figure 1.2 shows the most recent contamination data originating from the EU monitoring database (updated in June 2006). From this figure, it becomes clear that there is a large variability in acrylamide contamination within each food category. This variability was moreover found between different lots and even between different packaging units of the same lot (Dybing et al., 2005). Recently and rather unexpectedly, acrylamide was also detected in (unheated) black olives and prune juice (Roach et al., 2003; Stadler and Scholz, 2004).

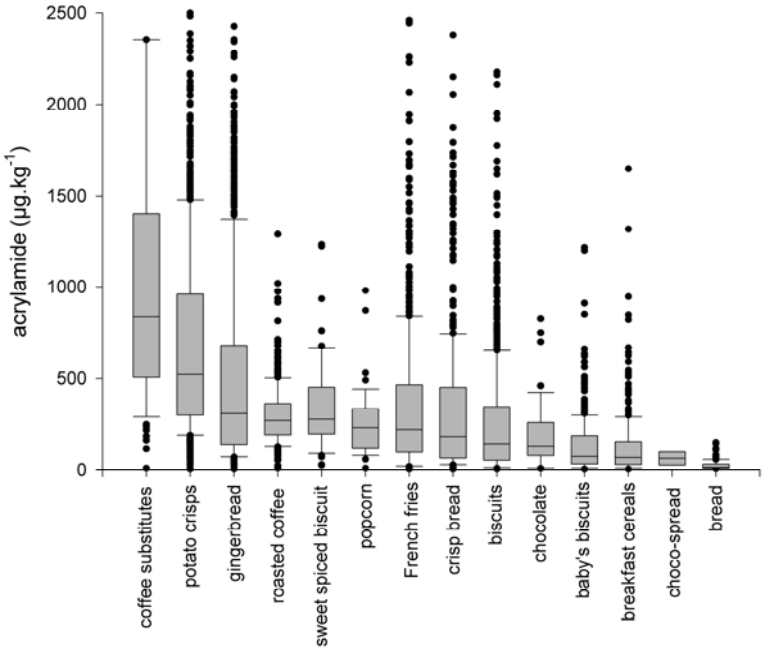


Figure 1.2. Box plot diagram of acrylamide contamination data in the EU monitoring database [updated from Stadler and Scholz (2004); IRMM (2006)]

1.3 Analysis techniques of acrylamide in foodstuffs

To quantify the degree of contamination and to measure possible mitigation strategies of acrylamide formation, reliable analytical methods are needed. Several chromatographical methods, discussed below, were developed. The occurrence of acrylamide in a large number of food matrices, with distinct matrix interferences, makes the development of a universal analysis method a challenge. A more detailed description of the available methods can be found in various review articles (Wenzl et al., 2003; Castle and Eriksson, 2005; Zhang et al., 2005).

1.3.1 Extraction and clean-up procedures

Usually the isotope dilution technique is applied. This means that, before the extraction step, a stable isotopically labelled internal standard is added to the homogenized food sample in order to compensate for losses during clean-up and injection. For this, deuterium-labelled ($^{2}H_3$-) or carbon-labelled ($^{13}C_3$-) acrylamide, methacrylamide or propionamide are used (Castle and Eriksson, 2005; Owen et al., 2005; Zhang et al., 2005). Acrylamide, being highly water soluble, is subsequently extracted using mostly water (Tareke et al., 2002; Rosén and Hellenäs, 2002). The sample is then homogenized in the aqueous phase by mixing, stirring or shaking. According to the complexity of the sample matrix, organic solvents such as hexane have been used for defatting. Protein rich matrices are eventually clarified using Carrez I and II solutions or acetonitrile. Before continuing with the clean-up step, the aqueous phase is centrifuged or filtered (Riediker and Stadler, 2003; Delatour et al., 2004).

Most clean-up procedures consist of a combination of several solid-phase extraction (SPE) cartridges (Roach et al., 2003; Zhang et al., 2005). Eventually, the extract can be further concentrated by evaporation (Jezussek and Schieberle, 2003; Govaert et al., 2006). Difficult matrices such as coffee and roasted cocoa require more complex clean-up steps in order to avoid interference from co-extractives (Hoenicke et al., 2004; Mastovska and Lehotay, 2006; Gökmen and Senyuva, 2006; Ren et al., 2006; Bermudo et al., 2006a).

The obtained extract is subsequently analysed, mainly using GC-MS or LC-MS/MS (Wenzl et al., 2003; Zhang et al., 2005). Also other systems are available, but are still less widely employed. A brief summary of the chromatographic methods is given below.

1.3.2 Chromatographic analysis based on GC-MS

Although acrylamide can be analysed using gas chromatography without derivatization, the molecule is normally brominated. This step may usually be operated even before clean-up. Accordingly, a derivative is formed which has improved GC properties (such as higher volatility and lower polarity) and better MS characteristics (higher mass ions and characteristic $^{79}Br/^{81}Br$ patterns). Even though bromination is a time-consuming step, it thus leads to increased selectivity (Zhang et al., 2005). Acrylamide can also be derivatized through silylation. The volatile derivative can thereupon be extracted from the headspace using solid-phase microextraction (SPME) (Lagalante and Felter, 2004). The analyte separation is performed on standard GC capillary columns of middle to high polarity (Wenzl et al., 2003).

The major drawback of GC analysis without derivatization is the lack of characteristic ion peaks in the mass spectrum. Co-extractives can produce almost the same fragmentation pattern and may thus interfere (Biedermann et al., 2002a). As a consequence, the clean-up step is more demanding. The selectivity may be increased by means of chemical ionization using methane, ammonia or acetonitrile as the reagent gas and tandem mass spectrometric detection (Wenzl et al., 2003; Hoenicke et al., 2004; Lee et al., 2007).

1.3.3 Chromatographic analysis based on LC-MS/MS

An equally important acrylamide analysis method is a technique based on liquid chromatography. The LC method also has high sensitivity, but avoids the time-consuming derivatization step. Recently, techniques based on ultra performance liquid chromatography (UPLC) were developed, with improved resolution within a shorter retention time (Zhang et al., 2007). Reversed-phase columns are needed which offer sufficient retention and separation of the polar compounds in order to avoid co-elution of acrylamide with other compounds. In order to improve the sensitivity and selectivity, tandem mass spectrometry is needed, working in multiple reaction monitoring (MRM). In this data acquisition mode, the first quadrupole (MS 1) is set to transmit only the parent ion of interest, coming from the ion source (Figure 1.3). This parent ion is fragmented in the collision cell, filled with argon. The second quadrupole (MS 2) is subsequently set to transmit only a specific daughter ion (Zhang et al., 2005).

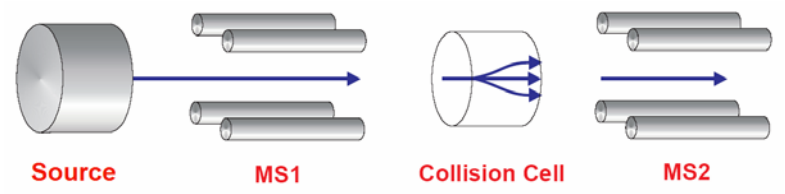


Figure 1.3. Schematic overview of the multiple reaction monitoring mode (Micromass, 1998)

1.3.4 Other analytical methods for the determination of acrylamide

Since the two-stage mass spectrometric detectors are quite expensive, attempts have been made to develop methods using cheaper detectors. An LC method was developed based on a derivatization of acrylamide with 2-mercaptobenzoic acid, requiring only a single-stage mass spectrometer (Jezussek and Schieberle, 2003). This detector was also successfully applied in combination with ion-exclusion chromatography, without the need to derivatize (Cavalli et al., 2004). In addition, LC-UV (Paleologos and Kontominas, 2005), LC-DAD (diode array detector) (Owen et al., 2005), LC-fluorescence (Schieberle et al., 2005), GC-ECD (electron capture detector) (Zhang et al., 2006) and GC-FID (flame ionization detector) (Pedersen and Olsson, 2003) were developed. Recently, a capillary zone electrophoresis method was described with a diode array detection system. The method required a derivatization step with 2-mercaptobenzoic acid (Bermudo et al., 2006b; Bermudo et al., 2006c). The robustness and sensitivity of these methods in complex matrices, such as coffee and cocoa, is however not always proven.

Finally, proton transfer reaction mass spectrometry (PTR-MS) has been considered as a suitable method for rapid and on-line measurements of acrylamide released into the headspace of the heated samples (Pollien et al., 2003). PTR-MS enables to measure time-dependent variations in the headspace with a time resolution of 0.1 s. Cook and Taylor (2005) also developed an online analysis technique, based on MS/MS detection.

1.3.5 Concluding remarks regarding acrylamide analysis

Mainly two analysis methods are used, being GC/MS with derivatization and LC-MS/MS. The results of interlaboratory proficiency tests indicate that both methods seem to be equivalent, corroborated by the fact that an excellent correlation between both techniques was found (Clarke et al., 2002; Ono et al., 2003; Castle and Eriksson, 2005; Owen et al., 2005; Zhang et al., 2005). A recent collaborative trial however revealed a superior performance of

the LC-MS/MS compared to the GC-MS method, due to inferior reproducibility and repeatability results of GC-MS (Wenzl et al., 2006). A direct comparison is however difficult since there is little evidence that the applied methods have been systematically validated for all relevant matrices according to international guidelines (Zhang et al., 2005). It is however important to investigate the validity of the used analysis technique in order to guarantee reliable results. Therefore, chapter 2 focuses on the efforts being put in the validation and in-house quality control procedures for the LC-MS/MS analysis method, used throughout this work.

1.4 Toxicology of acrylamide

The discovery of acrylamide in foodstuffs provoked a public debate among scientists, because of the relative lack of toxicological data for humans on the one hand and because of the knowledge that acrylamide in high doses is genotoxic in cell and animal studies on the other (Granath and Törnqvist, 2003). A wide range of studies spanning multiple disciplines was set up. This section summarizes the metabolic pathways of acrylamide in animals and humans, the current evidence for carcinogenicity, neurotoxicity and reproductive toxicity, and findings from epidemiological studies of occupational and dietary exposures. Finally the possible risk associated with the presence of acrylamide in foodstuffs is characterized.

1.4.1 Absorption, distribution, metabolism and excretion

1.4.1.1 Absorption and distribution

The absorption of acrylamide can occur through different routes. Several toxicokinetic studies were performed following oral and dermal exposure to acrylamide, or through inhalation. Because of its polarity and low molecular weight, acrylamide is readily absorbed, as demonstrated with ^{13}C and ^{14}C-labelled acrylamide (Doerge et al., 2005b; Shipp et al., 2006).

Due to its application in cosmetics, several studies were carried out on the dermal absorption of acrylamide, both *in vivo* and *in vitro* (Sumner et al., 2003; Fennell et al., 2005; Shipp et al., 2006). A controlled study on human volunteers (Fennell et al., 2005) revealed a mean dermal absorption of about 25-30% of the amount applied. Similar percentages were found in *in vivo* animal studies and *in vitro* (Shipp et al., 2006).

Compared to dermal exposure, the absorption of acrylamide via the gastrointestinal tract appeared to be more efficient, both in human volunteers (Fennell et al., 2005; Fuhr et al., 2006) and in test animals. When acrylamide was administered via drinking water to e.g. rats, absorption was 60 to 90% higher compared to administration via acrylamide-fortified food (28-47% absorption) (Doerge et al., 2005a; Doerge et al., 2005b; Doerge et al., 2005c). Model studies with Caco-2 cells attributed this reduced absorption to the interaction of acrylamide with proteins being present in the food matrix (Schabacker et al., 2004). These dietary proteins can bind acrylamide under physiological conditions in the intestine or under mild cooking conditions (80°C). Yet, a recent study found that the relative absorption of acrylamide from swine feed and from pure water was the same (Aureli et al., 2007).

After absorption, acrylamide is rapidly and widely distributed via the blood to various tissues, such as muscle tissue, testes, skin, liver, kidneys, brain, heart and lung. The distribution of acrylamide and its metabolites depends however upon the route of exposure and is species dependent (Ikeda et al., 1987; SCF, 2002; Sumner et al., 2003; Shipp et al., 2006). Acrylamide also readily crosses the placenta and was detected in human breast milk (Sorgel et al., 2002).

1.4.1.2 Metabolism

Once absorbed, acrylamide may react with cytochrome P450 2E1 oxidase in the liver to produce the chemically reactive epoxide glycidamide. Furthermore, the epoxide group can be cleaved by an epoxide hydrolase (EH) (Sumner et al., 1999; Kirman et al., 2003). Acrylamide and glycidamide may also conjugate with liver, kidney, brain or erythrocyte glutathione (GSH) (Paulsson et al., 2005). The major metabolic pathways are depicted in Figure 1.4. It was shown that the conversion to glycidamide in rats occurs more effectively at lower doses than at higher (Bergmark et al., 1991). Moreover, the percentage of acrylamide conjugated with glutathione or oxidized to glycidamide is species dependent, due to the different expression of metabolizing enzymes (Dybing et al., 2005; Shipp et al., 2006). The extent of acrylamide oxidation was the highest for mice, followed by rats and humans. In contrast to mice and rats, the majority of the metabolism of glycidamide in humans was via hydrolysis, with little via glutathione conjugation (Sumner et al., 2003; Fennell and Friedman, 2005; Fennell et al., 2005).

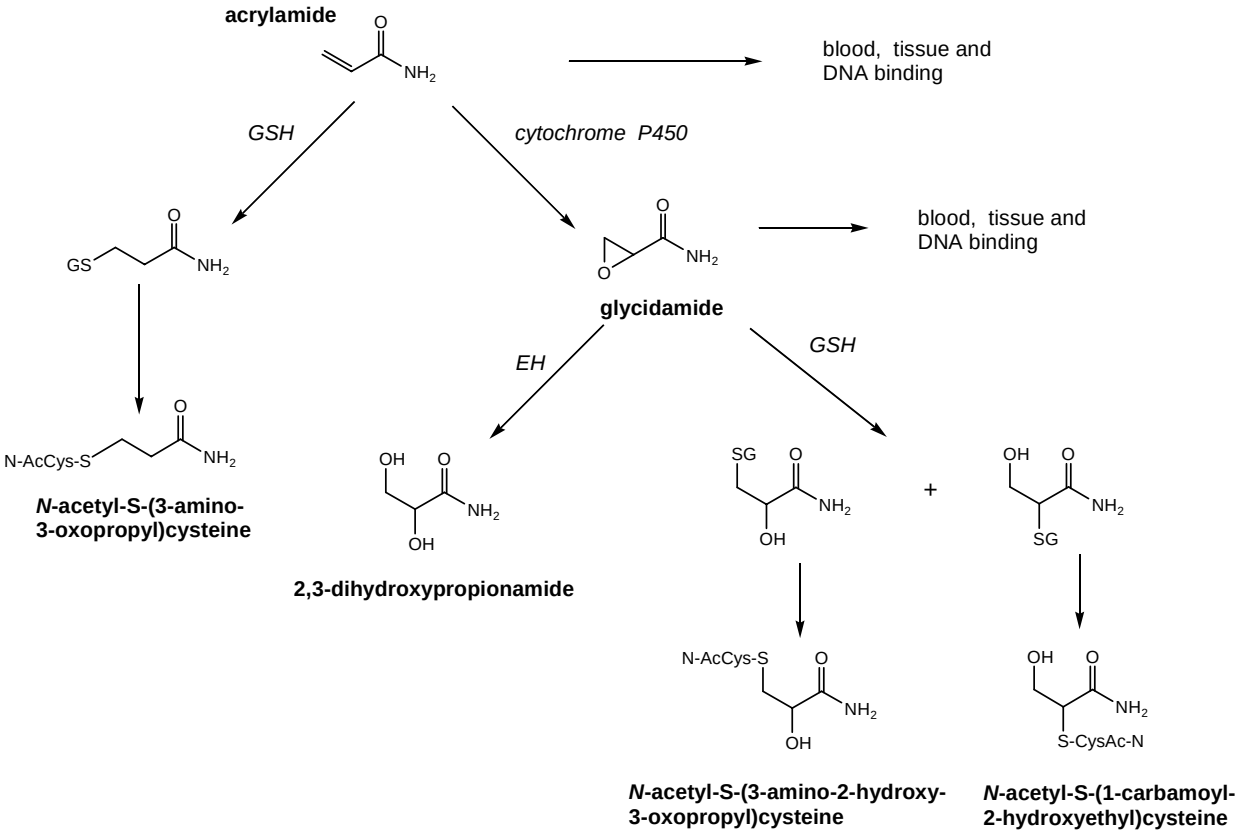


Figure 1.4. Proposed metabolism of acrylamide (Sumner et al., 2003)
GSH, glutathione; EH, epoxide hydrolase

Both acrylamide and glycidamide form adducts with thiol and amino groups on hemoglobin and other proteins (Bergmark, 1997; Paulsson et al., 2003). The α,β-unsaturated amide system reacts readily with nucleophiles by the Michael addition (Calleman, 1996; Shipp et al., 2006). Adducts with the α-amino group of *N*-terminal valine residue or with the thiol-group of cysteine residues in hemoglobin have been reported (Bergmark et al., 1993; Sumner et al., 2003; Fennell and Friedman, 2005; Fennell et al., 2005). These adducts represent the amount of acrylamide present in the blood circulation over the lifetime of the erythrocytes (approx. 125 days). Quantification of these biomarkers may therefore give a good estimate of the long-term acrylamide exposure (Bergmark et al., 1993; Bergmark, 1997; Hagmar et al., 2005; Baum et al., 2005; Aureli et al., 2007).

Glycidamide and to a much lesser extent acrylamide, also form adducts with guanine and adenine in DNA (Segerbäck et al., 1995; Besaratinia and Pfeifer, 2003; da Costa et al., 2003; Dybing et al., 2005; Maniere et al., 2005; Besaratinia and Pfeifer, 2005; Doerge et al., 2005a). These adducts are potential markers for the biologically active dose of acrylamide that reaches the DNA and may cause genotoxic effects, as discussed below.

1.4.1.3 Excretion

The majority (40-70%) of the absorbed radiolabeled acrylamide in rats is excreted via the urine (Shipp et al., 2006) after conversion of the GSH-conjugates of acrylamide and glycidamide to mercapturic acids. It has recently been shown that the excretion of acrylamide as urinary metabolites begins shortly after exposure and about 50% is excreted within 24 hours (Boettcher et al., 2006; Fennell et al., 2006). In contrast to hemoglobin adducts, the urinary mercapturic acid metabolites may thus give insight into the short-term exposure to acrylamide, as was recently reported (Bjellaas et al., 2007).

1.4.2 Acrylamide toxicity

As stated above, glycidamide and the α,β-unsaturated amide system of acrylamide may bind with biological nucleophilic groups of proteins and DNA. This reaction plays a significant role in the underlying biological events resulting in toxicity (Shipp et al., 2006).

1.4.2.1 Neurotoxicity

The neurotoxicity of acrylamide in humans is well-known from occupational and accidental exposures (FAO/WHO, 2005), characterized by skeletal muscle weakness and numbness of hands and feet. Acrylamide has been shown to be toxic to both the central and the peripheral nervous system (Calleman, 1996), although the nerve terminal is now considered to be the primary site of acrylamide action (LoPachin et al., 2003; LoPachin, 2004). Several competing theories have been proposed to explain its mechanism of action, including biochemical changes, such as the alteration in ion levels or the inhibition of glycolytic enzymes by acrylamide adduct formation. In such a way, the release of neurotransmitter could be reduced. Also morphological changes were demonstrated, such as changes in or degeneration of nerve structures, which could be visualized with microscopic examinations. However, the No Observable Effect Level (NOEL) for morphological changes is stated at 500 $\mu g.kg\ bw^{-1}.day^{-1}$, which is far above the known exposure levels due to food intake. Consequently, the risk of neurotoxic effects caused by dietary acrylamide is considered to be very low (Dybing and Sanner, 2003).

1.4.2.2 Reproductive toxicity

Acrylamide administered to drinking water of rodents at doses equal to or greater than 5 mg.kg bw^{-1}.day^{-1} resulted in significant decreases in number of live fetuses *per* litter (Shipp et al., 2006). At doses of 155 mg.kg bw^{-1}.day^{-1} or greater, signs of neurotoxicity and copulatory behaviour were noted, as well as effects on sperm motility and morphology. These exposure levels are however far above the known dietary acrylamide intake in order to pose such a risk.

1.4.2.3 Carcinogenicity

Already in 1994, acrylamide was classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC, 1994). This conclusion was based on positive bioassay results in rodents and supported by the evidence that acrylamide is transformed in mammalian tissues to its more reactive genotoxic metabolite, glycidamide (Figure 1.4). Currently, substantial laboratory evidence on experimental rodents shows that acrylamide is carcinogenic, causing tumours at multiple sites such as lungs, skin, brain, mammary gland, thyroid gland and uterus (Rice, 2005; Besaratinia and Pfeifer, 2005). Oxidation of acrylamide to glycidamide appeared to be a prerequisite for genotoxicity of acrylamide, due to the higher reactivity of glycidamide to form adducts with DNA (Besaratinia and Pfeifer, 2003; Besaratinia and Pfeifer, 2004; Rice, 2005; Puppel et al., 2005; Tareke et al., 2006). The major mode of carcinogenic action of acrylamide is genotoxic as it induces gene mutations, chromosomal aberrations and breaks in germ and somatic cells of mice *in vivo* and in cultured cells *in vitro* (IARC, 1994; Dearfield et al., 1995; Tritscher, 2004; Rice, 2005; Puppel et al., 2005). In addition, acrylamide may impair DNA repair (Blasiak et al., 2004) and cause unscheduled DNA synthesis in human mammary cells (Tritscher, 2004) and in target tissues of rats (Lafferty et al., 2004).

Extrapolation of the above-mentioned results to humans is however not obvious, among other things because of the much higher exposure levels used in animal and cell culture studies, differences in the routes of exposure and differences in metabolism between species (Wilson et al., 2006; Dybing et al., 2005; Vesper et al., 2005). So far, epidemiological studies could not find any significant association between acrylamide exposure and cancer incidence or mortality (Wilson et al., 2006). However, all studies had severe limitations, either in the

exposure assessment, confounders such as co-exposures or limited power to detect small increases in tumour incidence (Dybing and Sanner, 2003; Hagmar and Törnqvist, 2003; Mucci et al., 2003; Tritscher, 2004; Rice, 2005; Mucci and Adami, 2005; Mucci et al., 2006; Pelucchi et al., 2006). Based on the experiments with animals, the excess risk estimates (above the control group) are expected to be very small. A cohort of more than two million people would be needed to detect the additional risk (Mucci and Adami, 2005). Consequently, it is unlikely that epidemiological evidence will be able to prove or disprove an association. An accumulation of evidence through well-designed studies in multiple disciplines can shed light on this important public health concern (Wilson et al., 2006).

1.4.3 Acrylamide exposure assessment

Knowing the toxicological properties of acrylamide, many countries have estimated the dietary intake of acrylamide in order to evaluate the possible risks to human health (Table 1.1). Depending on the availability of contamination data at the time of calculation, different contamination databases were used for these simulations. Nevertheless, the calculations indicate an overall median acrylamide intake around 0.5 µg.kg $bw^{-1}.day^{-1}$. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated the average dietary acrylamide intake in the general population between 0.3 and 2.0 µg.kg $bw^{-1}.day^{-1}$, with intakes up to 5.1 µg.kg $bw^{-1}.day^{-1}$ for the 99^{th} percentile consumers (FAO/WHO, 2005). The major contributing foods to the exposure were French fries, potato crisps, coffee, biscuits and bread. Others food items contributed less than 10% of the total exposure (Wilson et al., 2006; Krokida et al., 2001d; Konings et al., 2003; Svensson et al., 2003; Boon et al., 2005; Dybing et al., 2005; FAO/WHO, 2005). Still, data for developing countries in Latin America and Africa are scarce (Arisseto et al., 2007).

As shown in Table 1.1, children and adolescents tend to be more exposed to acrylamide, about 2 to 3 times more compared to adults (FAO/WHO, 2002). This may be due to a combination of children's higher caloric demand relative to their body weight as well as their different dietary pattern, with higher consumption of certain acrylamide rich foodstuffs, such as French fries and potato crisps (Wilson et al., 2006; Dybing et al., 2005). It is thus clear that certain subgroups demonstrate especially high exposure to acrylamide.

Table 1.1. Results of acrylamide exposure estimations (µg.kg $bw^{-1}.day^{-1}$) in different countries

country	reference	population group	exposure (µg.kg $bw^{-1}.day^{-1}$)		
			mean	P 50	P 95/P 90*
Belgium	Matthys et al. (2005)	adolescents (13 – 18 y)		0.5	1.1
The Netherlands	Boon et al. (2005)	total population (1 – 97 y)		0.5	1.2
		young children (1 – 6 y)		1.1	2.0
The Netherlands	Konings et al. (2003)	total population (1 – 97 y)	0.5	0.2	0.6
		children - adolescents (7 – 18 y)	0.7	0.2	0.9
		young children (1 – 6 y)	1.0	0.3	1.1
Germany	Madle et al. (2003)	total population	0.6		
	Mosbach-Schulz et al. (2003)	young children (4 – 6 y)	1.2		
France	AFFSA (2003)	adults (> 15 y)	0.5		1.1
		children (3 – 14 y)	1.4		2.9
Norway	Dybing and Sanner (2003)	males (16 – 79 y)	0.5	0.4	1.2*
		females (16 – 79 y)	0.5	0.4	1.0*
Sweden	Svensson et al. (2003)	adults (18 – 74 y)	0.4	0.4	0.9
US	DiNovi (2006)	total population (> 2 y)	0.4		1.0*
		young children (2 – 5 y)	1.1		2.3*

Biomarkers such as hemoglobin adducts and excreted urinary metabolites could in some cases confirm the above-mentioned median acrylamide intake levels (Bjellaas et al., 2005; Urban et al., 2006; Ogawa et al., 2006; Paulsson et al., 2006; Bjellaas et al., 2007). However, a clear correlation between biomarkers and acrylamide intake was not always found. This may be attributed to the species and inter-individual difference in metabolic rates of acrylamide and glycidamide (Dybing et al., 2005; Vesper et al., 2005), to the uncertainty of dietary exposure assessments, the inherent variability of acrylamide contamination and the possibility of other acrylamide sources (Tareke et al., 2002; Urban et al., 2006). Taking the smoking habit already into account, this last assumption can however be ignored since exposure due to polyacrylamide use in cosmetics, food packaging and water treatment appears to be well below the intake via food (FAO/WHO, 2002; Ahn and Castle, 2003).

1.4.4 Acrylamide risk characterization and management

As discussed shortly under section 1.4.2, the risks of neurotoxicity and reproductive toxicity are unimportant when considering consumption of acrylamide-containing foodstuffs. The risk of carcinogenicity in humans is however uncertain. Yet genotoxic carcinogens, such as acrylamide, are considered to be without a threshold for their reaction with DNA and hence adverse effects (Dybing and Sanner, 2003; Tritscher, 2004; Barlow et al., 2006; O'Brien et al., 2006). Since acrylamide is an unavoidable contaminant in foodstuffs, the 'ALARA' principle can initially be applied, meaning that the levels should be 'as low as reasonably achievable'. However, this approach is solely based on hazard identification and does not take either carcinogenic potency or human exposure into account.

Another technique to characterize the risk for humans is the use of quantitative low-dose extrapolation of dose-response data from high doses in animal studies to low doses in humans (Figure 1.5) (EFSA, 2005; Barlow et al., 2006; O'Brien et al., 2006). The number of extra cancers caused by acrylamide exposure could be estimated accordingly. Lifetime cancer risks between 1 and 45 additional cases *per* 10000 people were calculated (Wilson et al., 2006; US EPA, 1993; Dybing and Sanner, 2003; FAO/WHO, 2005). The use of quantitative low-dose extrapolation raised however numerous scientific uncertainties related to the selection of mathematical extrapolation models. In addition, the technique does not take the underlying biological processes and bioavailability into account, probably giving rise to overestimation of the true risk.

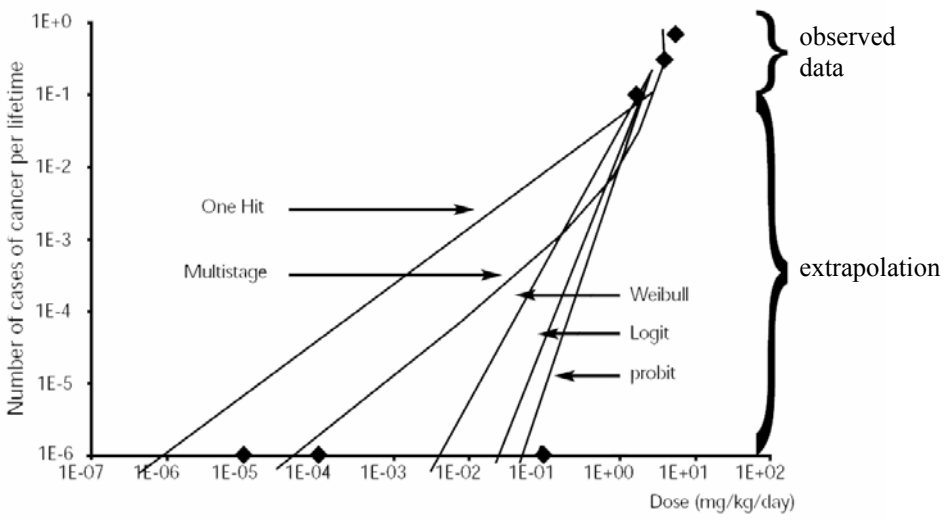


Figure 1.5. Example of a low-dose extrapolation from animal carcinogenicity data using various models (EFSA, 2005)

Recently, the margin of exposure (MOE) approach was put forward (FAO/WHO, 2005; EFSA, 2005; Barlow et al., 2006; Larsen, 2006; O'Brien et al., 2006). EFSA recommended the use of this method for assessing the risk of genotoxic carcinogens, since it requires little or no extrapolation outside the observed experimental data range. The MOE is defined as the ratio between a dose leading to tumours in experimental animals (BMDL10) and the human intake. BMD10 (benchmark dose) is defined as the daily dose at which 10% of the (animal) population develops a tumour above the control (Figure 1.6). BMDL10 corresponds to the lower limits of a one-sided 95% confidence interval on the BMD10 (Figure 1.6). A smaller MOE represents a higher risk than a larger one. Consequently, risk management can use this information for priority setting. However, it does not provide a quantitative estimate of risk (Barlow et al., 2006).

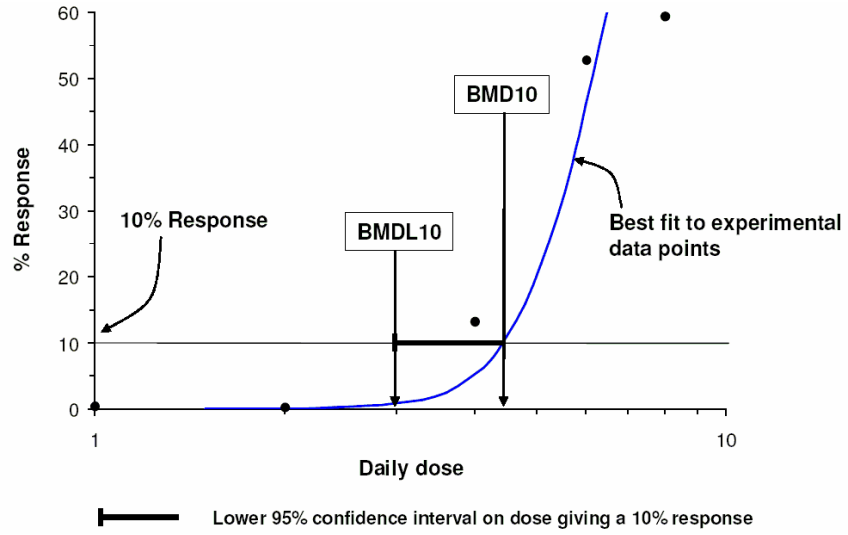


Figure 1.6. Hypothetic dose response data illustrating the concepts of BMD10 and BMDL10 for a 10% incidence response above the control (EFSA, 2005)

The MOE for acrylamide was calculated between 75 and 300 (FAO/WHO, 2005; O'Brien et al., 2006). In comparison, the margin of benzo(*a*)pyrene, a polyaromatic hydrocarbon and well known carcinogen formed in charbroiled food, was determined between 10000 and 25000. This clearly indicates the human health concern of acrylamide. Appropriate efforts to reduce acrylamide levels in foodstuffs should therefore continue. In order to achieve this goal, the different pathways of acrylamide formation should be elucidated. That is why the following section gives a state-of-the-art overview of the available information regarding the formation of acrylamide in foodstuffs.

As discussed under the next section, the Maillard reaction plays a major role in the formation of acrylamide. From toxicological point of view, this reaction does however not only generate products harmful for human health (Somoza, 2005), such as acrylamide. It was suggested that melanoidins (final products of the Maillard reaction) are able to reduce the absorption of mutagens, such as heterocyclic aromatic amines (Solyakov et al., 2002). Besides, the protective effect of melanoidins as antioxidants has also been demonstrated in several investigations (Tuohy et al., 2006; Summa et al., 2007). The challenge for future studies is however to chemically characterize these melanoidins, which consist of a complex mixture of polymers and copolymers. Accordingly, the structure-specific health effects of the harmful and beneficial compounds can be determined and the food processing technologies can be optimized towards a more selective formation of the health-beneficial Maillard reaction products. Upon lowering the formation of acrylamide in foodstuffs, the concomitant loss of valuable Maillard components should thus also be taken into account.

1.5 Pathways of acrylamide formation

Shortly after the announcement of the presence of acrylamide in heated foodstuffs (Tareke et al., 2002), numerous research groups started to investigate the potential sources and chemical reaction pathways. Below, the various acrylamide formation pathways are discussed, together with the key precursors and postulated intermediates.

1.5.1 The Maillard reaction and asparagine as source of acrylamide

In general, raw foodstuffs do not contain acrylamide, indicating that a heating process is required to form acrylamide. Pyrolysis experiments of model systems composed of amino acids and sugars demonstrated that acrylamide is indeed formed during the Maillard reaction. Free asparagine turned out to be the crucial participant in the production of acrylamide. Upon pyrolysis, asparagine alone may release acrylamide by thermally initiated decarboxylation and deamination. Yet, this yield increased a few hundredfold in the presence of (reducing) sugars. These findings were confirmed by the fact that acrylamide is formed in heated potato and cereal products, which contain reducing sugars and are particularly rich in free asparagine (Mottram et al., 2002; Weisshaar and Gutsche, 2002; Stadler et al., 2002).

By means of mass spectral studies, using ^{15}N-labelled asparagine and ^{13}C-labelled glucose, it was unambiguously demonstrated that the amide nitrogen and the three-carbon backbone of acrylamide originated both from the corresponding positions in the asparagine molecule. The sugar backbone and α-amino group of asparagine was thus not incorporated in the acrylamide molecule (Mottram et al., 2002; Weisshaar and Gutsche, 2002; Stadler et al., 2002).

The initial stages of the Maillard reaction involve the condensation of an amino acid (e.g. asparagine) with the carbonyl group of a reducing sugar to afford a *N*-glycosyl adduct (e.g. *N*-glycosyl asparagine), which is in equilibrium with the Schiff base (Figure 1.7). *N*-glycosyl-asparagine generated high amounts of acrylamide, suggesting that the early Maillard reaction is a major source of acrylamide (Stadler et al., 2002). Usually and preferably, this reaction will proceed to form Amadori compounds (pathway **I**). These Amadori products will degrade through the classical Maillard reaction pathways leading to the formation of flavour and colour compounds, instead of acrylamide. Since the Amadori compound does not easily decarboxylate, it can be concluded that the acrylamide formation pathway starts to follow another route prior to the Amadori rearrangement step (Stadler et al., 2004; Taeymans et al., 2004).

More concrete evidence on how the Schiff base can generate acrylamide was provided by several studies (Yaylayan et al., 2003; Zyzak et al., 2003; Stadler et al., 2004). According to Zyzak et al. (2003), the Schiff base undergoes heat-induced decarboxylation (Figure 1.7). This may proceed via the zwitterionic form to generate the azomethine ylide (pathway **II a**). Yaylayan et al. (2003) suggested a decarboxylation via intramolecular cyclization to the oxazolidin-5-one intermediate (pathway **II b**). A 1,2-prototropy determines the final location of the proton in the neutral imines (Stadler et al., 2004). The so-formed decarboxylated Schiff base (imine 1) can hydrolyze (pathway **III a**) upon heating to form 3-aminopropionamide which was proven to form acrylamide very efficiently via the elimination of ammonia (Granvogl and Schieberle, 2006). After tautomerization of imine 1 (pathway **IV**), the carbon-nitrogen covalent bond may break as a consequence of a β-elimination reaction. Although no direct evidence of the decarboxylated Amadori compound has been provided, the β-elimination reaction was proven to occur by means of experiments using model Amadori compounds (Stadler et al., 2004).

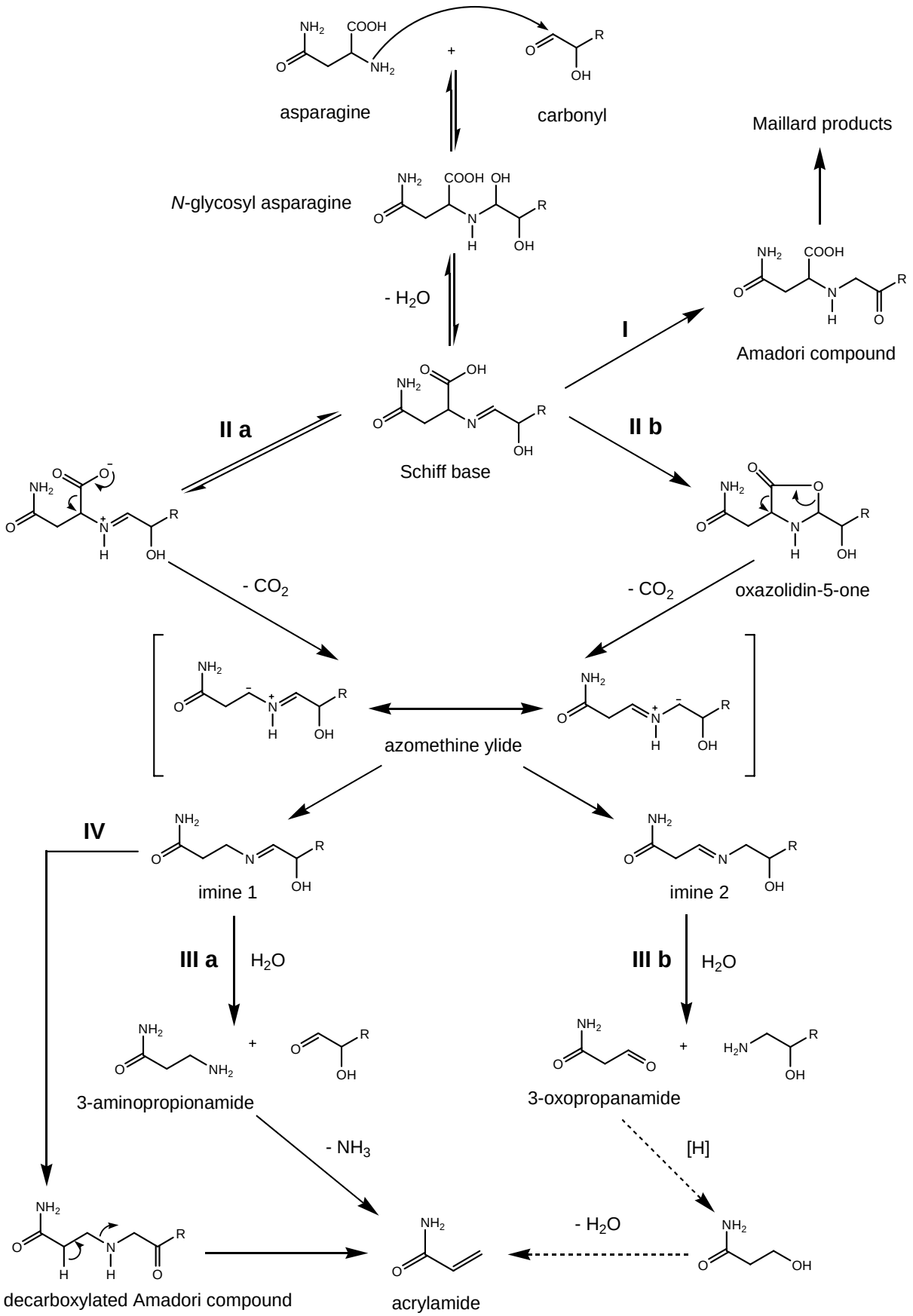


Figure 1.7. Proposed mechanisms of acrylamide formation through the Maillard reaction, starting from asparagine and a carbonyl source (Yaylayan et al., 2003; Zyzak et al., 2003; Stadler et al., 2004; Yaylayan and Stadler, 2005)

The 1,2-prototropic H-shift may also lead to the imine 2, which upon hydrolysis (pathway **III b**) furnishes the Strecker aldehyde of asparagine (3-oxopropanamide). However, this aldehyde did not release high amounts of acrylamide (Stadler et al., 2004; Blank et al., 2005).

Not only reducing sugars may act as a carbonyl source to form acrylamide. Also α-dicarbonyls or even any other carbonyl compound might generate acrylamide in the presence of asparagine (Mottram et al., 2002; Rydberg et al., 2003; Zyzak et al., 2003; Schieberle et al., 2005; Amrein et al., 2006a). A Strecker-type degradation of asparagine, initiated by α-dicarbonyl compounds and similar to pathway **II a** and **III a** (Figure 1.7 and 1.8) was reported (Schieberle et al., 2005; Granvogl and Schieberle, 2006). Yet, the type of carbonyl significantly affects the yields of acrylamide. The α-hydroxycarbonyl compounds, such as reducing sugars, generate much higher amounts of acrylamide compared to α-dicarbonyls or aldehydes. This can be explained by the fact that the presence of a hydroxyl group in β-position to the nitrogen atom (imine 1) favours the rearrangement (tautomerization) to the decarboxylated Amadori product (pathway **IV**). With α-dicarbonyls as precursor, it was however postulated that pathway **III b** is preferred above **III a**, due to the tendency of the carbonyl group in β-position to the nitrogen atom to delocalize the negative charge. As mentioned above, pathway **III b** does however not release high amounts of acrylamide (Stadler et al., 2004; Blank et al., 2005).

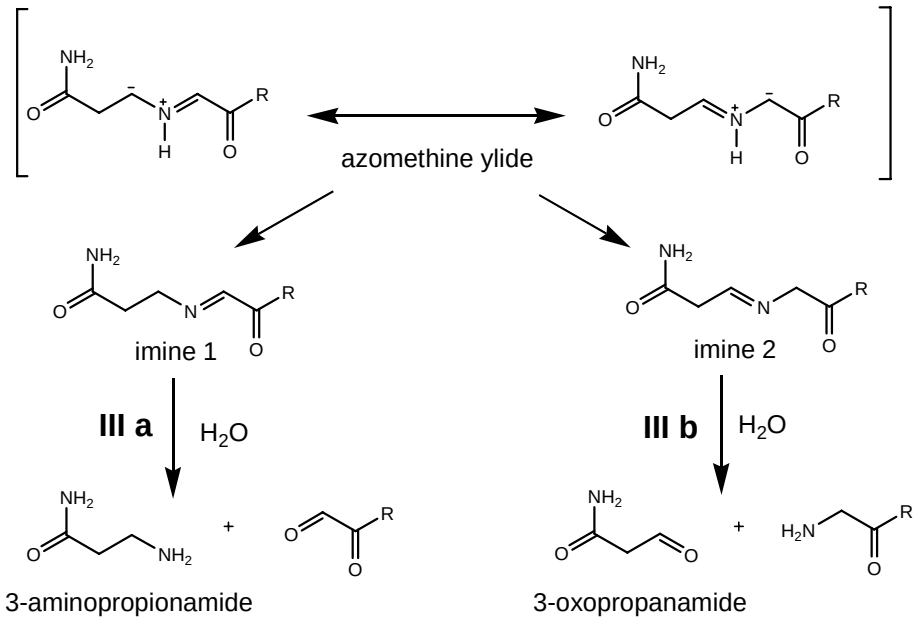


Figure 1.8. Resonance stabilized structures of the azomethine ylide after the condendsation of free asparagine with α-dicarbonyls (Blank et al., 2005)

To conclude, it may thus be assumed that the route via asparagine in the presence of reducing sugars is the major pathway to form acrylamide in foodstuffs. The yield is however not very high. On molar basis, less than 1% of free asparagine is converted to acrylamide (Mottram et al., 2002; Stadler et al., 2002; Becalski et al., 2003; Biedermann and Grob, 2003; Stadler et al., 2004; Surdyk et al., 2004; Amrein et al., 2004a). Yet, it is known that e.g. ammonium hydrogen carbonate might increase the conversion rate up to 5% (Biedermann and Grob, 2003; Amrein et al., 2004a; Weisshaar, 2004a; Amrein et al., 2006a). Reaction yields are however difficult to predict, since acrylamide is simultaneously degraded through polymerization reactions and Michael type addition reactions (Mottram et al., 2002; Stadler et al., 2002; Stadler et al., 2004). In this respect, kinetic modelling of acrylamide formation has been used as a way to quantify the amount of acrylamide formation and degradation, based on a simplification of the complex formation and degradation pathways (Knol et al., 2005; Wedzicha et al., 2005; Claeys et al., 2005a; Claeys et al., 2005b; Claeys et al., 2005c; Corradini and Peleg, 2006; De Vleeschouwer et al., 2006).

1.5.2 Pyrolytic acrylamide formation from other amino acids, peptides and proteins

Besides asparagine, other free amino acids such as aspartic acid, glutamine, methionine, cysteine and lysine might also liberate acrylamide to a much lower extent upon pyrolysis with a reducing sugar (Mottram et al., 2002; Weisshaar and Gutsche, 2002; Stadler et al., 2002; Becalski et al., 2003). Initially, this formation was ascribed to small asparagine impurities in the used amino acids. It was however demonstrated that β-alanine and aspartic acid can generate acrylic acid during their thermal decomposition (Figure 1.9), which can subsequently react with free ammonia to form acrylamide (Yaylayan et al., 2005; Ehling et al., 2005).

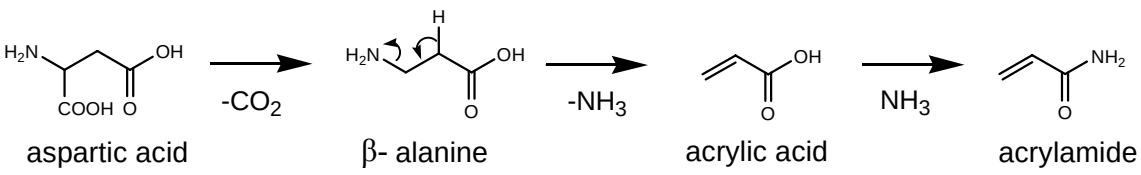


Figure 1.9. Formation of acrylamide starting from aspartic acid or β-alanine (Yaylayan et al., 2005)

Peptides having asparagine at the C-terminus can also produce acrylamide, but to a much lower extent than free asparagine (Schieberle et al., 2006). Also the dipeptide carnosine (*N*-β-alanyl-L-histidine) produced acrylic acid and acrylamide. It was postulated that the dipeptide bond hydrolyzes to release β-alanine, which can form acrylamide as discussed above. Also 3-aminopropionamide can be released from carnosine to yield acrylamide after its subsequent deamination. The presence of creatine (a major constituent of meat) however greatly suppressed the formation of acrylamide, which was expected since meat is not known to contain high levels of acrylamide (Friedman, 2003; Yaylayan et al., 2005).

Another acrylamide formation pathway in protein-rich foodstuffs was proposed by Claus et al. (2006). In this study, acrylamide was generated from purified wheat gluten by thermal degradation of an alanine-containing peptide (Figure 1.10).

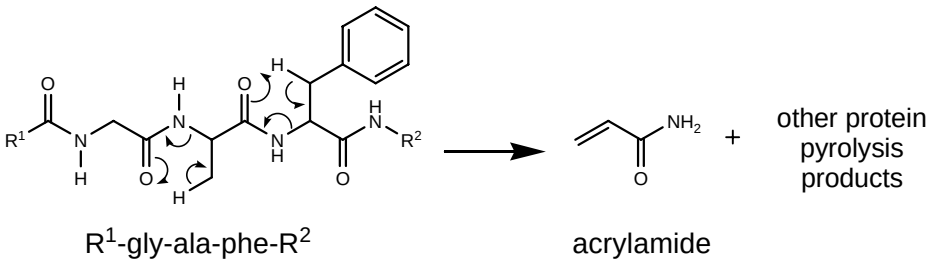


Figure 1.10. Proposed mechanisms of acrylamide formation upon protein pyrolysis (Claus et al., 2006)

In theory, more than one amino acid, and even bound amino acids, can thus generate acrylamide. The efficiency of the conversion of acrylic acid to acrylamide is however limited by the availability of free ammonia. Yet, this ammonia is extremely volatile at the applied heating temperatures (Yaylayan et al., 2005). Consequently, it is clear that these formation pathways are far less important compared to the ones described under the previous section. Furthermore, acrylamide, generated in these protein-rich foodstuffs, may readily react with nucleophilic amino acids present through Michael type addition reactions.

1.5.3 Acrylamide formed from 3-aminopropionamide

As described above, 3-aminopropionamide was detected as a transient intermediate of acrylamide during thermal degradation of free asparagine in the presence of a carbonyl compound (Schieberle et al., 2005; Granvogl and Schieberle, 2006). However, this amide was also detected in raw potatoes in different amounts (Granvogl et al., 2004; Bagdonaite et al., 2006). Low quantities of 3-aminopropionamide were moreover found in unprocessed Gouda cheese (Granvogl and Schieberle, 2006) and unfermented and fermented cocoa beans. A 7-day fermentation led to increasing 3-aminopropionamide contents (Granvogl and Schieberle, 2007). This "biogenic amine" is thus also present in raw or fermented food materials.

It was also demonstrated that 3-aminopropionamide can, in low amounts, be formed enzymatically from asparagine, without the need of a heat treatment or a carbohydrate source (Figure 1.11). At temperatures between 100 and 180°C, it was moreover shown that 3-aminopropionamide generated over 12-fold more acrylamide than the same reaction of asparagine and glucose, proving 3-aminopropionamide as a very effective precursor of acrylamide (Granvogl et al., 2004; Granvogl and Schieberle, 2006). Although 3-aminopropionamide is in many foodstuffs present in much lower levels as compared to free asparagine, the much higher effectiveness of 3-aminopropionamide in terms of yields as compared to asparagine suggests it as an additional precursor of acrylamide upon heating.

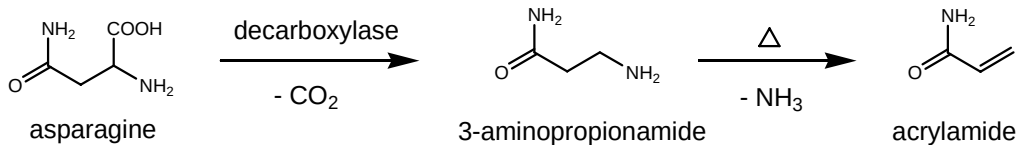


Figure 1.11. Enzymatic decarboxylation of asparagine leading to 3-aminopropionamide, which deaminates to acrylamide upon heating (Granvogl et al., 2004; Granvogl et al., 2006)

Recent studies however indicate that the thermal generation of 3-aminopropionamide during heating (Figure 1.7 and 1.8) is much more pronounced compared to its biochemical formation, as is shown in Figure 1.11 (Goldmann et al., 2006; Granvogl and Schieberle, 2006; Bagdonaite et al., 2006; Granvogl and Schieberle, 2007; Granvogl et al., 2007).

1.5.4 Oil degradation products as a source of acrylamide

Some researchers have ascribed an important role to oil hydrolysis products in the formation of acrylamide, particularly in lipid-rich foodstuffs (Gertz and Klostermann, 2002; Lingnert et al., 2002; Gertz et al., 2003; Ehling et al., 2005). Multistep heat degradation processes of triacylglycerols to acrolein and acrylic acid were suggested. Acrolein was shown to be formed from lipids in large amounts (Umano and Shibamoto, 1987) and may provide the reactive carbonyl function which generates acrylamide in the presence of asparagine (Becalski et al., 2003; Yasuhara et al., 2003; Weisshaar, 2004b). Another possible route is the oxidation of acrolein to acrylic acid, which reacts with ammonia to form acrylamide (Figure 1.12). The importance of these reaction pathways in foodstuffs relative to that of asparagine is however unclear (Stadler and Scholz, 2004). As a consequence, the impact of the deep-frying medium on the acrylamide formation was investigated in this work. The results are presented in chapters 3 and 4.

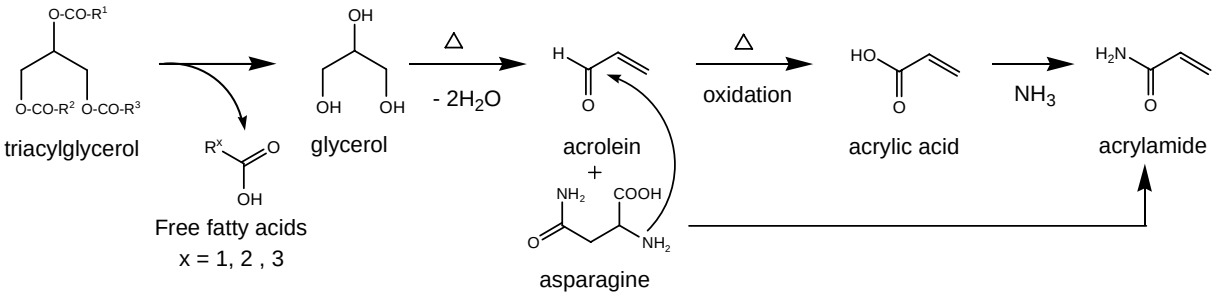


Figure 1.12. Formation of acrylamide after hydrolysis of triacylglycerol (Gertz and Klostermann, 2002; Gertz et al., 2003; Yasuhara et al., 2003)

1.6 Agricultural factors affecting the formation of acrylamide in potatoes

As mentioned under section 1.2.3, there is a high variability in acrylamide content between different product lots, but even between different bags of the same lot (Dybing et al., 2005). This substantial variability indicates that there are ways to reduce the degree of contamination. Research concerning fried potato products has indicated that the raw material quality and composition is of crucial importance in the mitigation of acrylamide. The strong susceptibility to acrylamide formation of potatoes is ascribed to the abundance of free asparagine, which is present in considerable excess compared to the amount of reducing sugars in the tuber. Consequently, the reducing sugar content can be considered as the limiting factor for acrylamide formation. Several studies could demonstrate a good linear correlation between

acrylamide formation and reducing sugar content, as illustrated in Figure 1.13. Yet, sucrose and free asparagine content did not correlate (Biedermann et al., 2002b; Amrein et al., 2003; Chuda et al., 2003; Haase et al., 2003; Becalski et al., 2004; Amrein et al., 2004b; De Wilde et al., 2005; Gökmen et al., 2006; De Wilde et al., 2006a; Brunton et al., 2007).

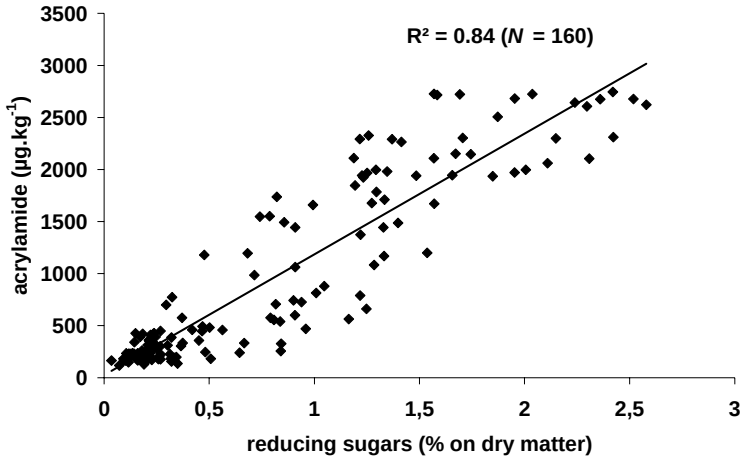


Figure 1.13. Acrylamide formation as a function of the reducing sugar content in the potato tuber [3 min par-frying and 2 min finish frying, both at 180°C] (De Wilde et al., 2005)

1.6.1 Potato cultivar

Different potato cultivars may have a different chemical composition. Some varieties, such as Tebina and Quincy, are considered to be unsuitable for frying purposes, because of too high reducing sugar contents. Cultivars used for the production of potato crisps, such as Saturna and Lady Claire, are bred and selected for low sugar content and are therefore less susceptible to acrylamide formation upon frying (Biedermann et al., 2002b; Amrein et al., 2003; Olsson et al., 2004; Amrein et al., 2004b; De Wilde et al., 2006a). However, it is obvious that other factors also have a considerable, if not more important impact on the reducing sugar content of the tuber, as discussed below.

1.6.2 Fertilization and soil

Since the potato is a crop with high nutrient requirements, heavy fertilization is routinely applied (Mondy and Koch, 1978). Due to environmentally related problems in various countries, there is however a growing legal pressure to decrease the amount of nitrogen fertilization applied in general agriculture. It was observed that the free asparagine content increases with increasing nitrogen fertilization (De Wilde et al., 2006b). This can be explained by the fact that the accumulation of free asparagine may be a mechanism by which the potato

tuber copes with excess of nitrogen fertilizer (Eppendorfer and Bille, 1996). The higher intake of nitrogen also leads to a reduction in available mono- and disaccharides, because these are used for the biosynthesis of amino acids (Kolbe, 1990). Decreasing nitrogen fertilization consequently leads to increased reducing sugar contents and subsequent acrylamide formation. Yet, the extent of sugar increase appears to be cultivar dependent. Another study reported that a moderate nitrogen fertilization in combination with a good provision of potassium resulted in low contents of free asparagine and reducing sugars (Heuser et al., 2005). On the other hand, increasing phosphorus nutrition gave rise to increased reducing sugar contents at harvest (Kolbe et al., 1995). Based on these studies, it seems thus important to find an appropriate balance between the level of fertilizers in order to obtain a tuber, low in potential to generate acrylamide, but on the other hand to consider the environmental impact and legal fertilization limits (De Wilde et al., 2006b).

The type of soil does however not seem to have a significant impact on the acrylamide formation during frying (De Wilde et al., 2006a).

1.6.3 Climatological conditions and harvest

Apart from differences in fertilization level and cultivar, potato growers and the potato processing industry are confronted with a year-to-year variation in the raw material characteristics. Due to the variability in the climatological conditions, a significant change in the reducing sugar and free asparagine content could be observed. Exceptional warm and dry summers gave rise to lower reducing sugar contents (on dry matter basis) and thus a lower susceptibility for acrylamide generation during subsequent frying (Davies et al., 1989; De Meulenaer et al., 2007). Exceptional rainfall in the final stages of the growth season gave rise to a lower dry matter content and increase in the nitrogen fraction (on dry matter basis), probably due to an extra uptake of available nitrogen fertilizer (De Meulenaer et al., 2007). Apart from changes in the reducing sugar content, other compositional parameters, such as the 3-aminopropionamide content (Granvogl et al., 2004), could also exert an influence on the complex mechanism of acrylamide generation.

The harvest time, linked with the maturity of the tuber, is often dependent upon the climatological conditions (Hertog et al., 1997a; Hertog et al., 1997b). During maturation, nutrients are transported from the leaves to the tuber. The reducing sugar content in immature

and thus smaller potatoes is higher since the degree of translocation of sugars from leaves to tuber still exceeds the degree of transformation of sugars to starch (Nelson and Sowokinos, 1983; Misra and Chand, 1990; Ohara-Takada et al., 2005). Consequently, smaller tubers have a higher potential to form acrylamide upon frying (De Wilde et al., 2006a). It is moreover known that the state of maturity at the time of harvest determines the storage behaviour, through the initial amount of enzyme or enzyme system, responsible for sweetening, as discussed below (Hertog et al., 1997a; Hertog et al., 1997b).

1.6.4 Potato storage

After harvest, potatoes are usually stored at 8°C for a period of several months. This storage temperature does not significantly influence the reducing sugar content and the acrylamide formation upon subsequent frying (Biedermann et al., 2002b; Noti et al., 2003; Olsson et al., 2004; De Wilde et al., 2005; De Wilde et al., 2006a) (Figure 1.13). Some cultivars are however more susceptible to senescent sweetening upon long-term storage, involving an increase in sugar content inside the tuber. This enzymatic process also depends upon climatological conditions prior to harvest, occurs more rapidly at higher storage temperatures (> 8°C) and is related to the start of sprout growth (Burton, 1989b; Amrein et al., 2004b). To avoid sprouting, chemical sprout suppressing agents may be used, although the use is not always permitted and desired by the consumer. Also low-dose irradiation can be applied. The use of sprout inhibitors does not result in a significant influence on the composition of the tuber and the potential of acrylamide formation during subsequent frying operation (Noti et al., 2003; De Wilde et al., 2005; Gökmen et al., 2006).

Due to climatic conditions, lower storage temperatures may however be unavoidable. These colder preservation conditions make potato tubers less susceptible to diseases (Burton and Wilson, 1978; Blenkinsop et al., 2002). Since sprouting can moreover be inhibited without the use of chemicals, this preservation technique is suitable for organic potato production (De Wilde et al., 2005). Presumably to protect themselves against frost, potatoes however start to mobilize sugars from the starch inside the tuber at temperatures below 8°C (Coffin et al., 1987; Davies et al., 1989; Burton, 1989b; Peshin, 2000; Sowokinos, 2001; Blenkinsop et al., 2002). This physiological reaction, also known as low temperature sweetening, makes the tubers more prone to undesired Maillard browning and dramatically increases the acrylamide

formation upon subsequent frying (Biedermann et al., 2002b; Noti et al., 2003; Olsson et al., 2004; De Wilde et al., 2005; Ohara-Takada et al., 2005; Matsuura-Endo et al., 2006). From Figure 1.14, it can moreover be observed that this susceptibility to low temperature sweetening is cultivar dependent. As indicated in previous section, the state of tuber maturity at the time of harvest also determines the storage behaviour, through the initial amount of enzyme responsible for sweetening (Hertog et al., 1997a; Hertog et al., 1997b).

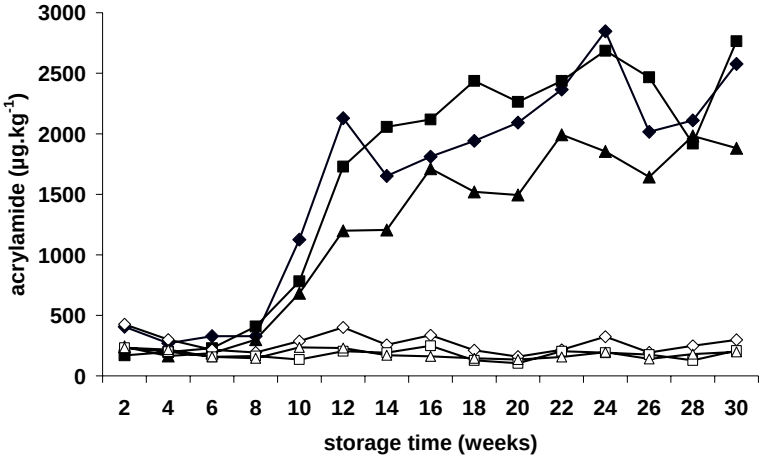


Figure 1.14. Influence of storage time and temperature on acrylamide formation during frying of three potato varieties (Bintje, Ramos, Saturna) stored at 4°C and 8°C over 30 weeks (♦ = Bintje, 4°C; ■ = Ramos, 4°C; ▲ = Saturna, 4°C; ◊ = Bintje, 8°C; □ = Ramos, 8°C; Δ = Saturna, 8°C) (De Wilde et al., 2005)

Unlike senescent sweetening, low temperature sweetening is at least partially reversible (Burton, 1989b). It is thus possible to achieve a significant reduction in the reducing sugar content after reconditioning of the cold-stored tubers for a period of 3 weeks at 15°C. These higher temperatures provoke an increased respiration rate inside the tuber. As a result, the reducing sugar content decreases through respiration and part of the sugars are again converted into starch. This operation step largely reduces the risk of later acrylamide formation, although this decrease in sugar content is not completely reversible (Peshin, 2000; Blenkinsop et al., 2002; Biedermann et al., 2002b; De Wilde et al., 2005).

Besides storage temperature, light also seems to activate potatoes, initiating the formation of reducing sugars and subsequent acrylamide formation upon frying (Biedermann et al., 2002b). In addition, there is an increased synthesis of glycoalkaloids (such as solanidine), with known toxic properties, at the periphery of the tuber (Burton, 1989b). So, potatoes should be stored at temperatures of about 8-10°C in the dark. In addition, the gaseous composition of storage

atmosphere has also been shown to affect the sugar content of potatoes. Depending on other agricultural factors, low oxygen levels suppressed sugar accumulation upon low-temperature storage, while increased carbon dioxide concentrations led to a rise in sugar content (Kumar et al., 2004). On large industrial scale, the control of these atmosphere conditions seems economically however less feasible.

To conclude, it can be stated that the raw material composition is of crucial importance to reduce the acrylamide formation during frying of potato products. This concerns mainly the reducing sugar content and to a lower extent the asparagine content. The potato variety should be well chosen, the cultivation and storage conditions should be well managed to obtain tubers with a minimum amount of sugars. Potatoes used for roasting or frying should contain less than 1 $g.kg^{-1}$ fresh weight of reducing sugars (Biedermann-Brem et al., 2003; De Wilde et al., 2005). However, adverse and moreover uncontrollable climatological circumstances make it sometimes difficult, if not impossible, to fulfil these requirements. Specific plant-breeding programmes could be set up, selecting specific cultivars which are more resistant to unfavourable climatological conditions before and after harvest. Since this is a lengthy process, genetic modification of plant materials could be an alternative, for example by inhibiting the enzymatic activity to reduce the formation of reducing sugars in the tuber (Friedman, 2003) or by lowering the free asparagine content. Recently, a genetically modified potato variety was produced in the US, by reducing the expression of specific genes, responsible for low-temperature sweetening (Rommens et al., 2006). Due to legal considerations and public acceptability it is however currently not possible to use genetically engineered products within the European Union (EFSA, 2003).

1.7 Research outline and objectives

Fried potato products constitute a large part of the acrylamide intake. Therefore, this study was specifically focused on these foodstuffs. As discussed in previous section, several agricultural factors have a major impact on the final acrylamide content. It was observed that the raw material should preferably be low in reducing sugars. This important parameter appeared however not to be perfectly controllable, due to unpredictable or uncontrollable conditions.

In the subsequent stages of potato processing, other factors may additionally influence acrylamide formation, allowing a (further) reduction of this process contaminant. This thesis aimed to investigate the influence of several process-bound parameters, in order to identify their importance to mitigate the final acrylamide content. Moreover, these measures were placed in the perspective of consumer acceptance, since it is known that any modification performed on the raw material composition or during processing may influence the Maillard reaction and its products, and concomitantly the organoleptic properties of the food. In order to combine significant acrylamide reduction with a guaranteed expected product quality for the consumer and finally a lower acrylamide intake, a multifactorial approach was essential. Figure 1.15 shows the detailed outline of this work, which was subdivided in the following, more specific objectives:

In order to ensure the quality and correctness of the analytical outcome, the primary objective was to monitor the performance of the applied research methodology. Accordingly, a better insight was gained into the reliability of the acrylamide analysis and the repeatability of the preceding heating or deep-frying methodologies (**chapter 2**).

With this knowledge, the impact of several factors on the formation of acrylamide was investigated. Since this study was focused on deep-fried potato products, the influence of the deep-frying medium was initially investigated. More specifically, the role of the deep-frying oil type was clarified in **chapter 3**, followed by a more in depth investigation of the impact of oil degradation products (**chapter 4**).

Subsequently, the effect of the raw material composition was studied. Water is a key component in foodstuffs, controlling the molecular mobility and thus the chemical reactions occurring upon frying. Therefore, the impact of this solvent on the Maillard reaction and acrylamide formation was investigated in **chapter 5**.

Besides, product colour is one of the most important food product characteristics. This colour is mainly caused by the Maillard reaction, occurring during deep-frying. As discussed in chapter 1, this Maillard browning is, together with the formation of acrylamide, mainly dependent on the reducing sugar content of the raw material. Therefore, **chapter 6** focused on the impact of the glucose/fructose ratio on the relationship between acrylamide and Maillard browning, using several colour measurement techniques.

Besides product colour, texture and taste are also essential parameters determining the final quality of fried potato products. Acrylamide-lowering pre-treatments may however influence these sensorial quality aspects of potato products. The impact of several treatments on both acrylamide formation and sensorial product quality was investigated in **chapter 7**. The latter was extensively evaluated with a sensory panel.

After evaluating several factors, influencing the formation of acrylamide in potato products, the dietary intake of this probable human carcinogen was studied in **chapter 8**. To decrease the human exposure, it is namely important to identify the major sources, contributing to the daily intake. In this context, the role of out of home eating was specifically investigated since this eating habit has considerably increased last decennia. Besides, the impact on the acrylamide intake of a nutritionally balanced diet was evaluated, providing extra fruit and vegetables to an intervention group.

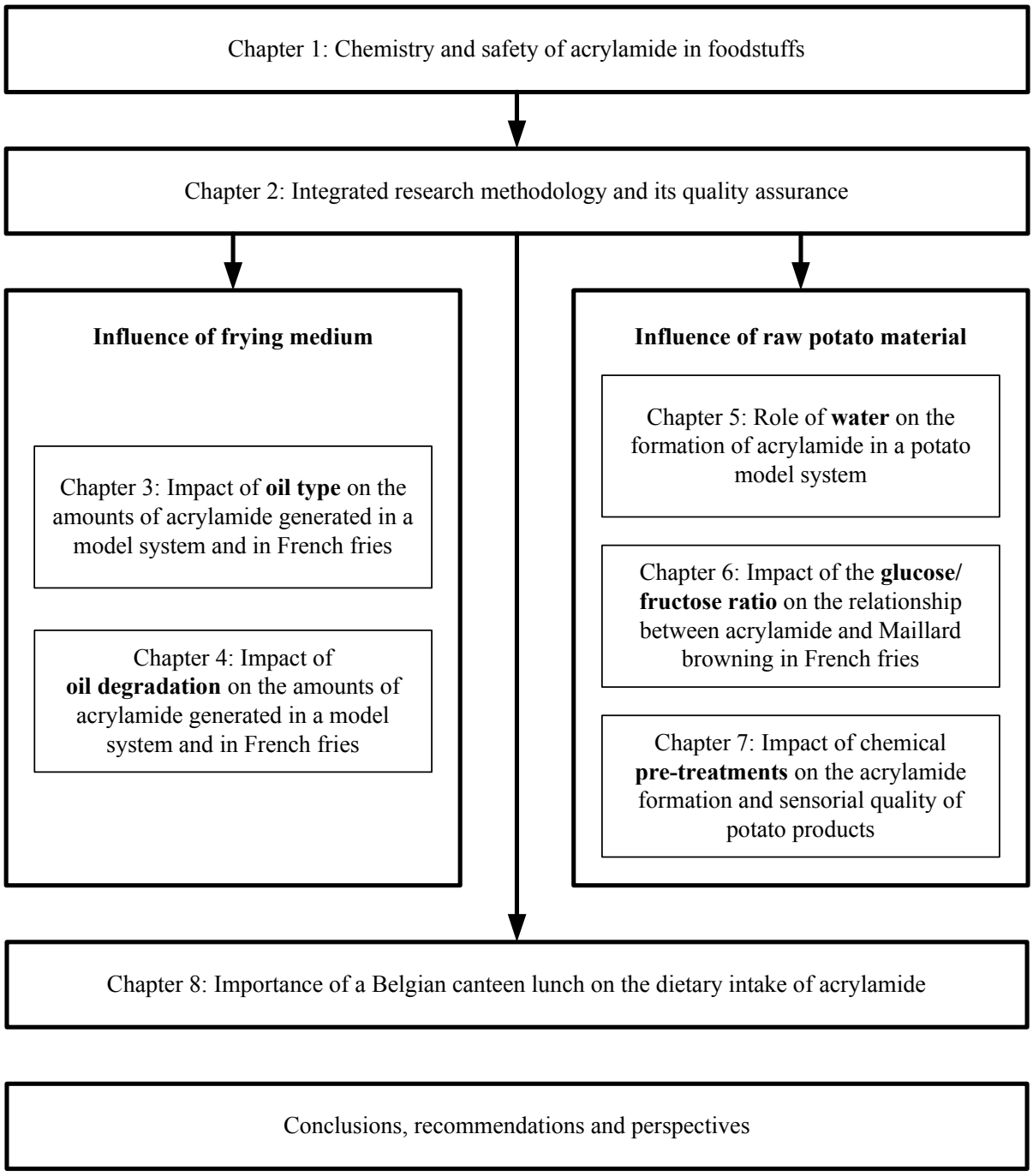


Figure 1.15. Outline of this study

Chapter 2

Integrated research methodology and its quality assurance

Partially redrafted after

Mestdagh, F., De Meulenaer, B., Van Poucke, C., Detavernier, C., Cromphout, C. & Van Peteghem, C. (2005). Journal of Agricultural and Food Chemistry, 53, 6170-6174.

Chapter 2 Integrated research methodology and its quality assurance

2.1 Abstract

A reliable LC-MS/MS method with positive electrospray ionization was applied for the quantification of acrylamide in heated foodstuffs. The analysis technique was validated in-house for linearity ($R^2 > 0.99$), specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability and recovery. The LOD and LOQ were 12.5 and 25 $\mu g.kg^{-1}$, while the relative standard deviation (RSD) was 10%. The quality of the analysis was furthermore assured using control samples during each sample run, together with a frequent participation in proficiency tests. In addition, a new heating methodology, based on a closed tubular reactor, was evaluated for its repeatability. It was shown that the potato powder mixtures, heated in this reactor, should be very homogeneous in order to obtain repeatable results with a RSD of 12%. For the preparation of French fries and potato crisps, a RSD of 15% was obtained, applying strict sample pre-treatment and temperature-controlled frying. Accordingly, it was shown that the entire research methodology should be strictly controlled, starting from the raw material preparation until the quantification of acrylamide in the heated samples.

Keywords: acrylamide; LC-MS/MS; validation; heating methodologies; quality assurance

2.2 Introduction

As discussed in the first chapter, many chromatographic techniques are available for detection and quantification of acrylamide in foodstuffs (Zhang et al., 2005). Although there have been reports of in-house validation, it was not always clear whether the applied methods were systematically validated according to international guidelines (Calbiani et al., 2004; Govaert et al., 2006; Ren et al., 2006; Senyuva and Gökmen, 2006). Yet, analysis methods need to provide accurate, repeatable and reproducible results within and between different laboratories. This is extremely important in view of possible legal actions and trade specifications, as well as for monitoring and risk assessment studies (Lauwaars and Anklam, 2004).

Besides the need for reliable acrylamide analyses, robust and consistent heating methodologies under well-controlled conditions are required to study acrylamide formation in foodstuffs. In this dissertation, acrylamide formation was studied in potato products. Two deep-frying methodologies were applied, being the preparation of French fries and potato crisps. In order to simplify the complex deep-frying process, another technique was developed using a closed stainless steel tubular reactor in which potato powder mixtures were heated. This method appeared very useful to investigate the specific impact of several factors on the formation of acrylamide in potato products, as elaborated in the following chapters.

In this chapter, it was the objective to check the performance of the acrylamide analysis in order to ensure the correctness of the analytical outcome. As a result, specific attention was paid to the validation of the method according to European guidelines (Eurachem, 1998; CD 2002/657/EC, 2002) and as prescribed by several ISO guidelines (ISO 5725, 1994; ISO 17025, 1999). Furthermore, the repeatability of the two deep-frying methodologies and the heating method with the tubular reactor was evaluated.

2.3 Materials and methods

2.3.1 Reagents and chemicals

Phosphate-buffered saline (PBS) (pH 7.4) consisted of 0.135 M NaCl, 1.5 mM KH_2PO_4, 8 mM $NaH_2PO_4.12H_2O$ and 2.7 mM KCl. These reagents as well as sulphuric acid were supplied by Chem-Lab, Belgium. Fructose, asparagine and trichloroacetic acid were from Acros Organics, Belgium. For the gas chromatographic determination of the sugars, the following aqueous solutions were prepared: Carrez I (14% $K_4Fe(CN)_6$, Merck, Germany) and Carrez II (30% $ZnSO_4$, Chem-Lab, Belgium). Furthermore, hexamethyldisilazane, trifluoroacetic acid from Chem-Lab (Belgium), an internal standard solution (6 mg mL^{-1} phenyl-β-D-glucopyranoside (Sigma-Aldrich, Belgium)) and an oximation reagent (2.5 g of hydroxylaminehydrochloride (UCB, Belgium) in 100 mL of dry pyridine (Merck, Germany)) were applied for the determination of sugars. For the acrylamide determination, [2,3,3-D_3]acrylamide (Polymer Source Inc., Dorval, Canada) and acrylamide (Sigma-Aldrich, Belgium) were used as standards. Acetic acid, formic acid, hydrochloric acid, methanol and *n*-hexane (BDH Laboratory Supplies) were supplied by VWR, Belgium. Deionised water (Milli-Q, Millipore Corp.) was used throughout. All reagents were of analytical grade, with purity > 99% (w/w) unless otherwise mentioned.

2.3.2 Acrylamide analysis

The acrylamide analysis method was based on a previously described FDA method (US FDA, 2003), with some modifications. A homogenized test portion of 1 g was weighed into a 50 mL centrifuge tube with cap and spiked with 40 µL of 10 ng.μL^{-1} [2,3,3-D_3]acrylamide internal standard. To defat the sample, 10 mL of *n*-hexane was added, followed by a 10 min shaking period. Subsequently the sample was centrifuged for 10 min at 4000*g* (4°C). After the hexane fraction was discarded, 10 mL of deionized water was added, followed by a 20 min shaking period, to extract the acrylamide from the food matrix. Next, the sample was centrifuged for 20 min at 4000*g* (4°C), followed by ultrafiltration through a 0.45 µm membrane filter. Further sample clean-up was performed on two solid-phase extraction columns. The Oasis HLB (6 mL, 200 mg; Waters, Milford, MA) column was conditioned by passing 5 mL of methanol followed by 5 mL of water through the column. The Bond Elut-Accucat column (200 mg

mixed mode packing: C_8, SAX and SCX) (Varian, Harbor City, CA) was conditioned with 3 mL of methanol followed by 3 mL of water. The filtrate (2 mL) was transferred onto the Oasis column and was allowed to pass first through the Oasis and subsequently through the Accucat column.

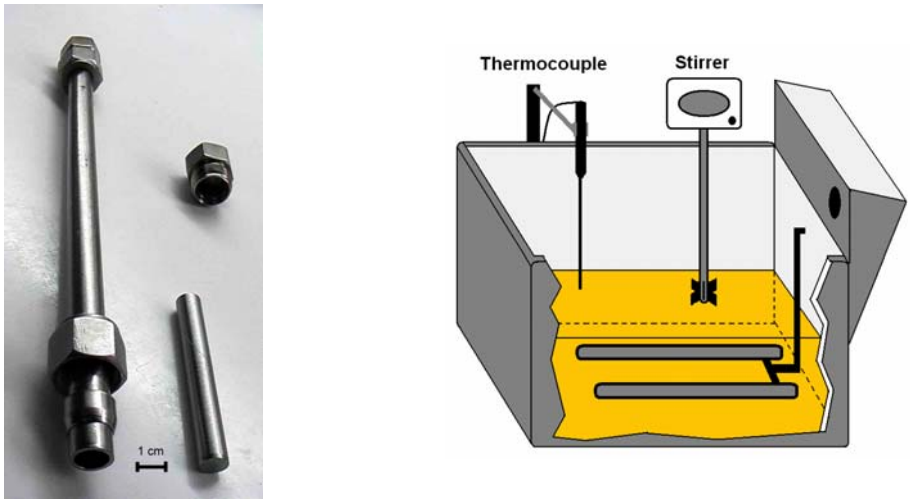
Finally, 20 µL of the purified extract or of a reference standard solution was injected into a Waters Alliance 2690 HPLC system, equipped with an Atlantis dC_{18} HPLC column (2.1 x 150 mm; 3 µm) (Waters, Belgium), which provide an optimal balance of retention for polar and non-polar compounds in reversed-phase chromatography (Ono et al., 2003; Zhang et al., 2005). The detection of acrylamide was performed on a Quattro LC (Micromass, UK) triple quadrupole mass spectrometer, operating in positive electrospray ionization (ESI^+). The mobile phase consisted of 92% water (containing 0.1% acetic acid) and 8% (v/v) water/methanol (35/65, with 0.3% formic acid), with an isocratic flow of 0.15 $mL.min^{-1}$. The capillary voltage was 2.8 kV, the cone voltage being at 22 V. The source block temperature was 120°C and the desolvation gas temperature was 350°C. The desolvation gas flow was 500 L of $nitrogen.h^{-1}$. The argon collision gas pressure was adjusted to 1.8×10^{-3} mbar for MS/MS. The collision energy was varied for each monitored transition in multiple reaction monitoring mode (MRM). The MS/MS transitions monitored for acrylamide were 72 → 72 at 5 eV and 72 → 55 at 10 eV collision energy, those for the internal standard were 75 → 58 at 10 eV and 75 → 30 at 20 eV. The dwell time for each monitored transition was 0.2 s. The quantification and calibration was based on the 72 → 55 and 75 → 58 transitions. Data interpretation was performed by use of the Quanlynx integration software (Micromass, UK).

2.3.3 Preparation of potato powder mixtures in tubular reactor

Two dried potato powders (*A* and *B*) were used to prepare the mixtures. The powders were sieved to obtain a product with a fine and homogeneous powder size distribution. The fraction between 90 and 160 µm was retained. Solutions containing dissolved compounds, such as reducing sugars or buffer solutions, could subsequently be added. The components were thoroughly mixed in a mortar to obtain a homogeneous blend. Similarly, oils could be added as well. In such a way, mixtures were prepared with a composition similar to that of finally prepared French fries, that is, 38% PBS, 21% oil and 41% potato powder, with a dry matter content of 58%, unless otherwise mentioned.

One gram of the homogenized mixture was introduced as a cylinder (diameter 1 cm) into the middle of a cylindrical stainless steel tubular reactor (internal diameter 1 cm, length 30 cm; Figure 2.1 A). The mixture was kept in place by two stainless steel supporting bars (diameter 1 cm), which were introduced at both sides of the reactor tube. Subsequently, the tubular reactor was sealed hermetically and was heated at selected temperatures and time intervals in a 5 L semi-professional thermostated deep-fryer (Fritel 2505, Belgium), equipped with a stirring mechanism to ensure a homogeneous temperature in the oil bath (Figure 2.1 B). The oil temperature was carefully monitored with a digital thermometer (Testo 925 with waterproof needle probe for measurements between -60 and 250°C). Immediately after heating, the tubular reactor was transferred to an ice bath (for 2 min), to enable a quick cooling of the artificial mixture. Subsequently, the reactor was opened and the 1-g mixture was analysed for its acrylamide content.

To assess the repeatability of the heating procedure in the reactor, mixtures with various compositions, including potato powder and PBS, were heated at 175°C for 2 or 3 min. These heating conditions were chosen since they are representative to the actual finish-frying operation of French fries. To boost acrylamide formation, fructose or asparagine was supplementarily added for some experiments, as these compounds are known acrylamide precursors (Mottram et al., 2002).



A **B**

Figure 2.1. (**A**) Sealable cylindrical stainless steel tubular reactor, 1 cm internal diameter, 30 cm length, with supporting bar, and (**B**) deep-fryer, equipped with thermocouple and stirring mechanism

2.3.4 Preparation of French fries

Potato tubers (*Solanum tuberosum* L., var. Spunta) were peeled and cut into pieces (1 cm x 1 cm x 3 cm) with a French fries-shaped cutter. To obtain potato strips with similar dimensions, parts that were in contact with the outer peel of the tuber were rejected for frying. Consequently, only the potato strips originating from the central part of the potato were washed five times (each for 1 min), followed each time by a 5 min rest under tap water. For this, approximately 7 L water was used for 1 kg of potato cuts. To eliminate variance between potatoes of the same variety, strips coming from different potatoes were thoroughly mixed together. Prior to frying, the potato strips were kept under distilled water at 4°C for a period of maximum 4 h. Just before frying, the potato cuts were patted dry with absorbing paper. The frying experiments were performed using the same equipment as mentioned under section 2.3.3. Ten potato strips (total weight ca. 33 g) were fried according to representative heating conditions, being 5 min at 175°C (± 1°C) in a heating basket, which was large enough to enable free movement of the strips in the frying oil. Potato-to-oil weight ratio was deliberately maintained low in order to stabilize the frying temperature (± 1°C). Directly after frying, the French fries were cooled at room temperature on an absorbing paper and homogenized. The samples were subsequently frozen until acrylamide analysis.

2.3.5 Preparation of potato crisps

Potato tubers (*Solanum tuberosum* L., var. Bintje) were peeled and cut in slices of 1.5 mm thickness and were washed in a similar way as the potato strips. After superficially drying on a paper towel, the potato slices (ca. 50 g) were fried at 170°C (± 1°C) for 3 min in above-mentioned deep-fryer (Figure 2.1 B). These characteristic heating conditions produced a palatable end product. Directly after frying, the potato crisps were cooled at room temperature on an absorbing paper and homogenized. The samples were subsequently frozen until acrylamide analysis.

2.3.6 Analysis of dry matter content

The dry matter content was determined by an oven-drying analysis technique, based on an official AOAC method (AOAC, 1990).

2.3.7 Sugar analysis

Mono- and disaccharides in homogenized potato sample were assessed by GC analysis upon aqueous extraction using phenyl-β-D-glucopyranoside as internal standard. After incubation for 30 min at 60°C, a clean-up step was carried out with Carrez I and II (5 mL each). The obtained solution was filtered and 1 mL of the solution was dried under nitrogen. The residue was derivatized in two steps, first an oximation with 100 µL of oximation reagents (30 min at 60°C), second to trimethylsilylesters with 100 µL of hexamethyldisilazane and 10 µL of trifluoracetic acid (10 min at ambient temperature). Analyses were carried out using a Varian 3380 gas chromatograph equipped with a flame-ionization detector (Varian Instrument Group, Walnut Creek, CA). The chromatographic parameters were: stationary phase (5%-phenyl)-methylpolysiloxane, film thickness 0.25 µm, 30 m x 0.32 mm inside diameter (Agilent Technologies, Palo Alto, CA, US); mobile phase: He at 1 $mL.min^{-1}$, split 1/40, injector temperature = 250°C; detector temperature = 340°C; injection volume = 1 µL; temperature program = 180°C for 1 min, ramp at 15°$C.min^{-1}$ to 290°C. The flame ionization detector was operated with hydrogen and air at respectively 30 and 300 $mL.min^{-1}$, and helium at 20 $mL.min^{-1}$ as make-up gas.

2.3.8 Starch analysis

The starch content was determined according to Browne and Zerban (1941). The procedure involved an acid hydrolysis of the starch with HCl, followed by a clean-up step with Carrez I and II. After filtration, the extent of polarization of the hydrolysate was measured in a polarimeter (Hilger and Watts, England).

2.3.9 Analysis of ash content

About 5 g of sample was carbonized in a crucible in an ashing oven at 500°C and weighed.

2.3.10 Analysis of crude protein content

The total Kjeldahl protein content was determined according to Egan et al. (1981). About 1.5 g of potato sample was transferred into a Kjeldahl tube to which 10 mL of H_2SO_4 and 1 Kjeltab CX (catalyst compound) were added. The digestion was done in a destruction block

(420°C) until a clear solution was obtained. Distillation was carried out with a 2200 Kjeltec Auto (FOSS Tecator, Sweden). The obtained distillate was titrated with 0.05 M HCl. For the calculation of the crude protein content a conversion factor of 6.25 was used.

2.3.11 Analysis of free amino acid content

The free amino acids were determined after incubation of the homogeneous potato sample in a 15% (v/v) trichloroacetic acid solution. After filtration and dilution, the filtrate was injected into a Biotronik LC3000 amino acid analyzer, according to De Wilde et al. (2005).

2.4 Results and discussion

2.4.1 In-house validation of acrylamide analysis

The analyses were integrated within the scope of an accredited laboratory controlled by the official Belgian organization for accreditation (BELAC). The method was validated in-house for linearity, specificity, limit of detection, limit of quantification, repeatability, intra-laboratory reproducibility and recovery. Two food matrices were tested being bread and French fries. Bread crumb and par-fried French fries were selected as blank matrix, since these did not contain acrylamide, as was determined before. This can be explained by the fact that respectively the temperature inside the baked bread does not exceed the temperature at which acrylamide is formed (120°C) (Ahrné et al., 2007) and due to the limited frying treatment applied to the potato strips.

Reference standard solutions of acrylamide were prepared in water from a 1 $mg.mL^{-1}$ stock solution and were stored at 4°C for a maximum of 3 months. An external calibration curve was established in the concentration range between 0 and 10000 $\mu g.kg^{-1}$, with a correlation coefficient > 0.99 (Figure 2.2). Depending on the concentration range of the measured samples, a calibration curve in a more specific range (0-1000 or 1000-10000 $\mu g.kg^{-1}$) was constructed, yielding similar correlation coefficients.

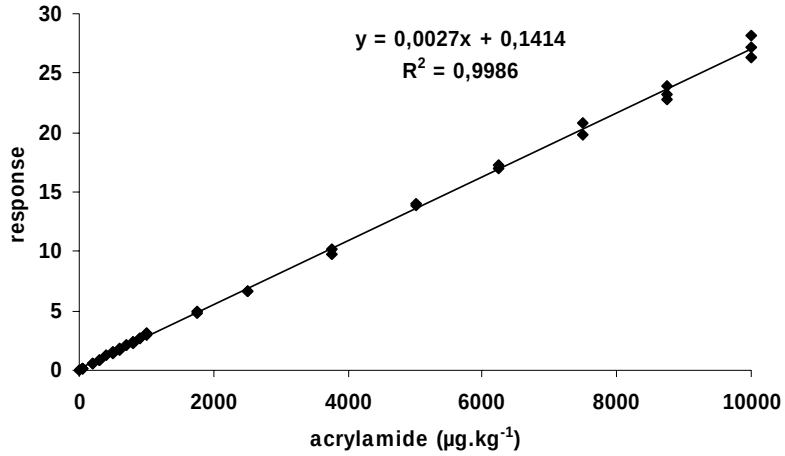


Figure 2.2. External calibration curve of acrylamide analysis in the concentration range between 0 and 10 000 µg.kg^{-1}

Confirmation of the identity of the response was based on four criteria (CD 2002/657/EC, 2002). First, the retention times of the acrylamide ions in the sample, as compared relatively to the internal standard ions, were within a 2.5% margin of the relative retention times of the ions in the reference standard solutions. Second, the abundances of the ions recorded, as compared relatively to the intensity of the most specific ion for quantification, corresponded with those of the ions in the reference standard solutions within fixed margins. Third, the signal-to-noise ratio of each ion was larger than 3. Finally, the signals of two daughter ions and one mother ion were followed to reach the four required identification points. For this, one identification point is attributed to each mother ion and 1.5 points to each daughter ion.

The limit of detection (LOD), defined as the mean value of the matrix blank readings plus 3 standard deviations (expressed in analyte concentration) was determined both in bread crumb and par-fried French fries. A similar result was obtained for both matrices, although the LOD was slightly higher for the French fries. Consequently, this value was chosen as LOD, being 12.5 µg.kg^{-1}. In a similar way, the limit of quantification (LOQ), being the mean value of the matrix blank readings plus 6 standard deviations, was determined at 25 µg.kg^{-1}. These limits correspond well with those indicated in a recent European collaborative trial validation study for the determination of acrylamide (Wenzl et al., 2006).

To assess the repeatability of the analysis method, the blank matrices were spiked with 1, 1.5 and 2 times the limit of detection concentration. These analyses were performed six times,

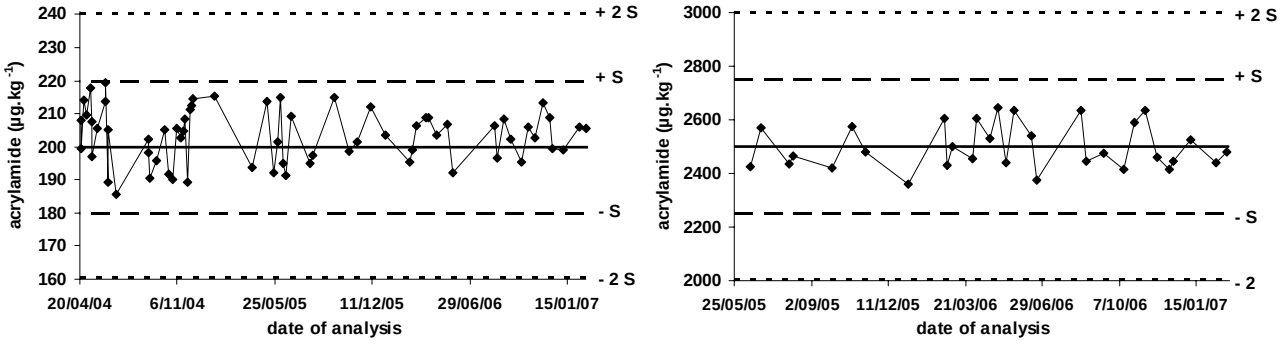
yielding a relative standard deviation (RSD) of 10%. The intra-laboratory reproducibility was assessed by repeating above-mentioned two more times, on several days, by several persons, yielding a similar RSD. The obtained precision parameters are situated well within the range of RSD values (5.4-13.2%), determined in a recent collaborative trial validation study (Wenzl et al., 2006). This indicates that the level of precision of the applied analysis method is comparable with other European and non-European laboratories, experienced in acrylamide analysis.

Since there was a lack of certified reference materials (CRM) for acrylamide at the time of research, the trueness of the analysis technique was assessed by means of a recovery study using blank matrices, fortified with 1, 1.5 and 2 times the limit of detection concentration (CD 2002/657/EC, 2002). Instead of adding the internal standard at the beginning of the sample clean-up, it was added just before the LC-MS/MS analysis. Again, the analyses were performed six times. The absolute recovery was calculated according to the EU CD 2002/657/EC (2002) and was about 65% for all concentration levels. To compensate this loss of analyte during sample clean-up, deuterated acrylamide was added as internal standard in the first step of the analysis method.

In order to assure the quality of each analysis run, one or more control samples were included, together with a blank sample. The control samples consisted out of pure water to which the internal standard and acrylamide were spiked in order to have a final acrylamide concentration of 200 or 2500 $\mu g.kg^{-1}$. The results are plotted in control charts (Figure 2.3). The standard deviation was taken from the repeatability experiments. As shown in Figure 2.3, the control samples never exceeded the warning limit (± 2 S), corresponding with Z = ± 2 (Galan, 2002). Because of this, the alarm limit of ± 3 S was not plotted. In addition, it can be observed that the measurements fluctuated randomly around the average value.

Furthermore, the accuracy of the method was demonstrated during participation in four inter-laboratory proficiency tests, organized by the IRMM and FAPAS. The results of the first European inter-laboratory comparison study on the determination of acrylamide in butter cookies and crisp bread (Wenzl et al., 2004) yielded Z-scores between -0.14 and -1.20. In 2004, IRMM organized another proficiency test, again on the determination in crisp bread samples, yielding Z-scores between 0.37 and 1.17 (Wenzl and Anklam, 2005). In 2007, a Z-score of -1.30 was obtained during a FAPAS proficiency test, once more on crisp bread.

Laboratory performance is considered to be satisfactory if the Z-scores are situated between -2 and +2, which was clearly fulfilled. This confirms the accuracy of the applied acrylamide analysis and the efficient functioning of the quality assurance system.



A **B**

Figure 2.3. Control charts of acrylamide analysis, for an average acrylamide concentration of 200 µg.kg^{-1} (**A**) and 2500 µg.kg^{-1} (**B**)

2.4.2 Repeatability of heating and deep-frying methodologies

2.4.2.1 Heating methodology with tubular reactor

A new heating approach to study acrylamide formation was developed, heating potato powder mixtures in a closed stainless steel tubular reactor (Figure 2.1 A). First, the temperature profile inside the tubular reactor was followed-up during heating at several preset and constant oil bath temperatures, as shown in Figure 2.4. In order to enable temperature measurement with a needle probe inside the reactor, the column was closed only at the side which was submerged in the oil bath. The open side was fixed a few centimetres above the hot oil surface. Instead of a potato powder mixture, the reactor was partially filled with sunflower oil prior to heating. This was done since the continuous evaporation of water from a water containing sample (such as potato powder) being heated inside the reactor would give a wrong image about the actual heat being transferred into the reactor. The temperature profile inside the tubular reactor, shown in Figure 2.4, is thus only an approach of the actual temperature profile of the potato powder mixture, heated inside the reactor. Each experiment was repeated several times, yielding similar temperature profiles. So, although the temperature inside the reactor only reached its preset level after more than 1 min, a repeatable heat transfer could be established upon heating at a fixed oil bath temperature.

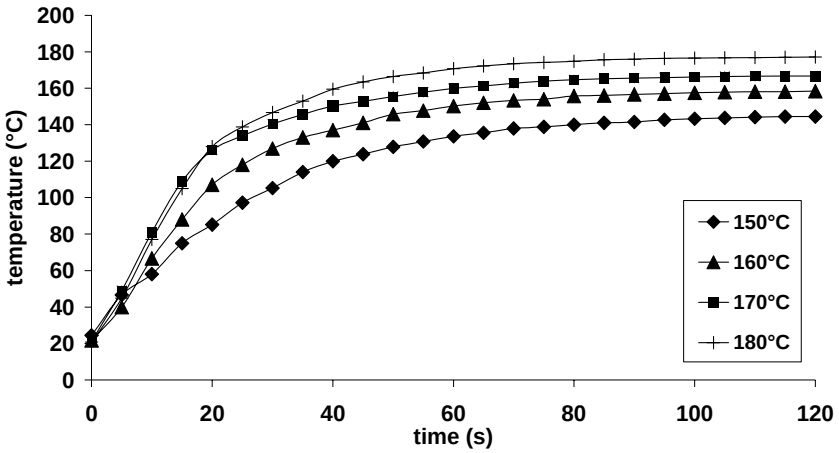


Figure 2.4. Temperature profile inside the tubular reactor upon heating in an oil bath at 150°C (♦), 160°C (▲), 170°C (■) and 180°C (+)

In a next step, the repeatability of the heating methodology was evaluated by heating different potato powder mixtures in the reactor. During the first series of experiments, the used potato powder (powder *A*) was not sieved. Fructose and asparagine were added to the potato powder as finely crushed crystals, without previous dissolution in the aqueous phase. The mixture had a final composition of 6.1% fructose, 6.1% asparagine, 48.4% potato powder and 39.4% PBS (w/w). It was heated for 2 min at 175°C, yielding an average acrylamide content of 7053 $\mu g.kg^{-1}$, with a RSD of 24% (N = 10) (Figure 2.5 A). In a second series of experiments, a lower amount of fructose (0.6% on total sample) was initially dissolved in the aqueous phase. Subsequently the aqueous solution was added to the potato powder (powder *A*). The asparagine was omitted, since it is not the limiting reaction partner in acrylamide formation in potatoes (Becalski et al., 2004). The final mixture composition was 0.6% fructose, 60% potato powder and 39.4% PBS (w/w). These experiments (N = 10, results not shown) yielded an average of 851 $\mu g.kg^{-1}$ acrylamide after 2 min of heating at 175°C, with a lower RSD of 18%, compared to the first series of experiments. A mixture with an identical composition was heated for 3 min at 175°C in a third series of experiments (Figure 2.5 B). However, in this third set of experiments the potato powder (powder *A*) was sieved before the aqueous fructose solution was added. On average, the acrylamide content was 6359 $\mu g.kg^{-1}$, with a RSD of only 12% (N = 7). Presumably, the repeatability of the heating experiments can be increased significantly if the potato powder used is sieved and thus a more homogeneous particle size distribution is obtained. Finally, the repeatability was assessed with omission of fructose from the mixture (Figure 2.5 C). Here the mixture consisted of 41% potato powder *B* (containing

0.03 g fructose/100 g powder, 0.03 g glucose/100 g powder and 0.89 g asparagine/100 g powder), 38% PBS and 21% rapeseed oil (w/w). It was heated for 2 min at 175°C. A lower average acrylamide content was obtained (88 $\mu g.kg^{-1}$), but the heating experiments proved again to be very repeatable (RSD = 6%, N = 10).

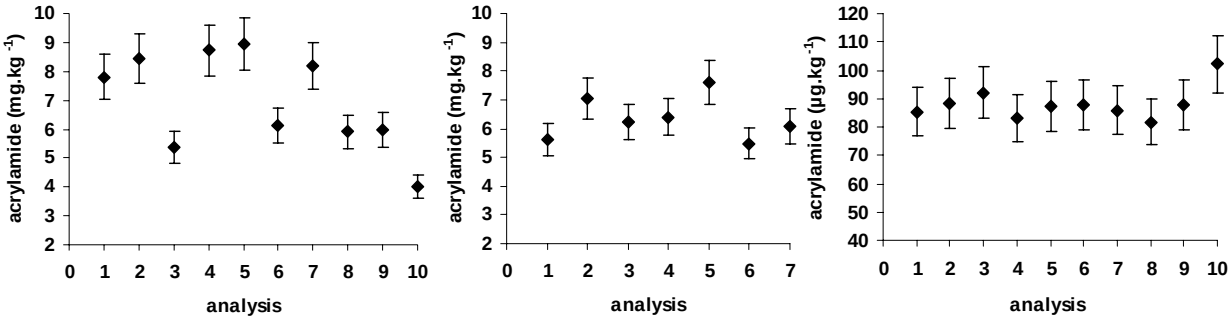


Figure 2.5. Repeatability tests of acrylamide formation upon heating of artificial mixtures at 175°C in the reactor. Error bars reflect 95% confidence intervals. (**A**) Mixture with 6.1% fructose, 6.1% asparagine, 48.4% potato powder *A* & 39.4% PBS (w/w), heated for 2 min. (**B**) Mixture with 0.6% fructose, 60.0% potato powder *A* & 39.4% PBS (w/w), heated for 3 min. (**C**) Mixture with 41.0% potato powder *B*, 21.0% oil & 38.0% PBS (w/w), heated for 2 min

From these preliminary experiments, it was found that the heated mixture should be very homogeneous in order to obtain repeatable results. Upon simply mixing different powders, no repeatable acrylamide levels could be generated. Therefore, it was essential to prepare homogeneous blends prior to heating. It seemed that acrylamide formation was particularly dependent upon heterogeneity in the potato powder model system. This problem could be solved by adding the other constituents to the potato powder in the form of aqueous solutions. Moreover, it appeared that the potato powder used should have a homogeneous particle size distribution. Sieving the powder before mixing together made the heating procedure even more repeatable (Figures 2.5 B and 2.5 C).

2.4.2.2 Characterization of potato powder

A large amount of potato powder *B* was bought in retail (Unilever, Belgium). This powder was used throughout the experiments with the model system in the following chapters. The detailed composition of the sieved potato powder is given in Table 2.1. Acrylamide was not detected in the powder (results not shown), which was industrially dehydrated by means of dryer drums. According to the manufacturer, this dehydration process is optimized so that the temperature of the potato powder does not rise above 100°C during processing. Due to the reduced amount of total thermal input, acrylamide formation is thus negligible.

As shown in Table 2.1, asparagine is the major free amino acid, present in the powder. The percentage of asparagine on total free amino acids is about 40%. This corresponds to earlier measurements (Lea et al., 2007). Compared to the reducing sugar content, free asparagine is present in large excess, as already indicated in chapter 1.

Table 2.1. Composition of potato powder *B* (% m/m), used throughout the model system experiments in chapters 3-7

major components		**free amino acid composition**		
	% (m/m)		**% (m/m)**	**% total free amino acid pool**
dry matter		aspartic acid	0.109	4.99
	90.33	threonine	0.047	2.15
starch		serine	0.026	1.17
	71.09	**asparagine**	**0.889**	**40.51**
		glutamic acid	0.181	8.26
reducing sugars		glutamine	0.163	7.42
glucose	0.03	proline	0.054	2.46
fructose	0.03	glycine	0.011	0.51
		alanine	0.056	2.55
non-reducing sugars		valine	0.098	4.47
sucrose	1.07	cysteine	0	0
		methionine	0.029	1.33
fat		isoleucine	0.053	2.43
	2.00	leucine	0.020	0.91
		tyrosine	0.068	3.10
ash		phenylalanine	0.085	3.89
	6.62	histidine	0.037	1.67
		tryptophane	0	0
protein		ornithine	0.003	0.12
total nitrogen	8.75	lysine	0.074	3.35
free nitrogen	3.63	arginine	0.191	8.71
		TOTAL	**2.194**	

2.4.2.3 Preparation of French fries and potato crisps

In addition to the heating experiments using the tubular reactor, the repeatability of the French fry and potato crisp preparation methods was assessed. The French fries or potato crisps used for each set of experiments originated from the same batch of potato cuts. Each frying test was repeated 10 times. An average acrylamide content (N = 10) of 729 $\mu g.kg^{-1}$ and 1041 $\mu g.kg^{-1}$ was obtained for French fries and potato crisps respectively (results not shown). A RSD of 15% was obtained for both preparation methods. Comparing this value with the RSD of acrylamide analysis, which was 10%, it is obvious that the frying procedure of French fries or potato crisps generated only a limited extra variability in the entire procedure of acrylamide generation and analysis.

Furthermore, the final dry matter content of the French fries was determined each time in duplicate. All values fluctuated around 60% ± 1% (N = 20) for French fries, again indicating the good repeatability of the applied frying procedure. So, although the artificial potato powder model system eliminated some variable factors occurring during deep-frying, such as oil and water transfer, it was demonstrated that preparation of French fries and potato crisps could also generate acrylamide in a repeatable way. It should however be stressed that these results could only be produced if sufficient attention was paid to sample preparation, such as potato cutting and washing, and to the frying procedure, that is, temperature control during the frying experiment.

2.5 Conclusion

In this chapter, it was shown that the quality of the applied research methodology was monitored starting from strict preparation of the raw material, through well-controlled heating or deep-frying methodologies and ending with an accurate quantification of acrylamide in the heated samples. This integrated approach allowed a better insight into the repeatability of both acrylamide analysis and the preceding heating or deep-frying methodology. With this knowledge, the impact of several factors on the formation of acrylamide could be evaluated, as elaborated in the following chapters.

Chapter 3

Impact of oil type on the amounts of acrylamide generated in a model system and in French fries

Redrafted after

Mestdagh, F., De Meulenaer, B., Van Poucke, C., Detavernier, C., Cromphout, C. & Van Peteghem, C. (2005). Journal of Agricultural and Food Chemistry, 53, 6170-6174.

Chapter 3 Impact of oil type on the amounts of acrylamide generated in a model system and in French fries

3.1 Abstract

Acrylamide formation was studied by use of a new heating methodology, based on a closed tubular reactor. Potato powder mixtures containing different oil types were homogenized and heated in the reactor, as discussed in previous chapter. By use of this experimental set-up, it became possible to study the acrylamide formation in the different mixtures, eliminating some physical and chemical variables during the frying process, such as heat flux and water evaporation from and oil ingress into the food. As a first application of this optimized heating concept, the influence on acrylamide formation of the type of deep-frying oil was investigated. The results obtained from the experiments with the tubular reactor were compared with standardized French fry preparation tests. In both cases, no significant difference in acrylamide formation could be found between the various heating oils applied. Consequently, the type of the deep-frying oils did not seem to affect the acrylamide formation in potatoes during frying. Surprisingly however, when artificial mixtures did not contain vegetable oil, significantly lower levels of acrylamide were detected, compared to oil-containing mixtures.

Keywords: acrylamide formation; food; modelling; oil type; LC-MS/MS

3.2 Introduction

The possible role of different factors influencing acrylamide formation has been extensively investigated. In this context, the eventual role of the deep-frying oil type was discussed on several occasions. It is believed that the type of oil affects acrylamide formation, due to the differing ability to transfer heat into foods (Gertz and Klostermann, 2002; Becalski et al., 2003; Gertz et al., 2003; Matthäus et al., 2004). Specifically, it was suggested that different quantities of substances such as mono- and diacylglycerols and short- and medium-chain fatty acids cause different surface tensions between the nonpolar oil and water-containing food, giving rise to different oil to food heat transfers. Therefore, much higher levels of acrylamide were found in foods heated in palm olein (Gertz et al., 2003). Moreover, the formation of acrylamide appeared to be more elevated in olive oil compared to corn oil (Becalski et al., 2003). It should be noted, however, that these findings could not be confirmed by others (Matthäus et al., 2004).

Apart from chemical reactions such as the Maillard reaction and acrylamide formation, physical transformations take place as well during deep-frying. For instance, water evaporates from the food and oil is absorbed (Vitrac et al., 2000). Mechanical deformations, such as development of porosity and surface roughness and physicochemical transformations, such as gelatinization, may also occur. These processes gradually change the physical environment in which chemical reactions occur. Furthermore, the heating medium progressively degrades as well, due to oxidative and hydrolytic processes, which may in their turn influence the heat transfer (Gertz et al., 2003; Kochhar and Gertz, 2004). All these events make the course of a frying experimental set-up dramatically complex and constantly changing. Moreover, not only is the foodstuff to be fried complex, but a food product (e.g., potato) is also extremely variable between and within species (Vitrac et al., 2000; Amrein et al., 2003; Amrein et al., 2004a). To cope with this complex situation, care should be taken during selection of raw materials, during sample preparation and at the frying stage. All these precautions are essential in order to avoid variability in acrylamide formation, occurring within the framework of a complex high-temperature environment. Possibly this inherent variability could be the cause of the conflicting data on acrylamide formation as mentioned above.

To simplify the complex frying process and thus to remove possible above-mentioned sources of variability, heating experiments were performed in a potato powder model system, which

was optimized in the previous chapter. Hereby, several artificial mixtures were heated in a closed tubular reactor, placed in a thermostated deep-fryer. Working with these artificial mixtures gave the opportunity to perfectly control the composition of the raw material, which was less achievable when working with raw potato tubers. Moreover, this tubular reactor simplified to some extent the variable heat and mass transfer. For instance, it was possible to conduct a heating process without fluctuation in the water or oil content of the food. In such a way, the heat transfer from the heating oil toward the food remained more constant. Moreover, the use of the tubular reactor prevented oil exchange between the artificial mixture and the progressively changing heating medium. So it was possible to estimate more accurately the specific impact of the type of frying oil on acrylamide formation mechanisms, without observing other above-mentioned and constantly altering physical processes. As there was no direct contact between the deep-frying oil and the food, it was additionally possible to determine the influence of the presence or absence of the deep-frying oil in the potato powder mixtures.

After the investigation of the repeatability of both the potato powder model system and the French fry preparation procedure in previous chapter, the aim of this chapter was to further clarify the role of deep-frying oil type on the formation of acrylamide in potato products. To evaluate the reliability of the model system, some results obtained from the model system were compared with parallel running French fry preparation tests. Finally, the effect of the presence or absence of oil in the artificial mixtures was investigated.

3.3 Materials and methods

3.3.1 Reagents and chemicals

Phosphate-buffered saline (PBS) was prepared in a similar way as described under section 2.3.1. HCl (25% w/w) and petroleum ether (bp 40-60°C, Chem-Lab, Belgium) were used for the determination of the oil content. Sodium hydroxide, sodium sulphate, ethyl alcohol, nonadecanoic acid and *p*-anisidine were from Acros Organics, Belgium, while the BF_3-reagent was delivered by Supelco, Belgium. Acetic acid, methanol and isooctane (BDH Laboratory Supplies) were supplied by VWR, Belgium. For the gas chromatographic determination of the sugars and for acrylamide determination, similar reagents and chemicals were used as described under section 2.3.1. All reagents were of analytical grade, with purity > 99% (w/w) unless otherwise mentioned.

3.3.2 Acrylamide analysis

Acrylamide analyses were performed exactly as described under section 2.3.2.

3.3.3 Preparation of potato powder mixtures in tubular reactor

The artificial mixtures were prepared, using a dried and sieved potato powder (Table 2.1). The potato powder was mixed with PBS and oil in order to obtain a homogeneous mixture with a final composition of 41% potato powder, 38% PBS and 21% oil. This composition approaches the final composition of a French fry. Subsequently, the mixture was heated as described under section 2.3.3. Two different heating conditions were applied in order to evaluate the impact of the oil type within two ranges of final acrylamide contents.

3.3.4 Preparation of French fries

Depending on the commercial availability at the time of research, *Solanum tuberosum* L., var. Spunta, harvest 2003 and var. Bintje, harvest 2004, were used. The acrylamide results within each table or figure were generated from the same batch of potatoes. The tubers were cut, washed and fried as described earlier under section 2.3.4. Specific attention was paid to this sample preparation and frying, in order to generate acrylamide in a repeatable way. For each experiment, two batches of potato cuts were fried simultaneously, so the reported acrylamide levels are the average of two batches unless otherwise mentioned.

3.3.5 Sugar analysis

The analysis of mono- and disaccharides was performed as described under section 2.3.7.

3.3.6 Determination of oil content

About 10 g of homogenized French fries was boiled for 15 min in a beaker containing 50 mL HCl (25%) and covered with a watch glass. The solution was filtered over a wet filter paper. The filter was rinsed with hot water until the filtrate reached a neutral pH and dried. The oil was Soxhlet extracted with 150 to 200 mL petroleumether during 4h. After solvent evaporation, the oil residue was dried until constant weight at 105°C (Egan et al., 1981).

3.3.7 Type of heating medium

Nine different types of lipids, including olive, rapeseed, corn, sunflower, grapeseed and soybean oil, palm fat and a highly hydrogenated soybean fat were purchased from retail sources. The oxidative status (*p*-anisidine value, PAV (AOCS, 1989b)) and the fatty acid profile (AOCS, 1989d) were determined for each lipid. As an alternative heat transfer medium, the influence on acrylamide formation of paraffin oil, with a melting section between 54 and 56°C, was investigated. The amounts of acrylamide generated by use of paraffin oil were compared to experiments with soybean oil and palm fat as heating media. The palm fat was a commercial mixture of 80% palm oil and 20% palm stearine (Vandemoortele, Belgium). The potato cuts were fried in the different heating media for 5 min at 175°C, to evaluate acrylamide formation. For every experiment, two batches of potato cuts were fried simultaneously, so the reported acrylamide levels are the average of two batches. As explained previously, each oil was mixed with PBS and potato powder to obtain a homogeneous mixture. These mixtures were heated for 2 min at 175°C. Some experiments were repeated at more severe heating conditions; that is, at 170°C for 5 min. All heating experiments with the potato powder model system were performed in duplicate, so the reported acrylamide levels are the average of two experiments.

3.3.8 Statistical analysis

The 95% confidence intervals, presented in the figures and table, are based on repeatability experiments obtained in chapter 2, yielding a RSD of 12% and 15% for the heating methodology of the potato powder mixtures and preparation of French fries respectively. Besides, comparison of means was performed using analysis of variance (One-way ANOVA) using SPSS 12.0 (SPSS Inc., Chicago, IL, US).

3.4 Results and discussion

3.4.1 Type of heating medium

To test the impact of the heating medium on acrylamide formation, experiments were performed with different vegetable oils. The fatty acid composition (results not shown) was in agreement with average fatty acid profiles for those vegetable oils (Bockisch, 1998). To ensure the freshness of the oils, the *p*-anisidine value (PAV) was determined (AOCS, 1989b).

The oils showed similar low PAV (below 15), except for palm fat (between 30 and 50). Rapeseed, olive, sunflower, soybean, corn and grapeseed oil were subsequently added as components in the potato powder model system, which was heated in the tubular reactor at 175°C for 2 min to assess acrylamide formation (Figure 3.1). Additionally, palm fat, olive oil, sunflower oil, soybean oil, saturated soybean fat and corn oil were separately added in the model system as well. These mixtures were heated at more severe heating conditions, being 170°C for 5 min (Figure 3.2). Accordingly, the effect of the oil type could also be evaluated in a different range of final acrylamide contents. Within each of these two figures, it can be observed that the acrylamide contents were not exactly the same for the different heating oils used in the artificial mixture. On the other hand, no significant differences could be demonstrated between the vegetable oils, for both heating treatments.

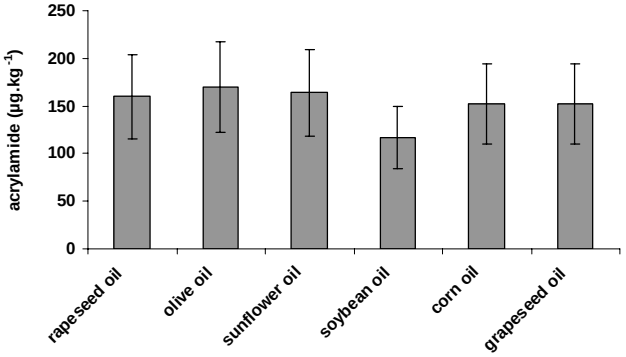


Figure 3.1. Influence of different oil types on acrylamide formation in the mixture, heated in the tubular reactor at 175°C for 2 min

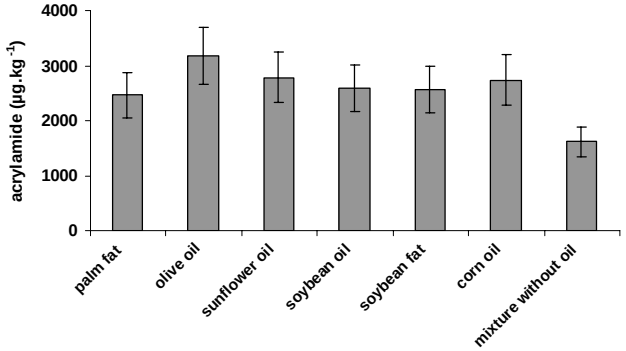


Figure 3.2. Influence of different oil types on acrylamide formation in the mixture, heated in the tubular reactor at 170°C for 5 min

During a second series of experiments, French fries were prepared for 5 min at 175°C as shown in Figure 3.3. The acrylamide contents did not significantly differ between the different deep-frying oils, confirming the results obtained from the potato powder model

system. Also the oil content did not markedly vary among the different French fries (results not shown), as already demonstrated previously (Mellema, 2003).

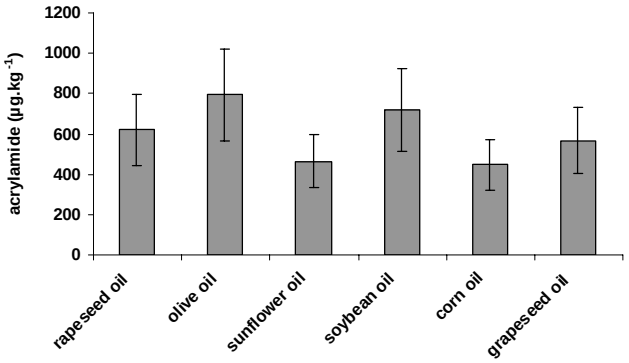


Figure 3.3. Influence of different oil types on acrylamide formation in French fries, prepared at 175°C for 5 min

In addition, paraffin oil was applied both in the potato powder model system and in French fry preparation experiments. The amounts of acrylamide generated by use of paraffin oil were compared to experiments with palm fat and soybean oil. The acrylamide contents are shown in Table 3.1. The acrylamide levels of the French fries in Table 3.1 cannot be compared with those in Figure 3.3 since different potato varieties were used for the two experiments. In Table 3.1, no significant differences in acrylamide formation were found between paraffin and the vegetable oils. Paraffin is chemically inert. Consequently, no oxidation reactions can occur and paraffin acts only as a heat transfer medium. Moreover, this oil is devoid of triacylglycerols, so acrolein, an oil degradation product and at the same time a suspected acrylamide precursor (Gertz and Klostermann, 2002; Becalski et al., 2003), cannot be formed starting from these compounds. From these results, another indication is presented that the heat medium as such did not influence the acrylamide formation.

Table 3.1. Influence of different heating media on acrylamide formation in French fries (prepared at 175°C for 5 min) and in the tubular reactor (heated at 175°C for 2 min)

	acrylamide content (µg.kg^{-1}) (*N* = 2)	
	French fries	**mixture heated in tubular reactor**
palm fat	436 (± 88)	103 (± 17)
soybean oil	335 (± 68)	109 (± 18)
paraffin	360 (± 73)	102 (± 17)

Previously, it was postulated that palm oil exhibits much higher acrylamide formation in French fries, compared to other deep-frying oils (Gertz and Klostermann, 2002). In addition, it was found that olive oil induced higher formation compared to corn oil (Becalski et al., 2003). However, other investigations (Matthäus et al., 2004; Williams, 2005) could not find any significant effect of the oil type. Obviously, there is still some confusion about the influence of the heating medium on acrylamide formation. The results shown in Table 3.1 and Figures 3.1-3.3 confirm the findings of Matthäus et al. (2004) and Williams (2005). Hence any significant influence of the heating medium could be demonstrated in the above-mentioned experimental set-up.

3.4.2 Influence of oil presence in the potato powder model system

In a final experiment, an artificial mixture without oil was heated in the tubular reactor. This mixture had a composition of 48% PBS and 52% potato powder (w/w). When the acrylamide formation of oil-containing artificial mixtures was compared to the mixture without oil (Figure 3.2), significantly higher levels were found in the oil-containing ones. Even in mixtures containing paraffin oil, higher contents were found. Consequently, it could be stated that the heat transfer in the mixture was changed when oil was added to the mixture. The different texture of the oil-containing mixtures, compared to those devoid of oil, could be a possible explanation for this phenomenon. It is plausible that the oil-containing mixture had better contact with the inner wall of the stainless steel tubular reactor. Consequently, the heat transfer may be facilitated. Moreover, it could be possible that heat was better distributed in more oily mixtures, due to oil convection flows in the foodstuff, and thus again resulting in a better heat transfer.

3.5 Conclusion

It could thus be concluded that the evaluated frying media, with different fatty acid composition, did not significantly influence acrylamide formation. The oil content on the other hand might influence the heat transfer and subsequent acrylamide formation, as demonstrated in the model system. To further clarify the role of deep-frying medium, the following chapter focuses on the influence of oil oxidation and hydrolysis on the formation of acrylamide.

Chapter 4

Impact of oil degradation on the amounts of acrylamide generated in a model system and in French fries

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Chapter 4 Impact of oil degradation on the amounts of acrylamide generated in a model system and in French fries

4.1 Abstract

Acrylamide formation in foodstuffs is subjected to different influencing factors. This chapter investigates the specific impact of both oil oxidation and oil hydrolysis on the formation of this probable human carcinogen. This was achieved using two heating methodologies, introduced in chapter 2. The first one was based on a closed tubular reactor, in which different homogenized mixtures were heated. Doing so, possible changes in the altered heat transfer properties of the oil upon degradation were excluded since direct contact between the food and the heat medium was eliminated. The results obtained from these experiments were compared with standardized French fry preparation trials. Using both heating methodologies, acrylamide formation was proven to be independent upon the oil oxidation and hydrolysis status in the experimental conditions used. More specifically, from the experimental results no evidence could be found that, due to oxidative or hydrolytic oil degradation, heat and oil transfer properties of the oil were changed to such an extent that acrylamide formation during French fry preparation would be significantly influenced. Finally, it could be concluded that the investigated oil degradation products, such as glycerol, mono- and diacylglycerols, acrylic acid and several aldehydes did not significantly influence acrylamide formation, especially in the presence of reducing sugars.

Keywords: acrylamide formation; food; modelling; oil oxidation; oil hydrolysis; LC–MS/MS

4.2 Introduction

To reduce acrylamide in foodstuffs, the formation mechanisms should be clarified, as well as the factors influencing it. As discussed in chapter 1, acrylamide formation is closely linked to the Maillard reaction. The free amino acid asparagine and reducing sugars are till now considered as the main precursors of acrylamide (Stadler and Scholz, 2004). Fried foodstuffs, such as potatoes, are susceptible for acrylamide formation because they contain these compounds in relatively high amounts.

Although it was demonstrated in previous chapter that the fatty acid composition of the deep-frying oils did not significantly influence the final acrylamide content, additional formation pathways, starting from lipids, may exist (Gertz and Klostermann, 2002; Becalski et al., 2003; Gertz et al., 2003; Yasuhara et al., 2003; Weisshaar, 2004b; Ehling et al., 2005). It was suggested that triacylglycerols partially hydrolyze during frying, followed by dehydration of glycerol to acrolein. This three-carbon compound may oxidize to acrylic acid, which can finally react with ammonia to form acrylamide (Figure 1.12). Acrolein could also be formed upon pyrolysis of triacylglycerols, without glycerol as an intermediary product. Moreover, monoacylglycerols decompose above 150°C in an elimination reaction to acrolein and a free fatty acid (Lin and Liou, 2000; Gertz and Klostermann, 2002). Consequently, oil hydrolysis products may act as acrylamide precursors. However, the importance of this pathway in the framework of acrylamide formation in foodstuffs is still not completely cleared out.

Apart from the fact that oil hydrolysis may create glycerol as acrylamide precursor, the generated mono- and diacylglycerols may also change the surface tension between the water-containing food surface and the non-polar oil (Dobarganes et al., 2000; Gertz and Klostermann, 2002; Gertz et al., 2003; Gertz, 2004). As a result more heat could be transferred from oil to food in a fixed period of time. In such a way it was stated (Gertz and Klostermann, 2002; Gertz et al., 2003; Gertz, 2004) that palm oil, being more sensitive to lipolytic degradation, would generate more acrylamide compared to oils containing lower amounts of diacylglycerols, like for example sunflower or rapeseed oil. Furthermore, it can be indicated that diacylglycerol-rich cooking oil has recently been marketed as edible oil for home use in the US and Japan. This oil would undergo hydrolysis more rapidly than triacylglycerol oil (Shimizu et al., 2004).

In the same way, oil oxidation could be an important variable, influencing acrylamide formation (Gertz et al., 2003; Gertz, 2004), because it may influence the surface tension between food and oil as well. Hence, the polar compounds, formed upon oil oxidation, could bind water in the frying oil for a longer period of time, leading to increasing heat transfer, heat conductivity and oil uptake. In this context, more oxidized oils would give rise to increased acrylamide formation. Accordingly, higher acrylamide formation was observed in partially hydrogenated rapeseed oil upon consecutive preparations of French fries and thus upon increasing contents of polar materials (Gertz et al., 2003).

As a conclusion, during frying, both oxidative and hydrolytic degradation processes gradually alter the deep-frying oil in which the foodstuff is prepared. These degradation processes may influence acrylamide formation during frying, because they change the heat transfer properties of the oil at the one hand and they may generate acrylamide precursors on the other. Therefore, the objective of this chapter was to elucidate the influencing mechanism of oil degradation on acrylamide formation. Similar to the previous chapter, heating experiments were conducted in the tubular reactor, as described under section 2.3.3. In such a way, the influence of heat transfer and other physical processes occurring during frying such as surface deformation, water evaporation from the food and oil ingress (Vitrac et al., 2000; Kochhar and Gertz, 2004) were eliminated because there was no direct contact between the heating medium and the food. To evaluate the reliability of the model system, some results obtained from the potato powder model system were again compared with parallel running French fry preparation tests.

4.3 Materials and methods

4.3.1 Frying oils

Different frying oils were used throughout this study. These oils were stored at 4°C in the dark. The fatty acid profile (AOCS, 1989d) was determined for each lipid and was in agreement with average fatty acid profiles for such vegetable oils (Bockisch, 1998). For the experiments regarding oil oxidation, soybean oil (GB, Belgium) was purchased from retail. Soybean fat (Vandemoortele, Belgium) was applied to investigate the influence of oil hydrolysis on acrylamide formation. Furthermore, corn oil (Copimex, Belgium) and a commercial palm fat mixture of 80% palm oil and 20% palm stearin (Vandemoortele,

Belgium), containing 3 $mg.kg^{-1}$ dimethyl polysiloxane (DMPS), were used to further elaborate the influence of oil degradation processes on the generation of acrylamide. The peroxide value (POV) (AOCS, 1989c) was for all fresh oils below 1 meq O_2/kg oil, except for soybean oil, with a value of 3.1 meq O_2/kg oil. The free fatty acid content (AOCS, 1989a) of the fresh oils was below 0.15%. Pure diacylglycerols (DAG) were obtained from Danisco (Belgium), while pure glycerol monostearate (94.1% w/w) was obtained from Oleon (Belgium).

4.3.2 Reagents and chemicals

Phosphate-buffered saline (PBS) was prepared in a similar way as described under section 2.3.1. Hydrogen peroxide, sodium hydroxide, sodium sulphate, sodium thiosulfate, ethyl alcohol, nonadecanoic acid, *p*-anisidine, acrylic acid, acrolein, crotonaldehyde, methacrolein, pentanal, hexanal, octanal, decanal, *trans*-cinnamaldehyde, glucose and asparagine were from Acros Organics, Belgium, while the BF_3-reagent and potassium iodide were delivered by Sigma Aldrich, Belgium. Acetic acid, methanol and isooctane (BDH Laboratory Supplies) were supplied by VWR, Belgium. Glycerol was obtained from Sigma–Aldrich, Belgium, ammonia 25% (w/v), HCl (25% w/w) and petroleum ether were from Chem-Lab (Belgium) and Grace Davision (Germany) delivered the silica gel (Davisil, 0.06-0.20 mm, containing 10% water). For the gas chromatographic determination of the sugars and for acrylamide determination, similar reagents and chemicals were used as described under section 2.3.1. All reagents were of analytical grade, with purity > 99% (w/w) unless otherwise mentioned.

4.3.3 Acrylamide analysis

Acrylamide analyses were performed exactly as described under section 2.3.2.

4.3.4 Preparation of homogenized mixtures in tubular reactor

The artificial potato powder mixtures were homogenized, as described under section 3.3.3 and heated as discussed under section 2.3.3. In a similar way, artificial mixtures were prepared, in which silica gel was used instead of potato powder. These mixtures consisted of 41% SiO_2, 20% fresh sunflower oil and 39% PBS. Prior to homogenization, several components were dissolved in the water or oil fraction, as mentioned in Table 4.4.

4.3.5 Preparation of French fries

Depending on the commercial availability at the time of research, *Solanum tuberosum* L., var. Spunta, harvest 2003 and var. Bintje, harvest 2004, were used. The acrylamide results within each table or figure were generated from the same batch of potatoes. The tubers were cut, washed and fried as described earlier under section 2.3.4. Specific attention was paid to this sample preparation and frying, in order to generate acrylamide in a repeatable way. For every experiment, two batches of potato cuts were fried simultaneously, so the reported acrylamide levels are the average of two batches unless otherwise mentioned.

4.3.6 Sugar analysis

The analysis of mono- and disaccharides was performed as described under section 2.3.7.

4.3.7 Determination of oil content

The oil content was determined as described under section 3.3.6.

4.3.8 Influence of oil oxidation

To test the impact of oil oxidation, soybean oil was used as heating medium. The oil (5 L) was constantly kept at 175°C for 8 h under continuous stirring. The stirring provided excellent mixing of air in the oil and a homogenous temperature distribution throughout the oil. Every 2 h during this period of heating, potato cuts (*S. tuberosum* L., var. Spunta) were fried in the oil for 5 min at 175°C. Finally, the acrylamide content in the finished fries was measured. Every 2 h, oil samples (100 g) were also taken from the fryer and kept for further analysis and use. Consequently, five samples were obtained in these 8 h of heating.

The oil sample taken from the fryer was partially used for determining the degree of oil oxidation, by assessing the PAV (AOCS, 1989b) and the POV (AOCS, 1989d), respectively. The remainder of the sample was used as an ingredient in the artificial mixture at a 21% w/w level, as discussed above. The obtained mixtures were heated in the tubular reactor as specified previously.

4.3.9 Influence of oil hydrolysis

To test the impact of oil hydrolysis on acrylamide formation, highly hydrogenated soybean fat (85.8% stearic acid, 12.8% palmitic acid w/w), which was supposed to contain mainly triacylglycerols (TAG), was mixed with diacylglycerols (DAG), monoacylglycerols (MAG) and glycerol (GLY), respectively, as shown in Table 4.2. The pure diacylglycerols added were produced from hydrogenated rapeseed oil, with 35% of the fatty acid chains on the 1,2-position of glycerol and 65% being at the 1,3-position, according to manufacturers specifications. Commercially available glycerol monostearate (94.1% w/w) was used to evaluate the influence of monoacylglycerols. Also an oil mixture of TAG with glycerol (GLY) was tested. The selected concentrations of these compounds were based on the composition of used frying oils (unpublished results). Potato cuts (*S. tuberosum* L., var. Spunta) were fried in the different oil mixtures for 5 min at 175°C. Each oil blend was moreover mixed with PBS and the potato powder, in order to obtain homogeneous mixtures, which were subsequently heated as explained previously.

4.3.10 Integrated experiment according to Gertz et al. (2003)

As a further elaboration of this study, 100 g of potato cuts (*S. tuberosum* L., var. Bintje) were fried (5 min, 175°C) every 10 min in corn oil for a period of 50 min. Subsequently, the oil was kept at 175°C for exactly 1 h without preparation of French fries. This was followed by another two consecutive frying experiments. A similar experiment was performed in a commercial palm fat mixture of 80% palm oil and 20% palm stearin, containing 3 $mg.kg^{-1}$ dimethyl polysiloxane (DMPS). These experiments are similar to those performed Gertz et al. (2003). The experiments were performed only once.

4.3.11 Statistical analysis

The 95% confidence intervals, presented between brackets in Tables 4.1 and 4.2 (experiments performed in duplicate) are based on repeatability experiments obtained in chapter 2, yielding a RSD of 12% and 15% for the heating methodology of the potato powder mixtures and the preparation of French fries respectively. The average acrylamide contents were compared using analysis of variance (One-way ANOVA) and post hoc multiple comparison of means (Tukey). Data in Table 4.3 were subjected to a linear regression to determine significant difference in acrylamide content within the period of oil heating. Differences were considered

as significant if the slope of the fitted straight line (representing oil heating time as function of acrylamide formation) was significantly different from zero. SPSS 12.0 (SPSS Inc., Chicago, IL, US) was used throughout. The chosen level of significance was 0.05 ($P < 0.05$).

4.4 Results and discussion

4.4.1 Influence of oil oxidation

Soybean oil was expected to be highly sensitive to oxidation, because of the high content of linolenic (8% w/w) and linoleic (52% w/w) acid. Therefore, the oil was kept for 8 h at 175°C under steady stirring in order obtain several oils with a different degree of oxidation.

Oil oxidation was monitored every 2 h by assessing the POV and PAV (Table 4.1). These parameters are typically used to determine the content of primary and secondary oxidation products present in oil, respectively. Both parameters were measured immediately after an oil sample was collected. As can be observed from Table 4.1, no significant build-up of peroxides occurred during the total experiment. Probably, peroxides were in situ degraded because it has been previously reported (Kochhar and Gertz, 2004) that peroxides are already instable at 80°C, which is well below the temperature used during the applied frying operations. As expected however, due to the decomposition of the peroxides, aldehydes and other secondary oxidation products were produced, as could be concluded from the constantly increasing PAV during the heating experiment.

Table 4.1. Influence of soybean oil oxidation on acrylamide formation in French fries, prepared at 175°C for 5 min, and in the tubular reactor, heated at 175°C for 2 min and at 170°C for 5 min (means ± corresponding 95% confidence intervals)

heating time (h)	soybean oil oxidation parameters		acrylamide content (µg.kg^{-1}) ($N = 2$)		
	peroxide value (POV) meq O_2 /kg oil	*p*-anisidine value (PAV)	French fries	mixture heated in tubular reactor	
				175°C, 2 min	170°C, 5 min
0	3.1	1.3	380 (± 77)	544 (± 90)	2607 (± 434)
2	2.5	149.3	357 (± 72)	-	-
4	1.5	189.6	330 (± 67)	-	-
6	2.6	320.2	305 (± 62)	-	-
8	3.4	455.3	372 (± 75)	476 (± 79)	2587 (± 430)

-: experiments not performed

Acrylamide formation as function of progressive oil oxidation was studied in two manners (Table 4.1). At the one hand, French fries were prepared in the heated soybean oil every 2 h for a total period of 8 h. In addition, the oxidized oil was used as an ingredient in a potato powder mixture with a similar composition as finished French fries, which was heated in the tubular reactor.

Considering the acrylamide content of the French fries prepared in the progressively oxidizing soybean oil (Table 4.1), it could be concluded that oil oxidation did not significantly influence acrylamide formation upon frying. Because of these results, the acrylamide content of the heated potato powder mixtures only containing the fresh and most abused oil was determined, respectively. Results were in agreement with those obtained during preparation of the French fries, since no significant difference in acrylamide content between the two heated mixtures could be observed if the samples were heated for 2 min at 175°C. Moreover, similar results were obtained if a more intense heating experiment (170°C for 5 min) was conducted and thus higher acrylamide levels were obtained.

These acrylamide results are in conflict with earlier published data (Gertz et al., 2003; Gertz, 2004), but in agreement with Williams (2005). Besides, the final oil content of the French fries slightly increased from 7.8% in fresh soybean oil to 8.3% after 8 h heating, as observed in a previous study (Dobarganes et al., 2000). Although oil oxidation may thus indeed affect the transfer of oil and heat to the French fries, above-mentioned results indicate that these possible changes ware not sufficient to alter the acrylamide formation in a significant way during preparation of French fries. In addition, the experimental results with the tubular reactor confirmed that oil oxidation did not significantly influence acrylamide formation.

4.4.2 Influence of oil hydrolysis

Despite the fact that previous results indicated that fat oxidation did not significantly influence acrylamide formation during frying, a fully saturated soybean fat (85.8% stearic acid, 12.8% palmitic acid w/w) was used in order to exclude significant oxidative degradation and to focus the study on oil hydrolysis products only. As mentioned under section 4.3, and as indicated in Table 4.2, four mixtures containing various hydrolysis products were evaluated.

Table 4.2. Influence of oil hydrolysis compounds on acrylamide formation in French fries, prepared at 175°C for 5 min, and in the tubular reactor, heated at 175°C for 2 min and at 170°C for 5 min (means ± corresponding 95% confidence intervals)

fat composition (w/w %)				acrylamide content (µg.kg^{-1}) (N = 2)		
TAG	DAG	MAG	GLY	French fries	mixture heated in tubular reactor	
					175°C, 2 min	170°C, 5 min
100	-	-	-	317 (± 64)	436 (± 72)	2555 (± 425)
85	15	-	-	315 (± 64)	318 (± 53)	1843 (± 306)
98	-	2	-	372 (± 75)	408 (± 68)	2150 (± 358)
99	-	-	1	261 (± 53)	335 (± 56)	2182 (± 363)

French fries were prepared in the different oil mixtures at 175°C for 5 min. These French fry preparation experiments (Table 4.2) indicated that acrylamide formation was not significantly influenced by the presence of any of the hydrolysis products incorporated in this study. Interestingly however, a lower trend in acrylamide level was found for the French fries, prepared in the deep-frying oil containing 1% glycerol, although statistically it was not significant. The final oil content of the French fries did not markedly change and fluctuated around 8.8% (results not shown). Each oil mixture was furthermore mixed with PBS and potato powder, as explained in section 4.3. Considering these heating experiments performed in the tubular reactor (Table 4.2), again no significant difference could be observed in the acrylamide content of the various heated mixtures studied, also if more stringent heating conditions were applied. Presumably none of the studied oil hydrolysis products were relevant precursors of acrylamide under heating conditions as applied during normal frying operations. Interestingly, a trend to lower acrylamide contents was observed for the heated artificial mixtures containing the DAG oil, although it was not statistically significant.

Despite the fact that glycerol was supposed to be a possible precursor for acrylamide during frying, our experiments (Table 4.2) indicate that during frying of potato products this hypothesis is not very likely. On the contrary, if glycerol was added to the frying oil used for French fry preparation, lower acrylamide levels were found.

The above-mentioned results are in disagreement with earlier published data (Gertz and Klostermann, 2002; Yasuhara et al., 2003). Oil hydrolysis products may, similar to oxidation products, influence the transfer of heat and oil to the food, but as shown in Table 4.2, these changes were not to that extent that it affected acrylamide formation during preparation of

French fries. Moreover, the results using the tubular reactor confirmed that the oil hydrolysis products under investigation did not significantly influence acrylamide formation. Consequently, the hypothesis that acrylamide formation is significantly influenced by the investigated oil hydrolysis products, could not be corroborated, neither in French fries nor in the model system.

4.4.3 Integrated experiment according to Gertz et al. (2003)

In order to further clarify the influence of oil degradation processes on acrylamide formation, consecutive French fry preparation tests were performed, similar to the experiments performed by Gertz et al. (2003), and as explained in section 4.3. In such a way, an intensive deep-frying process was simulated. This experiment should be considered as an integrated system, in which both the influence on acrylamide formation of oil oxidation and hydrolysis were investigated. The acrylamide contents of the prepared French fries are shown in Table 4.3.

Table 4.3. Formation of acrylamide in consecutive French fry preparation experiments, each one performed at 175°C for 5 min. Both oils remained at 175°C for the whole experiment (120 min)

	acrylamide content (µg.kg^{-1}) (*N* = 1) in French fries prepared in	
oil heating time (min)	**corn oil without DMPS**	**palm fat with DMPS**
0	1306	1190
10	1235	-
20	1385	1246
30	1344	-
40	1281	1199
50	1177	1224
110	1237	1268
120	1449	1279

-: experiments not performed

Considering the acrylamide contents of the French fries prepared in corn oil, no significant difference in acrylamide formation could be observed throughout the period of oil heating (Table 4.3). This is in contrast to results published previously (Gertz et al., 2003; Gertz, 2004). There it was suggested that the amount of polar compounds in a partially hydrogenated

rapeseed oil (containing 1.5 mg.kg^{-1} DMPS) increased after a series of 4 frying experiments. Consequently, these polar compounds could bind water in deep-frying oils, giving rise to enhanced oil to food heat transfer and thus increased formation of acrylamide. It was moreover stated that the water content decreased again after a period of 60 min at 170°C without frying. This would again be linked with decreased acrylamide formation. However, these results could not be confirmed (Table 4.3). For a similar experimental set-up, no decrease in acrylamide formation could be observed. However, corn oil without DMPS (E 900) was used for these experiments. Yet, DMPS tends to accumulate at the oil surface, protecting the oil against oxidation and forming a monolayer (Márquez-Ruiz et al., 2004). Consequently, it may be likely that this monolayer decelerated the water evaporation from the oil, giving rise to an increased polarity of the oil and increased acrylamide formation. To test this hypothesis, similar frying experiments were performed using above-mentioned palm fat, containing DMPS. The acrylamide contents are also shown in Table 4.3. No significant difference could be noticed between the two deep-frying oils applied. The final oil content of the French fries was not determined anymore, since a similar experimental outcome was expected as under section 4.4.1.

Consequently, above-mentioned results are another indication that oil degradation processes do not have a significant impact on acrylamide formation in French fries, even if the frying procedures succeed each other very quickly.

4.4.4 Comparison of acrylamide levels obtained in the different experiments

Finally, the acrylamide contents in the French fries, mentioned in Tables 4.1 and 4.2 at the one hand and in Table 4.3 at the other hand cannot be compared, since a different potato variety with different harvest season and storage condition was used. However, when observing the acrylamide contents generated within the same potato variety, the type of deep-frying oil did not significantly affect acrylamide formation in both heating methodologies, as demonstrated in the previous chapter. More specifically, no significant difference in generated acrylamide was found between the two vegetable oils applied in Table 4.3, neither was a difference between the acrylamide contents in Tables 4.1 and 4.2 for the same experimental method applied. Namely, in Tables 4.1 and 4.2, experiments were performed using unsaturated and highly hydrogenated soybean oil, respectively. On the other hand, the

acrylamide contents presented in Table 4.3 are rather high. On basis of previous research in our and other laboratories (Amrein et al., 2003; Grob et al., 2003; Becalski et al., 2004; De Wilde et al., 2005), it seems obvious that these higher acrylamide levels are mainly due to a higher content in reducing sugars in the raw material.

4.4.5 Concluding experiments performed in the silica gel model system

From above-mentioned results in potato products, it could be concluded that oil degradation precursors did not significantly contribute to the formation of acrylamide. However, these potato products also contained reducing sugars, which are considered as one of the major acrylamide precursors, together with free asparagine (Stadler et al., 2002). In these concluding experiments, it was therefore questioned whether oil degradation products could contribute more significantly to the formation of acrylamide in the absence of reducing sugars.

To investigate this, it was essential to dispose of a model system free of sugars. Therefore, the sugars were completely extracted from the previously used potato powder. Several cold water extraction steps were performed until no sugars were detected anymore in the lyophilized powder. Similar to previous experiments, a model system was reconstituted, composed of 41% sugar free potato powder, 20% fresh sunflower oil (with a PAV < 1) and 39% PBS. Since it could be assumed that the free asparagine was also largely extracted from the powder, it was again added to the model system in a similar amount as in potatoes, being 0.46 mmol/10 g mixture. Since the glucose content in potatoes is in general about 10 times lower (De Wilde et al., 2006b), this reducing sugar was added in a concentration of 0.046 mmol/10 g mixture. Preliminary heating experiments in the tubular reactor were performed, adding only asparagine and asparagine in combination with glucose to the model system. Surprisingly, no significant difference in acrylamide formation was found between both model systems (results not shown). Although no reducing sugars were detected in the heated mixtures, it was assumed that the starch, present in the sugar free powder, was hydrolyzed to some extent during heating in the tubular reactor, liberating sugars which could directly react with the present asparagine to form acrylamide in a similar way as in the model system to which glucose was additionally added. Because of this, the sugar free potato powder appeared to be unsuitable for the current experiments.

Therefore, the potato powder in the model system was replaced with inert silica gel. In order to simulate the real food matrix as much as possible, fresh sunflower oil, PBS and free asparagine were again added in similar amounts as in above-mentioned experiments. The pH of all mixtures was stabilized around 6 by means of PBS. A silica gel model system with only asparagine being added in the liquid phase was used as a control (Table 4.4). Other probable acrylamide precursors, mentioned in Table 4.4, were added in the same molar amount as glucose in the above-mentioned experiments to have a precursor/asparagine ratio of 0.1.

Table 4.4. Formation of acrylamide in a silica gel model system containing asparagine and several probable acrylamide precursors, upon heating at 170°C for 5 min in the tubular reactor

reactant added to asparagine in model system	**acrylamide content ($\mu g.kg^{-1}$) ($N \geq 3$)***
control (PBS)	360[a]
glucose	9925[b]
DAG	386[a]
MAG	364[a]
GLY	360[a]
acrolein	1430[c]
acrylic acid	316[a]
methacrolein	1745[c]
crotonaldehyde	408[a]
cinnamaldehyde	623[a]
pentanal	319[a]
hexanal	370[a]
octanal	348[a]
decanal	250[a]

* Different letters indicate significant difference ($P < 0.05$) by Tukey test

As shown in Table 4.4, an acrylamide content of 360 $\mu g.kg^{-1}$ was found in the model system containing only asparagine as acrylamide precursor. It was indeed previously demonstrated that free asparagine may release acrylamide by thermally initiated decarboxylation and deamination (Weisshaar and Gutsche, 2002; Yasuhara et al., 2003; Zyzak et al., 2003). In contrast to the model system with the sugar free powder, addition of glucose dramatically increased acrylamide formation till 9925 $\mu g.kg^{-1}$. This content is markedly higher than in the previous experiments with the sugar free powder and than the results in Table 4.2. This can be explained by the fact that the silica gel model system did not contain other free amino acids

(besides asparagine) or proteins, which are known to mitigate the formation of this carcinogen (Rydberg et al., 2003). Based on the reaction mechanism proposed in Figure 1.12, several oil hydrolysis compounds were evaluated, including diacylglycerols (DAG), monoacylglycerols (MAG), glycerol (GLY), acrolein and acrylic acid.

The addition of DAG, MAG, GLY or acrylic acid to the asparagine-containing model system did not lead to a significant increase in acrylamide formation compared to the control, containing only asparagine. Yet, according to Figure 1.12, ammonia is required in order to form acrylamide out of acrylic acid. It was however not known how much ammonia was liberated from asparagine in the silica gel model system upon heating. Therefore, an additional heating experiment was performed where acrylic acid and ammonia were heated in the tubular reactor without the presence of asparagine. No acrylamide was however formed (results not shown). These results are in contrast to earlier studies (Yasuhara et al., 2003; Schieberle et al., 2006), where more intense heating treatments were applied (170 or 180°C for 30 min). Under the current and practically more relevant heating conditions, the postulated formation mechanism of acrylamide via acrylic acid could however not be confirmed.

Interestingly, the heated model system containing acrolein and asparagine showed a significantly higher acrylamide content compared to the control, only containing asparagine. Yet, this increase was less pronounced compared to the model system with asparagine and glucose (Table 4.4). Heating of acrolein with ammonia in the absence of asparagine did not lead to acrylamide formation (results not shown). Consequently, it was postulated that a nucleophilic 1,2-addition of the α-amino group of free asparagine to the carbonyl function of acrolein would lead to the formation of acrylamide, via the Schiff base **2** (pathway **A** in Figure 4.1). After decarboxylation, the imine **3** is formed, which hydrolyzes to 3-aminopropionamide **4**, which is known to efficiently release acrylamide (Zyzak et al., 2003). This pathway is similar to the reaction route, starting from asparagine and a reducing sugar, as discussed in chapter 1 (Figure 1.7). However, the α-amino group of asparagine could also react with acrolein via the so-called Michael addition (1,4-addition, pathway **B** in Figure 4.1). After tautomerization, the intermediate **6** is formed. Due to the absence of the imine in β-position of the carboxylic acid group, this compound would however not easily decarboxylate, as was demonstrated for the structurally similar Amadori compound in Figure 1.7 (Stadler et al., 2004; Taeymans et al., 2004). Consequently, pathway **B** is not very likely to generate acrylamide.

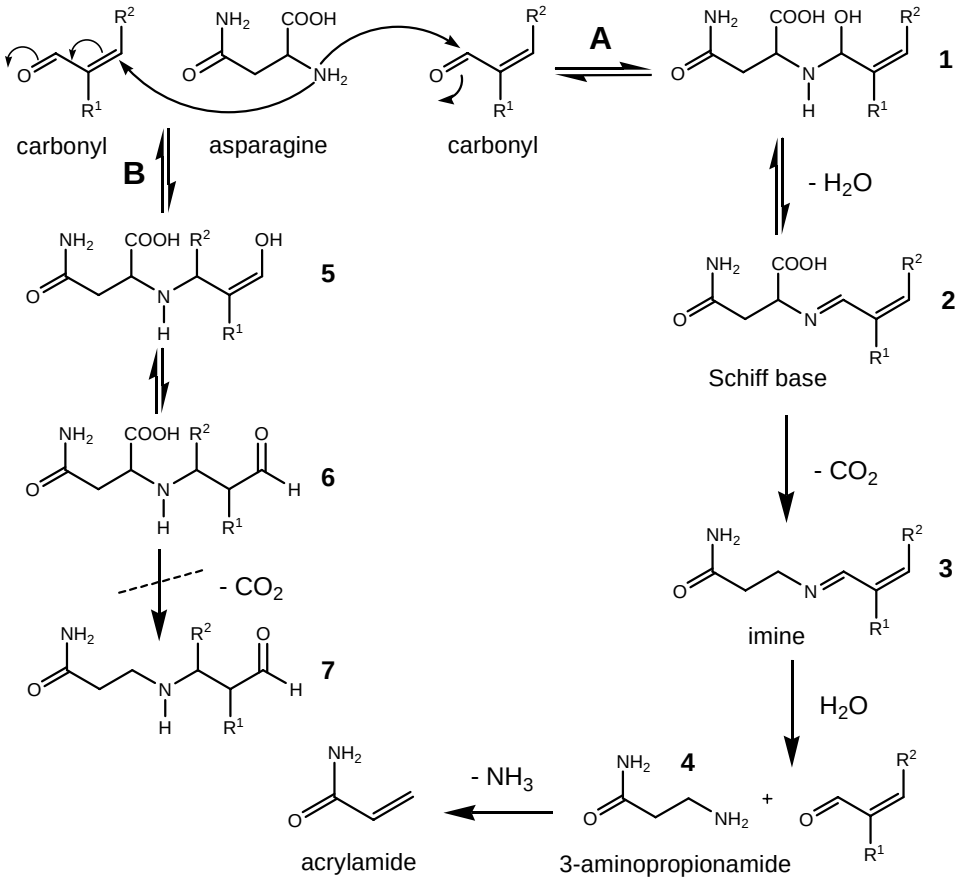

Figure 4.1. Reaction mechanisms, starting from asparagine and an α,β-unsaturated aldehyde ($R^1 = R^2 = H$, acrolein; $R^1 = CH_3$, $R^2 = H$, methacrolein; $R^1 = H$, $R^2 = CH_3$, crotonaldehyde and $R^1 = H$, R^2 = phenyl, cinnamaldehyde)

In order to further investigate this hypothesis, several α,β-unsaturated aldehydes with different substituents on the α (R^1) and β-carbons (R^2) were heated with asparagine in the model system (Figure 4.2). The heated mixtures containing asparagine and methacrolein showed a similar increase in acrylamide formation as the mixtures with asparagine and acrolein (Table 4.4). Also crotonaldehyde and cinnamaldehyde increased, in combination with asparagine, the formation of acrylamide, compared to the control containing only asparagine, although not significantly. For cinnamaldehyde, this could be explained by the fact that the water solubility of this reactant is lower compared to acrolein. The reaction with asparagine, present in the aqueous fraction of the model system, would thus be less probable. On the other hand, it was expected that the β-methyl group of crotonaldehyde would hinder the 1,4-addition, which could possibly promote the 1,2-addition (Clayden et al., 2001) and thus acrylamide formation. On the contrary, the mixture containing crotonaldehyde had a lower acrylamide content compared to the mixture to which methacrolein was added, suggesting that the 1,2-addition was not stimulated. Of course, also other reactions between the several intermediary

components may occur and complicate the unravelling of the overall reaction mechanism. A more thorough investigation of these intermediates would be necessary to further investigate above-mentioned hypotheses.

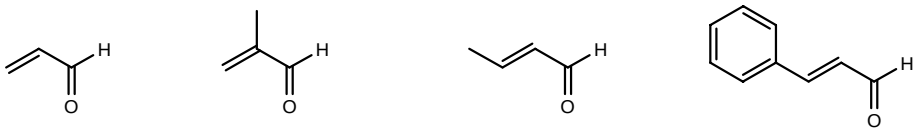

Figure 4.2. Chemical structures of acrolein, methacrolein, crotonaldehyde and cinnamaldehyde

Furthermore, several known oil oxidation products, such as pentanal, hexanal, octanal and decanal were evaluated in the asparagine-containing silica gel system (Frankel, 2005). Yet, these compounds did not increase the formation of acrylamide compared to the control (Table 4.4). Similar to cinnamaldehyde, the poor water solubility of these aldehydes might explain this outcome. Previously, it was however postulated that octanal and decanal were able to react with asparagine to form acrylamide (Becalski et al., 2003; Zyzak et al., 2003). Our results indicate that the formation of acrylamide in the presence of these aldehydes and asparagine could merely be attributed to decarboxylation and deamination of asparagine, at the applied heating conditions.

In the presence of asparagine, it was thus observed that acrolein significantly enhanced acrylamide formation, compared to the control containing only asparagine. In contrast to a common deep-frying process, these experiments were performed in a closed reactor. Since frying temperatures are moreover far above the boiling point of acrolein (51°C), this compound may readily evaporate from the foodstuff being fried (Umano and Shibamoto, 1987; Ehling et al., 2005). The importance of this chemical pathway in real foodstuffs such as French fries remains thus questionable. In addition, the significance of this pathway should be evaluated in the presence of other acrylamide precursors, such as glucose. Therefore, the glucose concentration was gradually increased, from 0 to 0.1 mole glucose/mole asparagine, in a silica gel model system containing 0.046 mmol acrolein and 0.46 mmol asparagine/10 g mixture. Similar experiments were performed with a model system containing only 0.46 mmol asparagine/10 g mixture. The results (experiments performed in quadruplicate) are presented in Figure 4.3 and show that acrylamide gradually increased as the glucose content was enhanced. The difference between the model system with and without acrolein however

disappeared when 0.01 mole glucose/mole asparagine was added, indicating that the contribution of acrolein to the overall formation of acrylamide was negligible in the presence of reducing sugars in the food matrix.

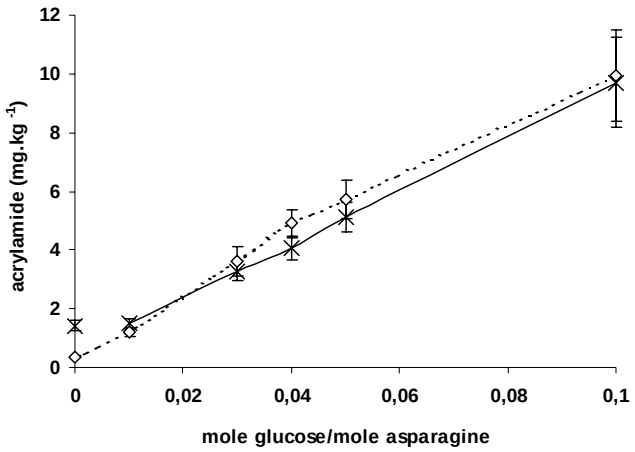

Figure 4.3. Acrylamide formation as function of several molar glucose/asparagine ratios of a silica gel model system containing 0.1 mole acrolein/mole asparagine (x) and a model system containing initially only asparagine (◊), heated in the tubular reactor at 170°C for 5 min

4.5 Conclusion

From above-mentioned results, the importance of the suspected acrylamide precursors acrolein and other oil degradation products in general appeared to be negligible compared to reducing sugars, as evaluated by the potato powder and silica gel model system and as corroborated by the French fry preparation experiments.

Chapter 3 and 4 focused on the impact of the heating medium on the formation of acrylamide. Besides oil as a heating medium, water is another important constituent in fried foodstuffs. The Maillard reaction is influenced by the water activity and moisture content. Since this reaction is moreover linked with acrylamide formation, the following chapter deals with the impact of water on the formation of acrylamide in the heated potato powder model system.

Chapter 5

Role of water on the formation of acrylamide in a potato model system

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Chapter 5 Role of water on the formation of acrylamide in a potato model system

5.1 Abstract

Moisture sorption isotherms of the potato powder were investigated at 20°C for water activities (a_w) ranging from 0.11 to 0.97. The sorption isotherms were typical type-II sigmoidal curves, with a steep increase in moisture content for water activities above 0.9 and exhibiting hysteresis over the whole water activity range. On the basis of the isotherms, the influence of initial water activity and moisture content on both Maillard browning and acrylamide formation was determined by heating potato powder mixtures, containing 21% oil, in a closed tubular reactor. Maillard browning, as determined spectrophotometrically, showed an optimum at intermediate water activities. The yields of acrylamide, expressed relatively to the molar amount of asparagine, remained constant below 0.8 a_w and below moisture contents of about 20% (on dry matter basis). For the more severe heat treatments, an increased acrylamide yield was however observed at higher moisture contents, with an optimum at water contents of about 100% (on dry matter basis). However, this increase and optimum was not observed at less severe heat treatments. At moisture contents above 100%, a significant decrease in acrylamide yields was assessed, although the water activity increased only marginally in this area of the sorption isotherms. It was thus observed that the acrylamide yield was rather dependent upon moisture content than upon water activity in the high-moisture potato powder model system. Similar to chapter 3, acrylamide formation was lowered upon decreasing oil content from 21 to 0%, coinciding with a shift in optimal acrylamide formation to lower moisture contents.

Keywords: sorption isotherms; hysteresis; water activity; acrylamide; Maillard browning; LC-MS/MS

5.2 Introduction

Deep frying has been defined as the submersion of a food product in edible oil or fat heated above the boiling point of water. Therefore, this operation may be considered a dehydration process, which comprises evaporation of water from the product, in the form of vapour. Simultaneously, oil penetrates the product and major transformations in the (porous) microstructure occur, which determine the final physical and sensorial food properties. In addition, chemical interactions between food components and frying oil give rise to non-volatile and volatile compounds (Krokida et al., 2001b; Pedreschi et al., 2005a). The nonenzymatic browning reaction generates a vast range of odour and flavour molecules, as well as brown pigments (Ames, 1990). In relation with this Maillard reaction, acrylamide is formed, starting from reducing sugars and free asparagine (Yaylayan et al., 2003; Zyzak et al., 2003).

Furthermore, it has been shown that water activity (a_w) is a key factor to consider in the Maillard reaction (Eichner and Karel, 1972; Ames, 1990; Robert et al., 2005). Optimal rates of Maillard browning at intermediate a_w are reported (Ames, 1990). At lower water activity levels, the molecular mobility or solubility is hindered. At higher levels, the reaction rates decrease because of a dilution effect of the reactants. Because water is produced during the Maillard reaction, the law of mass action plays an inhibiting role, as well at high a_w. The consistency of other components within the matrix, such as polymers and humectants, may also affect the mobility of the reactants, complicating the unravelling of the Maillard reaction and the factors influencing it (Eichner and Karel, 1972; Ames, 1990; Mustapha et al., 1998).

The above-mentioned optimal rate of browning can best be explained by reference to the moisture sorption isotherms. Moisture sorption isotherms represent the relationship between the equilibrium moisture content and water activity at constant temperatures and pressures (Kaymak-Ertekin and Gedik, 2004). Because food materials have complex compositions and structures, sorption isotherms actually describe the integrated hygroscopic properties of the various constituents. They express the sorption mechanism and interaction of food biopolymers with water (Kaymak-Ertekin and Gedik, 2004).

Because acrylamide formation is linked to the Maillard reaction (Mottram et al., 2002; Stadler et al., 2002), water activity and moisture content may also have an impact on the generation of

acrylamide. Sadd and Hamlet (2005) developed a mathematical dough model, which showed that acrylamide formation increased upon decreasing the moisture content. However, the Maillard reaction showed an optimal level of browning at intermediate moisture levels, indicating that the Maillard reaction and acrylamide formation do not always concur. The impact of the water activity was however not investigated in this study. On the other hand, Robert et al. (2005) proved that a_w is not a critical parameter for acrylamide formation in low-moisture model systems ($0.07 < a_w < 0.22$) based on asparagine and glucose. However, the acrylamide amounts were correlated with physical changes occurring during the reaction, such as melting of sugars and the physical state of the reaction system (crystalline *versus* amorphous).

On the other hand, model studies revealed a dramatic drop of acrylamide yield when lowering the water content of the heated mixture from 10 to 0% (Schieberle et al., 2005). The yields further increased when experiments were performed at a water content of 25% but dropped again when tests were performed in an aqueous buffer.

Recently, a kinetic study was published (Amrein et al., 2006b) in which the influence of moisture content on colour and acrylamide formation in a closed potato model system was investigated at three heating temperatures (119, 143 and 167°C). At low- and high-moisture contents, lower Maillard browning and acrylamide formation rates were determined. Increasing activation energies for both parameters at low-moisture contents (< 25 g/100 g of dry matter) were demonstrated. Also here, the impact of a_w was not considered.

After investigating the impact of the deep-frying medium on acrylamide formation (chapter 3 and 4), this chapter focused on the relationship between water activity and moisture content of a commercial potato powder. Through the construction of sorption isotherms, a better insight into the integrated hygroscopic properties of the foodstuff could be acquired. On the basis of this knowledge, the subsequent step was to determine whether initial water activity or water content was the crucial factor on the formation of brown Maillard pigments and on acrylamide generation in a closed and heated potato powder model system.

5.3 Materials and methods

5.3.1 Materials

Potato powder was purchased in retail (Unilever, Belgium). The powder was sieved and the fraction between 90 and 160 μm was retained, as discussed in chapter 2. The initial dry matter content and water activity was 90.33 ± 0.24% and 0.37 ± 0.015, respectively. The fructose, glucose, sucrose and asparagine contents are mentioned in Table 2.1.

5.3.2 Sorption experiment

For the determination of the adsorption isotherm, the potato powder was initially dried for about 48 h in a vacuum oven at 30°C until no appreciable weight loss was noted. Sulphuric acid (96%, Chem-Lab, Belgium) was used to trap water vapour inside the oven. The powder was thereupon used for the development of the adsorption isotherm by the static gravimetric method. For the desorption isotherm, potato powder was initially placed over pure distilled water until no appreciable weight gain was observed before use. Pure distilled water and nine saturated salt solutions of different relative humidity were selected to get different a_w values in the potato powder, ranging from 0.110 to 0.972. The relative humidity/ a_w values of the salt solutions were taken from previous studies (Labuza, 1984; Resnik and Chirife, 1988; McLaughlin and Magee, 1998) and include LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, NaCl, KCl, $BaCl_2$ and K_2SO_4 (purity > 99% (w/w), Acros Organics, Belgium). About 10-15 g of potato powder was placed in a glass jar (V = 2.5 L) containing a saturated salt solution or distilled water. An internal platform was used to raise the samples off the floor. Experiments were carried out in triplicate. A small amount of toluene was placed in each jar to prevent growth of fungi (Labuza, 1984; Mcminn and Magee, 2003). The jars were then placed in a constant temperature cabinet at 20 ± 1°C.

The samples were left to equilibrate until the weight was constant over at least three consecutive days. The total weighing time was maintained at less than 30 s to reduce the sorption of atmospheric moisture. The dry matter content of the potato powder was determined by the oven-drying method (AOAC, 1990) and the exact value of the corresponding a_w was confirmed by the Novasina Thermoconstanter TH200 (Axair Ltd. Systems, Switzerland).

5.3.3 Modelling of sorption isotherms

The mathematical sorption isotherm models, shown in Table 5.1 (Halsey, 1948; Van den Berg and Bruin, 1981; Peleg, 1993), were fitted to the experimental data. These include one four-parameter Peleg equation, one three-parameter equation (Guggenheim-Anderson-de Boer, GAB) and one two-parameter equation (Halsey). These sorption models are among those most widely used to describe sorption isotherms for various food materials. The parameters of the sorption models were estimated using the nonlinear regression function of SPSS version 12.0 (SPSS, Inc., Chicago, IL, US). Goodness of fit of the models was evaluated by means of the mean relative percentage deviation modulus (P), defined as

$$P(\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{\left|M_{ei} - M_{ci}\right|}{M_{ei}}$$

where M_{ei} and M_{ci} are the experimental and predicted moisture content values, respectively, and N is the number of experimental data. A model is considered acceptable if it has a P value less than 10% (Lomauro et al., 1985a).

Table 5.1. Isotherm equations for experimental data fitting

name model	equation
Peleg (1993)	$M = K_1 a_w^{n_1} + K_2 a_w^{n_2}$
GAB (Van den Berg and Bruin, 1981)	$M = \frac{m_0 C K a_w}{\left[(1 - K a_w)(1 - K a_w + C K a_w)\right]}$
Halsey (1948)	$a_w = e^{(-k/M^n)}$

K_1, K_2, n_1, n_2: equation parameters
a_w: water activity
M: moisture content (g/100 g dry matter)
m_0: monolayer moisture content (g/100 g dry matter)
C, K: GAB model parameters
k, n: constant

5.3.4 Preparation of potato powder mixtures in tubular reactor

Immediately after concluding the water activity measurements, the 10 potato powders, having a different a_w, were mixed with sunflower oil in a mortar. Potato powder mixtures with different final oil content were evaluated, as described in the text. The total mixing time was maintained at less than 1 min to reduce sorption of atmospheric moisture. Besides the static gravimetric method, during which potato powder adsorbed or desorbed water through the

vapour phase, potato powder mixtures were also prepared by adding distilled water to dry potato powder. A total of 1 g of homogenized mixture was thereupon heated at 170°C for 3, 5 or 7 min in the tubular reactor as explained under section 2.3.3.

5.3.5 Acrylamide analysis

Acrylamide analyses were performed as described under section 2.3.2. The 95% confidence intervals, presented between brackets in Figures 5.3-5.5, are based on repeatability experiments reported in chapter 2, yielding a RSD of 12%. The molar acrylamide yields in Figures 5.3-5.5 are expressed relatively to the molar amount of asparagine, present in each mixture.

5.3.6 Colour analysis

The colour of the clear aqueous acrylamide extracts, obtained after 20 min of shaking, centrifugation and ultrafiltration, was measured at 420, 450, 470 and 490 nm (Eichner and Karel, 1972; Mustapha et al., 1998; Martins and van Boekel, 2003) using a Varian Cary 50 Bio spectrophotometer (Mulgrave, Victoria, Australia). The extracts were diluted to obtain absorbance values mainly between zero and one. Absorbances *versus* different sample masses (0.50-1.25 g) of the same heated sample gave a linear relationship ($R^2 > 0.99$). The results in Figure 5.2 are expressed in relative absorbance, being the absorbance of the clear extract divided by the amount of dry potato powder present in each mixture.

5.4 Results and discussion

5.4.1 Sorption isotherms

The adsorption and desorption isotherms of the potato powder at 20°C are shown in Figure 5.1. The isotherms have a sigmoidal shape, depicting an increase in equilibrium moisture content with a_w. This is typical for type-II isotherms and has been reported for starchy products such as potatoes (Mazza, 1982; McLaughlin and Magee, 1998; Mcminn and Magee, 2003), cookies and corn snacks (Palou et al., 1997). Both adsorption and desorption isotherms could roughly be divided into two regions. In the region with $a_w < 0.9$, the moisture content only changed limitedly and reached about 30% (on dry matter basis). However, a steep

increase in the moisture content, up to 140%, was assessed for a_w > 0.9, both for the adsorption and desorption isotherms (Figure 5.1), because of the increasing amount of free water in the mixture. Hysteresis could also be observed over the total water activity range investigated, because the equilibrium moisture content for desorption was higher than that for adsorption.

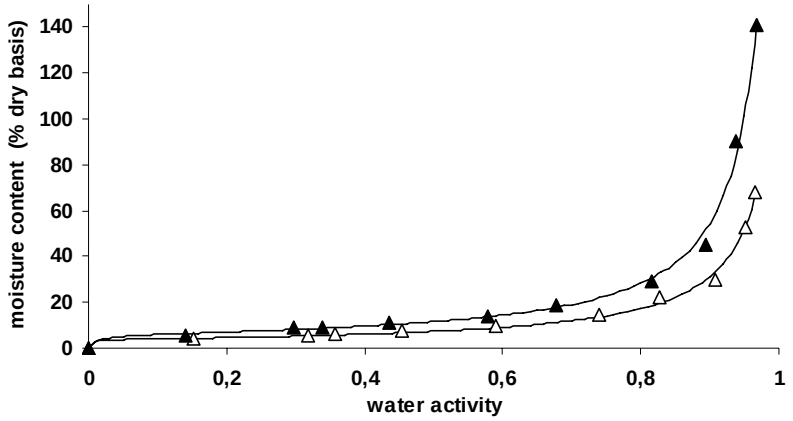

Figure 5.1. Adsorption (Δ) and desorption (▲) isotherm of potato powder at 20°C. The smooth curves represent the GAB isotherm curves, fitted to the data

5.4.2 Fitting mathematical sorption models to isotherm data

Table 5.2 shows the coefficients of the three sorption isotherm equations fitted to the experimental adsorption and desorption data, respectively, and *P* (%), the mean relative percentage deviation modulus. Other sorption isotherm equations were also considered. However, only the equations best fitting to the experimental data are mentioned here. For the adsorption data, the Halsey, Peleg and GAB model gave a *P* value below 10% and could therefore be considered to be adequate for describing experimental adsorption data for the potato powder (Lomauro et al., 1985a). For the desorption data, the Peleg equation showed the lowest *P* value, closely followed by the GAB and Halsey equation. Because the *P* value for the Halsey equation for the desorption data was initially slightly above 10% (10.21%), a_w values below 0.3 were omitted upon fitting the data. The omission of extreme water activity points was already done previously (Al Muhtaseb et al., 2004) to obtain a better mathematical fit. Doing so, a *P* value below 10% was obtained. This was also done for the Peleg equation of the adsorption data, which had initially a *P* value of 10.66%. It should be realized that no single mathematical model can be considered accurate over the entire a_w range, because water is associated with the food matrix by different mechanisms in different a_w regions (Labuza,

1975). Moreover, the goodness of fit of a sorption model to experimental data does not describe the nature of the sorption process. It only reflects on the mathematical quality of a model (Labuza, 1975).

The GAB (plotted in Figure 5.1 as a smooth line for both adsorption and desorption data) (McLaughlin and Magee, 1998; Mcminn and Magee, 2003; Al Muhtaseb et al., 2004) as well as the empirical Peleg equation (Al Muhtaseb et al., 2004) have already been previously shown to be good models for predicting potato and potato starch isotherms. However, the findings for the Halsey model are somewhat contradictory. Al Muhtaseb et al. (2004) and Wang and Brennan (1991) have found the Halsey model to be inadequate for representing the sorption isotherms for starch powders and potatoes, respectively. This is in contrast to Mcminn and Magee (2003) and Kaymak-Ertekin and Gedik (2004).

Table 5.2. Estimated parameters and *P* (%) values of sorption equations fitted to the adsorption and desorption isotherm data of the potato powder

model	**constants**	**adsorption**	**desorption**
Peleg	K_1	25.703	26.761
	K_2	113.601	226.583
	n_1	1.499	0.980
	n_2	27.638	19.971
	P (%)	7.88*	7.89
GAB	m_0	3.747	5.867
	C	473.169	91.027
	K	0.977	0.992
	P (%)	8.53	7.96
Halsey	k	13.218	12.062
	n	1.411	1.186
	P (%)	3.32	9.19*

* for $a_w > 0.3$

The estimated monolayer moisture content (m_0) from the adsorption isotherm using the GAB equation was 3.747 g/100 g of dry matter. A slightly higher monolayer moisture content of 5.867 g/100 g of dry matter was determined from the desorption isotherms. The estimated values are comparable to values reported by Al Muhtaseb et al. (2004), being 3.1 and 5.6

g/100 g of dry matter, respectively, for adsorption and desorption isotherms of potato starch powder at 30°C. The values are somewhat lower compared to those reported by Mcminn and Magee (2003) and McLaughlin and Magee (1998) for potatoes. However, m_0 values between 3.2 and 16 g/100 g of dry matter have been reported for starchy foods (Lomauro et al., 1985b).

5.4.3 Influence of initial water activity on Maillard browning

To investigate the influence of initial a_w on the Maillard reaction, the potato powders with different a_w, obtained from the adsorption and desorption experiments, were heated in a hermetically sealable tubular reactor. This was done to obtain constant food moisture content during heating, by eliminating water evaporation. It should however be realized that the measured initial a_w was not constant during the heating process, because a_w changes with temperature. The increasing vapour pressure in the sealed reactor upon heating additionally influenced a_w. It can however be assumed that the course of this change upon heating was similar for each mixture. Consequently, a difference in a_w remained between mixtures with distinct initial water activity levels at a specific point of the heating process. Sunflower oil was mixed with the different potato powders to obtain an oil content of 21% (w/w). In such a way, a more realistic mixture composition compared to, e.g., French fries was obtained without influencing a_w. To check possible sorption of atmospheric moisture upon mixing potato powder and oil, a_w values of three potato powders (0.14, 0.44 and 0.94) before and after oil addition were measured. These differences were < 0.005. The heating experiments were carried out at 170°C for 3, 5 and 7 min, which are representative heating conditions for a finish-frying process of French fries and which enable to compare the effect of water activity in several ranges of final acrylamide content.

The basis for the assessment of the rate of the Maillard reaction has been to monitor colour formation as assessed in aqueous extracts, containing water-soluble melanoidins (brown, nitrogenous polymers and copolymers), which are the final products of the Maillard reaction. Previously, the absorbance of heated reaction mixtures was determined spectrophotometrically at different wavelengths (420, 450, 470 and 490 nm) to assess the degree of the Maillard reaction (Mustapha et al., 1998; Martins and van Boekel, 2003).

Figure 5.2 shows the relative absorbance of the different potato powder mixtures, obtained from the construction of the desorption isotherm, for the three heating times. Only the 420 nm measurements are plotted, because the measurements at other wavelengths gave a similar trend and lower absorbance. To correct for the different moisture content between the heated mixtures, the results are expressed on the amount of dry potato powder. The relative absorbance values in Figure 5.2 increased with increasing heating times and thus progressive Maillard browning. A maximum was observed around 0.7 a_w for 3 and 5 min heating at 170°C. For 7 min heating, the highest relative absorbance value was already reached at 0.4 a_w and remained at this level until 0.7 a_w. In addition, a decrease in colour formation occurred at lower ($a_w < 0.3$) and higher ($a_w > 0.9$) initial a_w levels. This shows that a difference in initial water activity of the potato powders had an impact on the Maillard reaction in the closed tubular reactor upon heating. These results confirm previous studies in which an optimum of Maillard browning was observed at intermediate water activities (Eichner and Karel, 1972; Ames, 1990; Mustapha et al., 1998). In addition, Sadd and Hamlet (2005) also found optimum browning at intermediate moisture contents in a closed dough model system. However, a recent study using a closed potato model system revealed optimum browning rates at moisture contents of about 68.4 g/100 g of dry matter (Amrein et al., 2006b). On the basis of the developed sorption isotherms, this roughly corresponds to 0.9 a_w. In this particular study, Maillard browning was however measured at the product surface as relative brightness (L/L_0), which may explain the different experimental outcome.

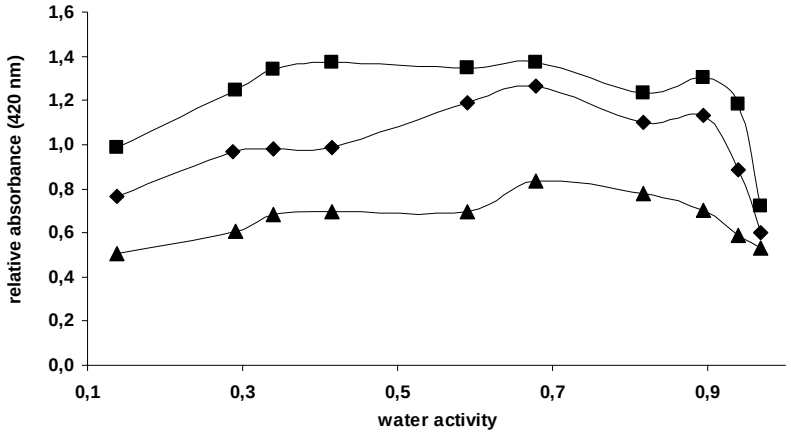

Figure 5.2. Relative absorbance values (420 nm), expressed relatively to the amount of dry potato powder, of aqueous extracts of potato powder mixtures, heated at 170°C for 3 (▲), 5 (♦) and 7 (■) min in the closed tubular reactor

5.4.4 Influence of initial water activity and water content on the amounts of acrylamide

The results presented above show that the Maillard reaction and colour formation in the model system had an optimum at intermediate a_w. Because acrylamide is linked to the Maillard reaction (Mottram et al., 2002; Stadler et al., 2002), the impact of initial a_w on the final acrylamide content was investigated accordingly. For this, the mixtures with different initial a_w, which were heated inside the reactor as discussed above, were analysed on their acrylamide content. Acrylamide contents of the heated potato powders originating from both adsorption and desorption isotherms (Figure 5.1) were determined. Because acrylamide is formed from the amide side chain of asparagine (Zyzak et al., 2003), the molar amounts of acrylamide in Figures 5.3 and 5.4 are expressed relatively to the molar amounts of asparagine, present in each heated mixture. In such a way, the molar yield of acrylamide was calculated as a function of the initial water activity and water content. The results represent the average of three replicates.

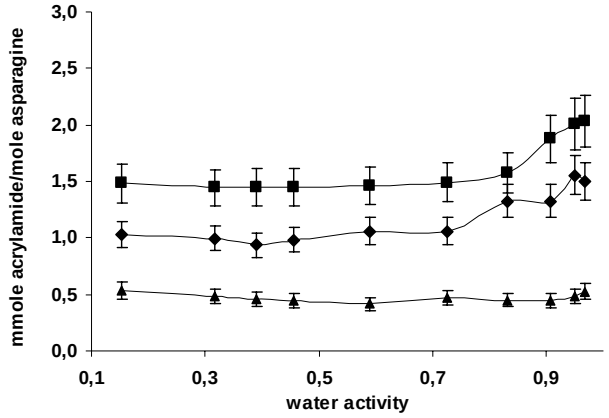

Figure 5.3. Effect of initial a_w on the molar yield of acrylamide in potato powder mixtures, obtained after a water adsorption experiment. Mixtures were heated for 3 (▲), 5 (♦) and 7 (■) min in the closed reactor

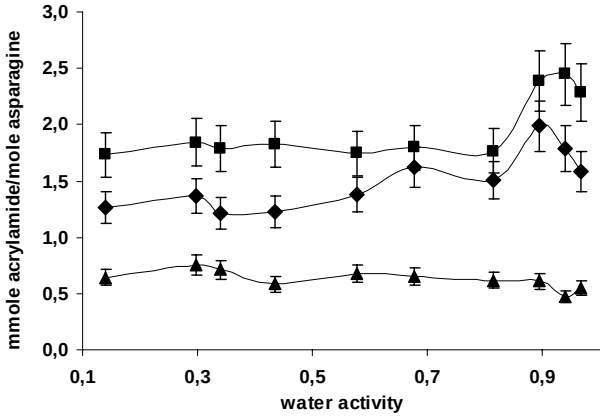

Figure 5.4. Effect of initial a_w on the molar yield of acrylamide in potato powder mixtures, obtained after a water desorption experiment. Mixtures were heated for 3 (▲), 5 (♦) and 7 (■) min in the closed reactor

This experimental set-up did however not allow for a quantification of acrylamide degradation upon heating. However, Figures 5.3 and 5.4 show increasing acrylamide contents upon longer heating times. Because no decreasing trend for longer heating times was assessed, this indicates that formation still exceeded elimination (Amrein et al., 2006b). In addition, acrylamide levels in Figure 5.4 tended to be higher than those in Figure 5.3, although these

differences were not significant. Furthermore, it could be observed that the final acrylamide yield in the heated potato model systems, originating from both adsorption and desorption experiments, remained constant between 0.14 and 0.8 a_w. Previously (Robert et al., 2005), it was also concluded that a_w did not seem to be a critical parameter for acrylamide formation in asparagine/glucose model systems under low-moisture conditions ($0.07 < a_w < 0.22$). Even initial a_w levels higher than 0.8 barely changed the acrylamide yields in Figure 5.3 for the mixtures heated for 3 min at 170°C; in fact, a small decrease was observed at 0.94 a_w in Figure 5.4. For the mixtures heated for 5 and 7 min, however, the acrylamide yields in Figures 5.3 and 5.4 clearly increased with an increasing initial a_w above 0.8. In potato mixtures obtained after the water desorption experiment (Figure 5.4), this increase was followed by a decrease in acrylamide yields when the initial a_w of the mixtures further approached 1.0. However, this reduction was not obvious in the heated mixtures, obtained after the water adsorption experiment (Figure 5.3). Consequently, it is clear that, depending upon the adsorption/desorption status of the potato powder, the acrylamide yields upon heating were different, despite the fact that the initial a_w of the powder was similar. When evaluating these data against the sorption isotherms (Figure 5.1), it becomes clear that powders experiencing water desorption had a much higher moisture content compared to powders going through a water adsorption phenomenon, for similar a_w. This hysteresis effect was much more pronounced at $a_w > 0.9$. A slight increase in a_w caused a steep increase in moisture content in this region of the desorption isotherm, leading to a decrease in final acrylamide yield upon heating (Figure 5.4). This acrylamide-lowering effect at $a_w > 0.9$ was not observed for the heated mixtures originating from the adsorption isotherm (Figure 5.3), because moisture contents did not reach the same level for the investigated a_w levels. Consequently, it is most likely that the moisture content, rather than a_w, played a role in acrylamide formation in current experimental set-up.

To further investigate the impact of initial a_w and water content on the acrylamide yield upon heating at a higher a_w and water content range, the evolution in acrylamide formation was evaluated in heated potato powder mixtures with moisture contents above 100% (expressed on dry potato powder). For this, different amounts of distilled water were added to the dry potato powder. Three mixtures were prepared accordingly, with moisture contents of 114.1, 192.5 and 213.5% (expressed on dry potato powder) and water activities of 0.964, 0.977 and 0.980, respectively. These high-moisture content levels could not be achieved during the adsorption experiment, using the static gravimetric method (Figure 5.1).

Because this experiment can be considered as an adsorption process, the three points were evaluated against the adsorption isotherms (Table 5.2). Using the three measured a_w values, these equations resulted in moisture content values of about 65, 83 and 90%, respectively, for the three potato powder mixtures. Consequently, it was observed that the measured moisture content values were situated above the predicted adsorption curves. In contrast to the static gravimetric method, however, the three mixtures were only left overnight to equilibrate between the addition of water and the a_w measurement. The static gravimetric method indeed allowed the potato powders 3 weeks to equilibrate above saturated salt solutions, before measuring a_w. In addition, the different manner of water administration to the potato powder could be another reason for this different sorption behaviour. In the static gravimetric method, water adsorption occurred through the vapour phase, while for the second method, liquid water was added. It was stated as well (Labuza, 1975) that not any model could correctly predict the sorption isotherm over the whole range of water activities, because water is associated with the food matrix by different mechanisms in different a_w regions. Therefore, it could indeed be possible that the calculated adsorption equations were not valid for water activities above 0.96.

To the three potato powders obtained accordingly, 21% of sunflower oil was added, followed by homogenization. Similar to the previous experiments, the mixtures were thereupon heated in the closed tubular reactor for 3, 5 and 7 min. Figure 5.5 shows the molar acrylamide yields, together with the acrylamide yields originating from the two highest a_w points of the adsorption and desorption isotherm experiments, respectively. Because a_w levels only varied marginally in this region of the sorption isotherm (Figure 5.1), the data are now plotted against the moisture content, expressed in terms of percentage on the amount of dry potato powder. The water activity of each mixture is mentioned as labels (0.943-0.980) in Figure 5.5. From this figure, it is clear that the acrylamide yields further dropped as the moisture content further increased above 100%, confirming the decreasing trend in acrylamide yield for high a_w levels in Figure 5.4. This decreasing trend was now also visible for the high-moisture content (> 150%) mixtures heated for 3 min. From Figure 5.5, it is also clear that the formation of acrylamide was more likely dependent upon the moisture content rather than a_w.

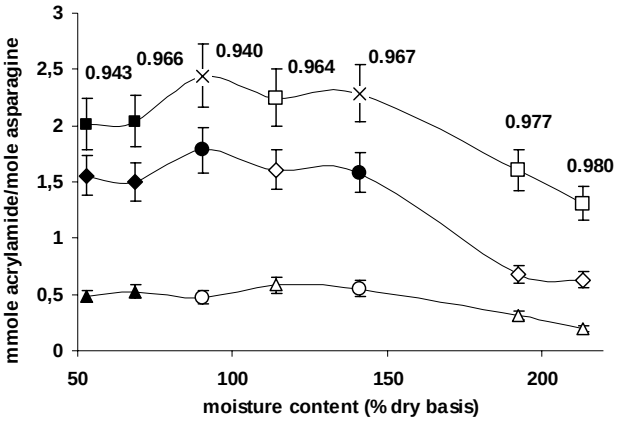

Figure 5.5. Effect of high moisture content (> 50%) on the molar yield of acrylamide in potato powder mixtures, containing different amounts of water. Mixtures were heated for 3 (Δ), 5 (◊) and 7 (□) min in the closed reactor. Data from the adsorption experiment are also plotted for 3 (▲), 5 (♦) and 7 (■) min heating. The desorption experiment data, with initial a_w values of 0.940 and 0.967, are included as well, for 3 (o), 5 (●) and 7 (x) min in the closed reactor. The labels show the a_w level corresponding to the moisture content of each mixture

In addition, the absorbance at 420 nm of the aqueous acrylamide extracts was measured, for the three heat treatments. A further reduction in absorbance was assessed, confirming an additional decrease in Maillard browning at higher a_w and moisture content (results not shown). Here, this decrease in Maillard browning occurred in parallel with a decrease in acrylamide formation (Figure 5.5). For lower a_w (< 0.9) and moisture content levels, however, these two phenomena were not related. Although a maximum browning at intermediate a_w was observed (Figure 5.2), the final acrylamide content remained constant between 0.14 and 0.8 a_w (Figures 5.3 and 5.4). However, the molar acrylamide yields of the heated mixtures correlated linearly with the relative absorbance values (R^2 > 0.93) for all investigated moisture contents. These correlations were however only observed when samples were compared with the same initial a_w but not between mixtures of different a_w or moisture content. Earlier studies (Surdyk et al., 2004; Cook et al., 2005; Sadd and Hamlet, 2005) also suggested that, in some systems, colour may be a good marker of acrylamide content but the two are not implicitly linked. Consequently, the sample colour could not unambiguously predict the acrylamide content. The link between acrylamide formation and Maillard browning in French fries is however further elaborated in the following chapter.

In contrast to our findings, it was previously demonstrated that acrylamide formation only occurred to any extent when final moisture levels in potato, rye and wheat cakes had fallen below 5% (w/w). Below 5% moisture, acrylamide formation was inversely proportional to the moisture content (Elmore et al., 2005). However, this significant increase in acrylamide

formation was also caused by a more prolonged heat treatment, resulting in lower final moisture contents of the prepared products. Probably, this steep increase was also linked to this extra thermal input, in connection with physicochemical transformations occurring simultaneously (Pedreschi et al., 2005a). Moreover, because water evaporation takes place during the initial phase of heating, the inner temperature of the product did not exceed 100°C until all free water was evaporated. Because acrylamide is not generated at temperatures below 100°C, it is obvious that a significant increase in formation was only observed when all free water was evaporated, coinciding with a dramatic increase of the inner product temperature. Consequently, the findings of Elmore et al. (2005) are more likely linked to a different thermal input of the foodstuff upon heating, rather than to a different moisture content. In our closed model system, however, the parameter water evaporation was eliminated. Consequently, both thermal input and moisture content were kept more constant upon the entire heating duration, allowing for an assessment of the actual impact of moisture content and revealing no significant acrylamide increase upon decreasing the moisture content to about 4%, the lowest point tested.

Sadd and Hamlet (2005) performed experiments in a closed dough model system also with a constant thermal input and found gradually decreasing acrylamide levels upon increasing moisture content. Four moisture contents were tested: 2.7, 10, 19 and 45%. The water activity was however not measured. On the basis of sorption isotherm studies on cereal products (Lomauro et al., 1985b; Palou et al., 1997), it is likely that these moisture contents correspond to a broad a_w range, roughly between 0.1 and 0.9. Consequently, it could be stated that the final acrylamide concentrations in this dough model system decreased gradually upon increasing a_w and moisture content. A dilution effect of acrylamide precursors for increasing moisture contents could have caused this effect. For cereal products, the dilution of free asparagine in the free aqueous phase could be a controlling mechanism because free asparagine is the rate-limiting factor in these products (Surdyk et al., 2004). The difference in moisture content could also lead to changed kinetics of acrylamide formation. At very low-moisture contents, no decrease in acrylamide formation could however be demonstrated in this dough model system.

In potato products, however, reducing sugars are the rate-limiting factor. A similar dilution effect and changing kinetics could be expected upon increasing the moisture content and a_w. This was indeed demonstrated very recently in a kinetic study, where potato powders with

different water content were heated inside a closed reactor system (Amrein et al., 2006b). In contrast to our experiments, no oil was however added to this model system. An optimum rate of acrylamide formation was found at 13.3 g/100 g of dry matter. On the basis of the developed sorption isotherms, this corresponds roughly to 0.7 a_w. The results in Figure 5.5 however show an optimum at a higher moisture content of about 100% (on dry basis) for the mixtures heated for 5 and 7 min at 170°C. Nevertheless, this optimum was not observed for the mixtures heated for 3 min, indicating that a different (lower) thermal input into the model system may also play a role in the kinetics and thus the final molar acrylamide yield. In addition, increasing activation energies for acrylamide formation at low-moisture contents (< 25 g/100 g of dry matter) were demonstrated (Amrein et al., 2006b). Our potato model system did however not reveal a significant decrease in molar acrylamide yields at the lowest moisture contents investigated. This somewhat different experimental outcome between the Amrein study (Amrein et al., 2006b) and above-mentioned results may be attributed to a different thermal input applied to the model systems, as well as the different oil content of the model systems.

In chapter 3, the oil content indeed appeared to influence the final acrylamide content. Therefore, some additional experiments were performed with 0, 7 and 14% oil, as shown in Figure 5.6. In these experiments, water was again manually added in different amounts to dry potato powder. All mixtures were heated for 5 min at 170°C in the closed reactor. In order to keep a clear view over the results, the 95% confidence intervals in Figure 5.6 were only plotted for the mixtures containing 14% oil. Maillard browning was also assessed. No significant difference was however assessed between mixtures with diverse oil content (results not shown). From Figure 5.6, it is clear that the acrylamide yield decreased when the oil content was lowered, as was discussed in chapter 3 (section 3.4.2). Moreover, there appeared to be a shift in optimum acrylamide yield to lower moisture contents upon decreasing oil content. To be more precise, an optimum at about 20% moisture content was assessed in the oil-free mixtures, which is quite comparable to the results (13.3%), obtained earlier (Amrein et al., 2006b). Furthermore, a more pronounced decrease in acrylamide yield could be assessed for the low-moisture mixtures, as compared to the mixtures containing 21% oil (Figures 5.3-5.5). Consequently, from previous studies (Sadd and Hamlet, 2005; Amrein et al., 2006b) and above-mentioned results, it appears that low-moisture contents lead to a levelling off or a decrease in acrylamide formation, depending on the lowest moisture content investigated and the experimental set-up. Therefore, it could be stated that at least a certain

amount of water is needed for acrylamide formation to occur upon thermal treatment (Schieberle et al., 2005; Granvogl et al., 2007).

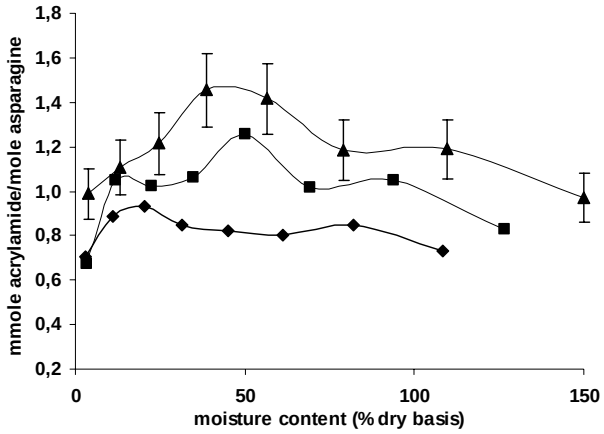

Figure 5.6. Effect of moisture content on the molar yield of acrylamide in potato powder mixtures, containing 0 (♦),7 (■) and 14% (▲) oil. Mixtures were heated for 5 min at 170°C in the closed reactor

5.5 Conclusion

Upon deep-frying of potato strips, the moisture content decreases from 400 to about 30-80 g/100 g of dry matter, depending upon the oil temperature (Krokida et al., 2001b). Frying of potato slices even causes a more pronounced drop of the final product moisture content (Pedreschi et al., 2005a). According to the results presented in Figures 5.3-5.6, there is a phase throughout the deep-frying process during which the acrylamide formation may be stimulated, due to the observed optimum in acrylamide yield. As shown in Figures 5.3-5.5 for the mixtures heated for only 3 min, a different (lower) thermal input may however have a decreasing impact on the final acrylamide yield and thus acrylamide kinetics, as also suggested previously (Amrein et al., 2006b). However, when this reducing measure is applied in real foodstuffs, other aspects such as product quality characteristics should then also be safeguarded.

As previously mentioned in this chapter, a correlation was found between acrylamide formation and Maillard browning, in model systems with a similar initial a_w. This relationship might however also be influenced by other factors. Therefore, following chapter focuses on the impact of the glucose/fructose ratio on this relationship, since it is known that acrylamide formation and Maillard browning are mainly dependent upon the reducing sugar content of the raw potato material.

Chapter 6

Impact of the glucose/fructose ratio on the relationship between acrylamide and Maillard browning in French fries

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Chapter 6 Impact of the glucose/fructose ratio on the relationship between acrylamide and Maillard browning in French fries

6.1 Abstract

The relationship between the acrylamide content and Maillard browning in French fries was studied as a function of different glucose/fructose ratios in the raw product using several colour measurement methods. An exponential correlation was found between acrylamide formation and surface colour, as evaluated by the parameters a*, delta E and Agtron. As a consequence, small differences in product colour could result in more pronounced differences in acrylamide contamination. This relationship however appeared to be dependent upon the glucose/fructose ratio of the raw material. An excess of fructose compared to glucose stimulated acrylamide formation to a higher extent than Maillard browning. The opposite effect was established with an excess of glucose. In addition, a linear relationship was found between the absorbance of aqueous French fry extracts and acrylamide content, which moreover appeared to be less affected by the addition of extra reducing sugars prior to frying. To predict acrylamide formation, quantitative and qualitative sugar analysis however remains an important instrument and is complementary to surface colour measurement.

Keywords: acrylamide; colour; Maillard browning; reducing sugars; French fries

6.2 Introduction

The appealing colours as well as the relatively fast creation of unique flavours and texture make the deep-frying process of e.g. French fries one of the most popular food preparation methods, both industrially and at home (Pedreschi et al., 2007b). On the other hand acrylamide is formed in these tasty foodstuffs. As discussed in chapter 1, it is known that the formation pathway of this probable human carcinogen is linked to the Maillard browning reaction and that reducing sugars and asparagine are crucial precursors (Stadler et al., 2002). Since the amount of free asparagine in potato tubers largely exceeds the amount of reducing sugars, it is the latter which determines the degree of acrylamide formation and Maillard browning upon frying (Márquez and Añón, 1986; Brierley et al., 1996; Amrein et al., 2003). Several pre- and post-harvest factors may influence the sugar content and thus acrylamide formation (Blenkinsop et al., 2002; Nourian et al., 2003; De Wilde et al., 2005; De Wilde et al., 2006b). In practice, reducing sugar contents below about 1 $g.kg^{-1}$ fresh weight are indicative for a suitable processing quality (Biedermann-Brem et al., 2003). In order to obtain this goal, several techniques such as hot water blanching and soaking have been applied to partially extract the accumulated sugars from the raw material (Haase et al., 2003; Pedreschi et al., 2005b; Pedreschi et al., 2006; Pedreschi et al., 2007b). Yet, these adjustments also affect the colour of the fried product (Krokida et al., 2001a).

Colour is however one of the most important food product characteristics as it is the first quality parameter evaluated by the consumer, even before the food enters the mouth (Pedreschi et al., 2005b). Generally, light yellow French fries are preferred above darker brown ones. The colour of fried potatoes has usually been measured in units L*a*b*, which is an international standard for colour measurement, adopted by the International Commission on Illumination (Commission Internationale de l'Eclairage, CIE) in 1976. On the other hand, Agtron analysers are widely used in the food industry as a process-specific method for the quick quality control of foodstuffs, such as fried potato products (Blenkinsop et al., 2002; Prange et al., 2005; Daniels-Lake et al., 2005; Rommens et al., 2006). These reflectance abridged spectrophotometers have a large measurement area to deal with the colour measurement of foodstuffs with irregular geometry, such as French fries.

Since Maillard browning and acrylamide formation are to some extent linked, colour measurement may be used as an easy assessable and fast indicator of acrylamide. Recently,

several researchers investigated the relation between the content of this probable human carcinogen and surface colour, as evaluated by the standard CIE L*a*b* parameters. In many cases, there was a good correlation between the acrylamide content of fried potatoes and their colour (Jackson and Al-Taher, 2005; Pedreschi et al., 2005b; Pedreschi et al., 2006; Viklund et al., 2007; Pedreschi et al., 2007b). However, it was observed that this correlation was less close for large surface-to-volume material, such as potato crisps, in comparison with small surface-to-volume material, such as French fries. Data for potato crisps could thus not be extrapolated for French fries and vice versa (Taubert et al., 2004). Very extensive frying caused dark coloured foodstuffs, but lower acrylamide contents due to degradation or polymerization phenomena. The applied processing conditions were however more severe than the usual domestic and commercial practice (Taubert et al., 2004). Infusion of potato slices with different glucose and asparagine levels also broke the correlation. While both components increased the acrylamide formation, only glucose caused a significantly different product colour (Granda et al., 2005). It was moreover reported that the addition of other amino acids such as glycine to potato flakes had a marked enhancing effect on the Maillard browning, while lowering the acrylamide content (Low et al., 2006). In previous chapter, the correlation between Maillard browning and acrylamide formation was also disrupted if the initial moisture content of the potato powder mixture was different.

The objective of this chapter was to investigate the correlation between the acrylamide content and Maillard browning in French fries, using several colour measurement techniques. Similar to the previous studies, the surface colour was evaluated spectrophotometrically using the CIE L*a*b* colour parameters. In addition, a quick process-specific Agtron analyser was used, which has to our knowledge not yet been applied to investigate the link between acrylamide formation and product colour. Moreover, the absorbance of aqueous French fry extracts was determined as an inexpensive and fast way to determine the degree of Maillard browning. Fructose and glucose were added in different ratios to the raw material prior to frying, in order to evaluate the impact on the relationship between acrylamide formation and product colour.

6.3 Materials and methods

6.3.1 Reagents and chemicals

Fructose and glucose (purity > 99.9%) were supplied by Acros Organics, Belgium. All reagents and chemicals used for the acrylamide and sugar analysis are described under section 2.3.1.

6.3.2 Raw material

Potatoes (*Solanum tuberosum* L.), var. Bintje, were used throughout the study. A series of experiments was performed with tubers from the 2005 harvest, which were sampled in April 2006 which is already quite late in the storage period (potato *A*). For the subsequent tests, potatoes from the 2006 harvest (potato *B*) were sampled in December 2006. In addition, the experimental outcome was compared with frying tests performed on potatoes, sampled and fried in November 2005 (potato *C*). For all experiments, potato strips with cross section of 1 cm x 1 cm were sampled from an industrial production line just after a blanching step of 5 min in water at 80°C.

6.3.3 Preparation of French fries

Blanched potato strips were soaked for exactly 5 min in tap water or solutions containing different ratios of glucose and fructose. The potato/solution ratio was deliberately kept constant and low (< 0.05 w/v). After superficially drying on a paper towel, about 180 g of potato strips were par-fried for 70 s at 165°C in a 5 L semi-professional deep-fryer, equipped with a stirring mechanism and digital thermometer as described under section 2.3.4 (Figure 2.1 B). Finish frying was carried out at several times and temperatures, as mentioned below. Directly after frying, the French fries were cooled on an absorbing paper and the surface colour was measured. Finally, the fries were thoroughly homogenized for subsequent acrylamide analysis.

6.3.4 Acrylamide analysis

Acrylamide was determined in homogenized potato sample by LC-MS/MS as described under section 2.3.2.

6.3.5 Sugar analysis

Mono- and disaccharides in homogenized potato sample were assessed by GC analysis as described under section 2.3.7. The results are expressed on dry matter content (section 2.3.6).

6.3.6 Colour evaluation techniques

The colour of the French fries was measured using a Konica Minolta spectrophotometer CM-2500d (Konica Minolta, Osaka, Japan), operating in the CIE L*a*b* colour space. The colour is defined by three orthogonal co-ordinates. L* is the lightness component, which ranges from zero (black) to 100 (white). Parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -120 to 120 (Hunt, 1991). Immediately after cooling down, the French fries were closely aligned in a plastic Petri dish. The readings were carried out in fifteen-fold placing the instrument measuring port (8 mm aperture) at both sides of the dish. In such a way, the reflectance of a representative part of the surface was measured. To exclude variable cover surface conditions, the specular reflectance was included in the colour measurement. Following measurement conditions were applied: UV 100%; standard illuminant D65 and observer angle 10°. The instrument was calibrated with a white calibration tile and black calibration box. Data acquisition was performed using the Spectramagic NX colour data software, version 1.52 (Osaka, Japan). In addition, the total colour change (ΔE) was calculated by following formula $\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2}$. The $L_0^* a_0^* b_0^*$ values correspond to the raw potato strips while the L*a*b* values correspond to the measurements of the different fried products.

The colour was also evaluated using an Agtron process analyser (model E15-FP, Nevada, US). Agtron values range from 0 (black) to 100 (white). The measurement was repeated three times by shaking up the fries in between each measurement. The apparatus was calibrated using a white tile (Agtron value of 100), according to manufacturer's instructions.

Finally, the extent of Maillard browning was evaluated measuring the absorbance of an aqueous French fry extract, containing the water-soluble melanoidins, similar to section 5.3.6. This extract was obtained after 20 min of shaking, centrifugation and ultrafiltration and the extent of browning was measured at 420 nm using a Varian Cary 50 Bio spectrophotometer (Mulgrave, Victoria, Australia) (Ajandouz and Puigserver, 1999; Martins and van Boekel, 2003). The samples were diluted to obtain absorbance values between zero and one. The

results are expressed in relative absorbance, being the absorbance of the clear extract divided by the mass of fried potato sample (about 1 g).

6.3.7 Statistical analysis

The repeatability of both the acrylamide analysis and the frying procedure was evaluated in chapter 2, yielding a RSD of 15% which was used to calculate the 95% confidence intervals. Curve fitting was performed using SigmaPlot 2000 for Windows (SPSS inc., Chicago, IL, US).

6.4 Results and discussion

6.4.1 Reducing sugar content

Since blanched potato strips were sampled from an industrial production line, a large homogeneous batch of raw material was obtained to perform each frying experiment in a repeatable manner. After soaking the potato strips in different solutions, the sugar and dry matter content were determined (Table 6.1).

Table 6.1. Sugar content (% on dry matter) of blanched potato strips after different soaking treatments

soaking treatment after blanching and before par-frying	**potato *A***			**potato *B***			**potato *C***		
	fructose	**glucose**	**sucrose**	**fructose**	**glucose**	**sucrose**	**fructose**	**glucose**	**sucrose**
water (reference)	0.39	0.61	0.56	0.20	0.32	0.29	0.17	0.24	0.33
glucose solution (10 $g.L^{-1}$)	0.40	0.94	0.59	0.19	0.72	0.28	-	-	-
fructose solution (10 $g.L^{-1}$)	-	-	-	0.75	0.33	0.19	-	-	-
fructose + glucose solution (4.34 + 5.66 $g.L^{-1}$)	-	-	-	0.50	0.64	0.20	-	-	-

-: experiments not performed

The sugar content in potato *A* was higher than in potatoes *B* and *C*, after soaking in water. This can be explained among other things by the fact that the storage period between harvest and processing of potato *A* was much longer compared to potatoes *B* and *C*. Due to senescent sweetening, it is known that potatoes accumulate sugars upon prolonged storage. Other factors, such as fertilization and seasonal variation might also have contributed to this

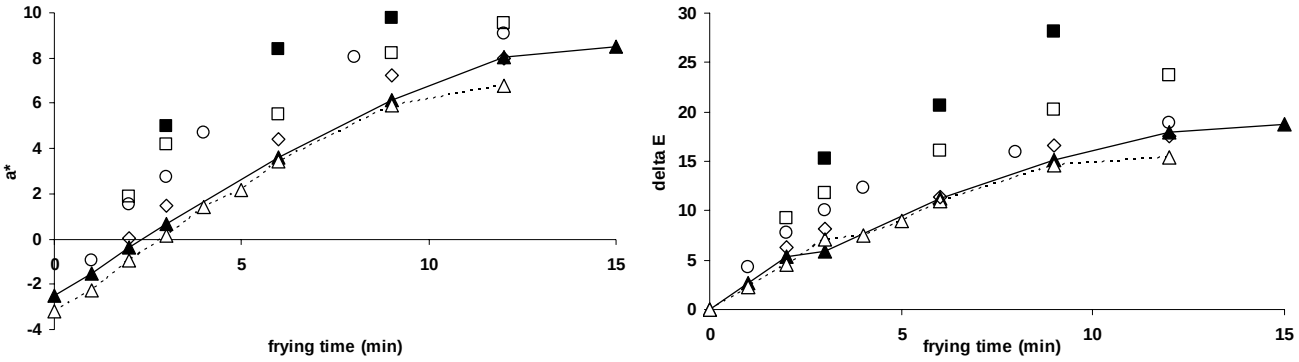
different raw material composition (Blenkinsop et al., 2002; De Wilde et al., 2005; De Wilde et al., 2006b).

In order to evaluate the impact of additional sugars on the relationship between Maillard browning and acrylamide formation, potato strips were soaked in glucose and fructose solutions, each with a total sugar concentration of 10 $g.L^{-1}$. The impact of a soaking solution containing both reducing sugars was also evaluated. For the latter, a glucose/fructose ratio of 1.3 (w/w) was selected, similar to the glucose/fructose ratio which was measured in previous research on Bintje potatoes (De Wilde et al., 2005; De Wilde et al., 2006a; De Wilde et al., 2006b). This value was moreover similar to the ratio, determined in the water soaked potato strips, as shown in Table 6.1. The glucose/fructose ratio is however subjected to some variation depending on the pre- and post-harvest enzymatic activity inside the tuber and on the tuber variety (Burton, 1989b; Hertog et al., 1997a). From Table 6.1, it can be observed that the sugar content in the potato strips increased and that the final glucose/fructose ratio differed depending on the used soaking solution.

6.4.2 Colour formation as a function of deep-frying time

After the soaking treatments, the potato strips were par-fried for 70 s at 165°C, followed by finish-frying at 170°C, common heating conditions applied for French fry preparation. In order to obtain final products with a different degree of browning, the finish-frying step was performed at several heating times, ranging from 0 to 15 min. Figures 6.1 to 6.3 show the change in final product colour as evaluated by the three colour measurement techniques, described above.

As shown in Figure 6.1, the parameter a* increased almost linearly as a function of frying time but tended to level off upon prolonged frying. Accordingly, the strips became more red as a result of the ongoing non-enzymatic browning reaction. On the other hand, the components L* and b* only changed slightly (results not shown). The lightness (L*) component decreased upon increasing frying time, meaning that the surface colour became darker. The b* value increased slightly, as a result of the more pronounced yellow tint. Similar to the parameter a*, the total colour difference, expressed as delta E (ΔE), also levelled off for longer frying times (Figure 6.1).



(I) **(II)**

Figure 6.1. Evolution of colour parameters a* (**I**) and delta E (**II**) as a function of frying time, for potatoes *A* and *B*, soaked in water (*A*: ▲, —; *B*: Δ, ---), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

Between potatoes *A* and *B* a similar trend can be observed as a function of frying time. The difference in colour between the applied soaking treatments is however striking. Soaking during 5 min in a 10 g.L^{-1} glucose solution dramatically accelerated the Maillard browning, as can be observed from a faster increase in redness (a*) and in ΔE as a function of frying time (Figure 6.1). Also the product lightness (L*) decreased more rapidly. However, the impact on the b* value was not univocal: soaking in glucose solutions did not lead to a consistent change in b* value (results not shown). These results confirm earlier findings, where potato slices were soaked in glucose solutions prior to frying (Granda et al., 2005). This increased Maillard browning effect was however not so obvious in fried potato strips which were soaked in glucose/fructose solutions (ratio 1.3). When glucose was fully replaced by fructose (10 g.L^{-1}), almost no difference in parameters a* and ΔE was noticeable compared to the water-soaked potato strips after frying. This is remarkable, since fructose is known as a more reactive reducing sugar compared to glucose in the Maillard reaction (Márquez and Añón, 1986). Consequently, a more pronounced browning was expected for the fructose-treated French fries.

Furthermore, the final product colour was evaluated using the Agtron process analyser (Figure 6.2). This spectrophotometer determines the ratio of product surface reflectance between two spectral modes, being near-infrared and green. The manufacturer selected specifically these spectral modes since the green colour is correlated with the Maillard reaction, similar to the parameter a* which is proportional to the greenness (or redness) (Granda et al., 2005). The narrow band measured inside the near-infrared spectrum was selected for its relationship with

thermal decomposition of sugars, according to the manufacturer. Recently, several studies were indeed published linking near-infrared spectra with reducing sugar content in potatoes (Mehrübeoglu and Coté, 1997), carbohydrate degradation (Claude and Ubbink, 2006), the development of some Amadori products (Yaylayan and Huyghuesdespointes, 1994) or even acrylamide formation (Segtnan et al., 2006).

A decrease in Agtron value was observed upon increasing frying time, as the French fries became darker coloured (Figure 6.2). This decrease tended to level off upon prolonged frying. Similar Agtron values were obtained for both potatoes *A* and *B*. Soaking in glucose solutions again speeded up the Maillard browning reaction. This effect was more pronounced in glucose compared to the fructose treated potato strips. Consequently, the conclusions are similar to those from Figure 6.1. This is not surprising since both colour measurement techniques measure among other parameters the product surface redness.

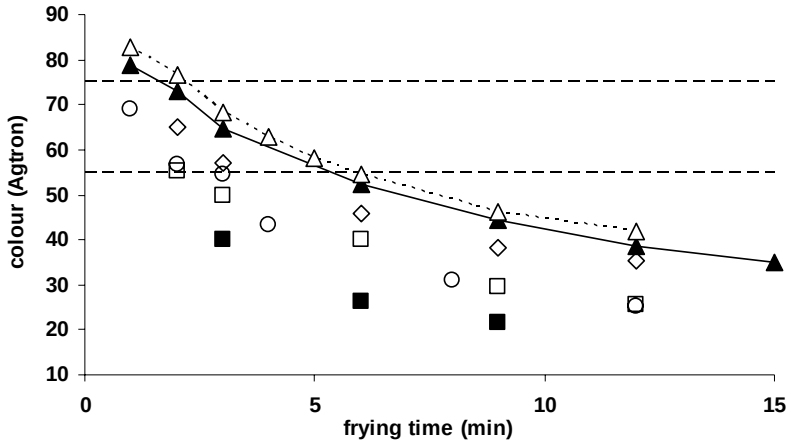


Figure 6.2. Evolution of the Agtron value as a function of frying time, for potatoes *A* and *B*, soaked in water (*A*: ▲, —; *B*: Δ, ---), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

In Figure 6.2, the dashed straight lines indicate the general limits of consumer acceptability. Generally considered, the final product colour is too bright at Agtron values above 75 and too dark below 55 (Sinha et al., 1992). Acceptable product colour could thus be obtained between 2 and 6 min finish frying at 170°C for the water-soaked potato strips, and below 3 min frying for the glucose and fructose soaked ones.

Finally, the Maillard browning of the French fries was evaluated measuring the absorbance of aqueous extracts (Figure 6.3). These extracts contain brown, nitrogenous polymers and

copolymers which are the final products of the Maillard reaction. According to the Lambert-Beer equation there is a direct linear relation between the absorbance and the concentration of these water soluble melanoidins. This technique is thus an easy, fast and low-cost alternative to the previous methods, requiring only a spectrophotometer, measuring the absorbance at 420 nm (Ajandouz and Puigserver, 1999; Martins and van Boekel, 2003). Figure 6.3 shows a linear increase in browning for increasing frying time for each soaking treatment. In contrast to Figures 6.1 and 6.2, the absorbance did not tend to stabilize upon longer finish frying. Where the previous techniques only measured the surface colour, this method evaluated the colour of the aqueous French fry extract, which is the average of both French fry surface and core browning. This could explain the different trend observed within the investigated frying time frame of 15 min.

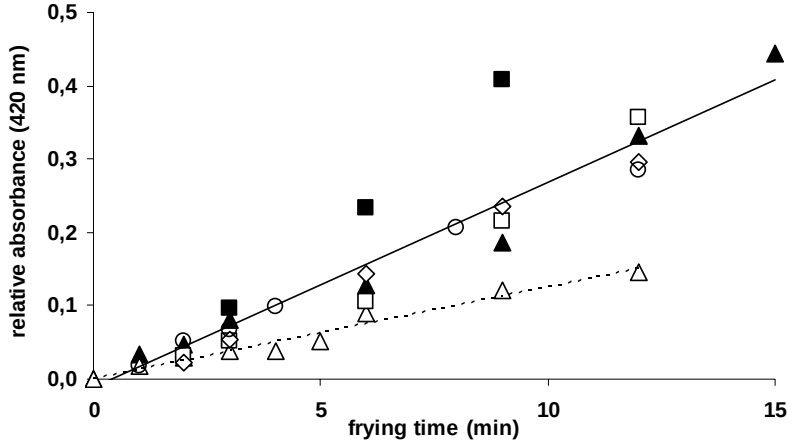


Figure 6.3. Evolution of the relative absorbance as a function of frying time, for potatoes *A* and *B*, soaked in water (*A*: ▲, —; *B*: Δ, ---), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

In Figure 6.3, the linear correlation curves for potatoes *A* and *B*, soaked in water, are plotted (R^2 = 0.97). In contrast to previous measurements (Figure 6.1 and 6.2), a different trend was observed between the two potatoes. However, as shown in Table 6.1, potato *A* had a higher reducing sugar content, due to longer storage. This could explain the difference in browning observed here. It was also observed that Maillard browning of potato strips, soaked in glucose solutions prior to frying was not consistently higher compared to the browning of those soaked in fructose, as was established in Figures 6.1 and 6.2.

6.4.3 Acrylamide formation as a function of deep-frying time

Figure 6.4 presents the acrylamide formation in the French fries for the different frying and soaking treatments. To keep clear view on the results, the 95% confidence intervals (error bars) are only indicated for the water-soaked potato strips. A linear increase in acrylamide content was assessed as a function of frying time ($R^2 \geq 0.98$). The acrylamide content in French fries prepared from potato *B* was however lower than in those originating from potato *A*, after soaking in water. This could be explained by the fact that last-mentioned contained more reducing sugars after soaking (Table 6.1). A parallel trend was already observed in the brown-coloured aqueous extracts, as shown in Figure 6.3.

From Figure 6.4, it can also be observed that the acrylamide formation was promoted by the previous soaking in both fructose and glucose solutions. It is moreover remarkable that the acrylamide content in fried fructose-soaked potato strips was comparable to glucose-soaked ones. This indicates a discrepancy between Maillard browning of the product surface (Figures 6.1-6.2) and acrylamide formation, upon addition of different reducing sugars to the raw material.

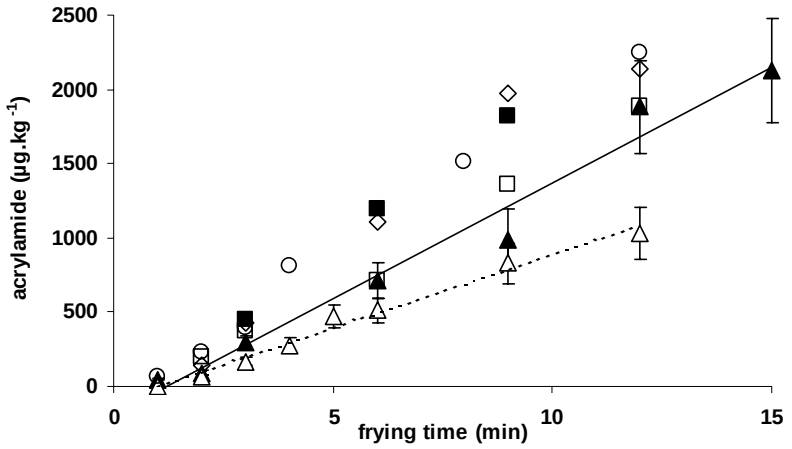
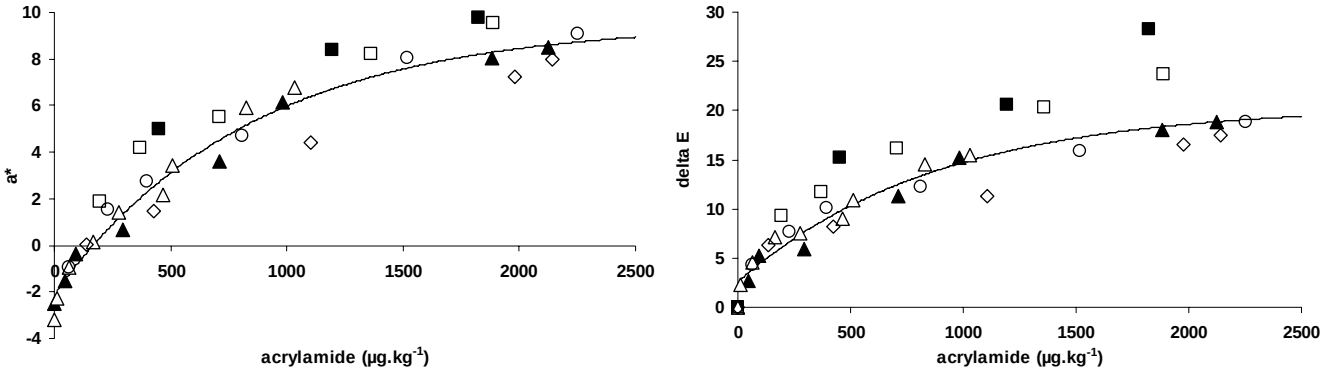


Figure 6.4. Acrylamide formation as a function of frying time, for potatoes *A* and *B*, soaked in water (*A*: ▲, —; *B*: Δ, ---), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

6.4.4 Link between colour and acrylamide formation

As shown in Figure 6.5, an exponential relationship was established between acrylamide formation and the a* and ΔE colour parameters, yielding a correlation coefficient (R^2) of 0.98 and 0.97 respectively. To construct this curve, only French fries (both potatoes *A* and *B*) previously soaked in water, were used. The potato strips, soaked in glucose-containing solutions, were generally situated above the fitted curve, while the strips submerged in fructose solutions were below the curve. The strips, soaked in the solution having a similar glucose/fructose ratio compared to the ratio naturally present in the tuber, had a similar relationship between colour and acrylamide formation as the strips soaked in water. With these observations, it could be concluded that glucose addition promoted the Maillard reaction to a higher extent than the acrylamide formation. The effect of the added fructose was exactly the opposite. The glucose/fructose ratio of the raw material had thus an impact on the relationship.



(I) **(II)**

Figure 6.5. Link between acrylamide content and colour parameters a* (**I**) and delta E (**II**) for potatoes *A* and *B*, soaked in water (*A*: ▲ & *B*: Δ, —), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

The link between acrylamide content and the colour parameters b* and L* (results not shown) was not univocal. Yet, Pedreschi et al. (2006; 2007b) found a linear correlation between acrylamide and the colour parameters L*, a* and ΔE in French fries. Smaller potato strips were however used (0.8 x 0.8 cm), in combination with several treatments such as immersion in citric acid and pyrophosphate solutions, which may have had an additional impact on the colour – acrylamide correlation. Jackson and Al-Taher (2005) found a linear relationship between the a* and L* values and the log of acrylamide levels, while no correlation was found with b*.

The relationship between acrylamide formation and the Agtron colour measurements is shown in Figure 6.6. In order to further elucidate the relationship, the results of another series of experiments, performed within a preceding investigation, were also included in the figure (potato *C*). In last-mentioned experiment, blanched potato strips were finish-fried at different oil temperatures (120-180°C) and frying times (1-10 min). In the framework of this study, only the Agtron colour parameter and acrylamide content were determined after frying. Between the Agtron values of the French fries (potatoes *A*, *B* and *C*), soaked in water prior to frying, a good exponential correlation was established ($R^2 = 0.96$), similar to Figure 6.5. Here, potato strips soaked in pure fructose solutions showed a quite similar relationship between Agtron colour and acrylamide formation. Addition of glucose on the other hand clearly broke the relationship, even in the glucose/fructose treated potato strips. Due to glucose addition, darker coloured French fries were produced for a certain acrylamide contamination.

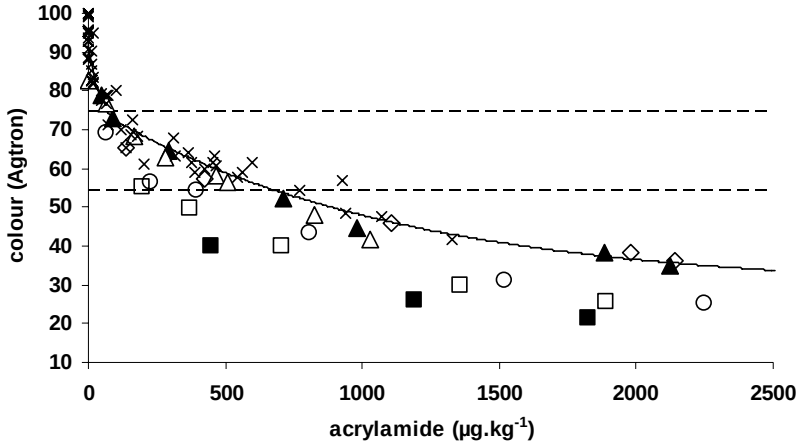


Figure 6.6. Agtron colour change as a function of acrylamide for the potatoes *A*, *B* and *C*, soaked in water (*A*: ▲, *B*: Δ & *C*: x, —), in 10 g.L^{-1} glucose (*A*: ■; *B*: □), in 10 g.L^{-1} fructose (*B*: ◊) and in 10 g.L^{-1} glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

As in Figure 6.2, the limits of product acceptability are shown (Agtron values between 55 and 75). It is remarkable that the acrylamide contamination in this region varied between 60 and 650 µg.kg^{-1}. Consequently, by optimising the frying conditions (time and temperature) and the reducing sugar content, a tenfold reduction in acrylamide contamination could be obtained. However, other quality characteristics such as crispness, final dry matter and fat content should of course be taken into account as well. From Figure 6.6, it becomes clear that a small difference in Agtron value gave rise to a more pronounced increase in acrylamide formation, due to the shape of the exponential curve, especially for Agtron values below 70. A similar

phenomenon could also be observed in Figure 6.5. It is moreover not surprising that Agtron and the parameters ΔE and a* showed a similar trend as a function of acrylamide content (Figures 6.5 and 6.6), since both parameters measured among other things the greenness of the product surface, as mentioned already previously. As a result, a good linear correlation (resp. $R^2 = 0.95$ and $R^2 = 0.96$) could be determined between Agtron and respectively ΔE and a* (results not shown).

Figure 6.7 shows the relationship between the acrylamide content and the absorbance of the aqueous French fry extracts. In order to focus on the more realistic acrylamide contamination levels for French fries, only the data below 1500 $\mu g.kg^{-1}$ are shown. In contrast to Figure 6.5 and 6.6, the correlation was not exponential but linear. As mentioned previously, this could be explained by the fact that this method evaluated the colour of aqueous extracts, which represents the average both surface and core browning. Previous techniques only evaluated surface browning.

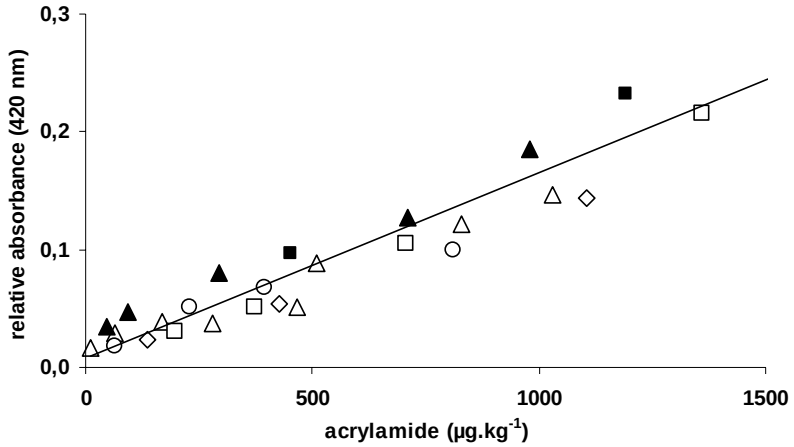


Figure 6.7. Acrylamide content as a function of the relative absorbance for potatoes *A* and *B*, soaked in water (*A*: ▲ & *B*: Δ, —), in 10 $g.L^{-1}$ glucose (*A*: ■; *B*: □), in 10 $g.L^{-1}$ fructose (*B*: ◊) and in 10 $g.L^{-1}$ glucose/fructose mixture, ratio 1.3 (*B*: o) prior to frying

Based on the French fries (both potatoes *A* and *B*) soaked in water prior to frying, a good linear regression line ($R^2 = 0.95$) was established (Figure 6.7). When the results of the fried potato strips, soaked in the different sugar solutions, were also included in the relationship still an acceptable linear correlation ($R^2 = 0.89$) was obtained (not shown in Figure 6.7). However, the variability in absorbance increased for higher acrylamide contents. From Figure 6.7, it could moreover be observed that the fructose-soaked French fries had a higher

acrylamide contamination for a particular relative absorbance compared to the water-soaked French fries. Glucose induced the opposite behaviour. This phenomenon, caused by a different glucose/fructose ratio of the raw material, was already observed by the other colour measurement methods. Nevertheless, this low-cost technique could be used as a primary, rough evaluation of acrylamide contamination in French fries, without the need for sugar or acrylamide analysis.

From above-mentioned results in French fries, an exponential relationship was demonstrated between acrylamide formation and Maillard browning, as evaluated by the parameters a*, ΔE and Agtron. Since the Agtron spectrophotometer is already widely used in the food processing industry as a method for the quick non-destructive quality control of fried potato products, its use could thus be extended for the prediction of acrylamide, besides the traditional CIE L*a*b* measurement. However, this relationship appeared to be dependent upon the glucose/fructose ratio of the raw material. Additional glucose stimulated the Maillard browning to a higher extent than the acrylamide formation. Extra fructose on the other hand promoted acrylamide formation more effectively than Maillard browning, although this last phenomenon was more obvious for the a* and ΔE parameters (Figure 6.5) compared to the Agtron measurements (Figure 6.6). On the other hand, the correlation between the relative absorbance and the acrylamide content appeared less sensitive to a variation in the glucose/fructose ratio.

6.5 Conclusion

Several factors may influence the relationship between Maillard browning and acrylamide, as discussed in the introductory section. The reducing sugar content is however the crucial parameter to follow up, since it is the limiting factor for both reactions and since its content in the raw material can vary easily by soaking in sugar-containing solutions. To improve Maillard browning, the potato processing industry applies in particular circumstances sugar soaking of blanched potato strips, similar to above-mentioned experiments. Based on the results presented in Figure 6.5 and 6.6, this soaking treatment should thus preferentially be performed with glucose, rather than with fructose. Besides these soaking treatments, the glucose/fructose ratio of the raw material can also be drastically influenced by many other sometimes less controllable pre- and post-harvest factors. This can confuse the relationship

between acrylamide formation and product surface colour. In addition to the measurement of the relative absorbance (Figure 6.7), the sugar analysis remains in this case an important instrument to predict acrylamide formation by means of the glucose/fructose ratio. This proves the relevance and the complementarity of quantitative and qualitative sugar analysis next to surface colour measurement.

Product colour is however not the only parameter for the final quality of fried products. Other factors such as texture and taste also play a major role. The following chapter further investigates these important aspects. In a first step, the effectiveness of several acrylamide mitigating treatments was studied. Subsequently, the impact of these treatments on the sensorial quality of potato crisps was evaluated.

Chapter 7

Impact of chemical pre-treatments on the acrylamide formation and sensorial quality of potato products

Redrafted after

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Chapter 7 Impact of chemical pre-treatments on the acrylamide formation and sensorial quality of potato products

7.1 Abstract

The impact on acrylamide formation of several additives was investigated as well as the mechanisms behind it. In the potato powder model system, sodium acid pyrophosphate, citric, acetic and L-lactic acid significantly reduced the final acrylamide content, due to the lowering of the pH. Free glycine and L-lysine also lowered acrylamide, while keeping the pH at its original level. Calcium ions induced a supplementary acrylamide reduction, not attributed to a lower pH. These results were largely confirmed upon dissolving the components in the blanching water of potato crisps. In contrast to the model system, also NaCl appeared to reduce acrylamide formation in the crisps, in parallel with a reduced oil uptake, suggesting that textural and compositional product changes may also influence acrylamide formation. By means of sensory analyses of these crisps, a successful combination was demonstrated between acrylamide mitigating treatments and crisps of acceptable or even superior product quality, compared to control crisps blanched in water. However, the applied components and concentration levels should be well chosen in order not to generate product-foreign flavours or undesired product colour.

Keywords: Acrylamide; pH; modelling; pre-treatments; sensory analysis; potato crisps

7.2 Introduction

Evidence of the widespread occurrence of acrylamide in fried and baked carbohydrate-rich foodstuffs has stimulated international research. As elaborated in chapter 1, these investigations have contributed to a better insight into the formation pathways, mainly originating from the Maillard reaction between asparagine and carbonyl compounds, such as reducing sugars (Stadler et al., 2004). This moreover led to an improved understanding of the impact of several pre- and post-harvest factors on the formation of this probable human carcinogen (De Wilde et al., 2005; De Wilde et al., 2006a; De Wilde et al., 2006b).

A number of raw material pre-treatments was investigated which could mitigate acrylamide formation. These techniques include the extraction of acrylamide precursors by soaking or blanching in water or in acidic solutions. The mitigating effect at low pH was attributed to protonation of asparagine amino groups. This would block the nucleophilic addition of asparagine with a carbonyl compound, preventing the formation of the corresponding Schiff base, a key intermediate in the Maillard reaction and in the formation of acrylamide (Jung et al., 2003; Rydberg et al., 2003; Kita et al., 2004; Pedreschi et al., 2004; Pedreschi et al., 2007b). Furthermore, mono- and divalent cations such as Na^+ or Ca^{2+} were indicated as efficient acrylamide reducing agents. It was postulated that these ions could interact with asparagine so that the Schiff base formation was again prevented (Park et al., 2005; Lindsay and Jang, 2005a; Lindsay and Jang, 2005b; Gökmen and Senyuva, 2007). Besides, it was suggested that NaCl accelerated the elimination of acrylamide via polymerization (Kolek et al., 2006a). The addition of proteins or free amino acids other than asparagine such as glutamine, glycine, lysine or cysteine was also studied. These components would reduce acrylamide formation by promoting competitive reactions and/or by covalently binding the formed acrylamide through Michael type addition reactions (Rydberg et al., 2003; Bråthen et al., 2005; Cook and Taylor, 2005; Kim et al., 2005; Hanley et al., 2005; Amrein et al., 2005; Claeys et al., 2005a; Low et al., 2006). Combined treatments with different amino acids (Hanley et al., 2005) or with an amino acid and organic acid (Low et al., 2006) could have an even greater potential to reduce acrylamide formation.

It is however not always clear what is the main mechanism provoking a reduction in acrylamide formation. For instance, addition of Ca^{2+} ions to potato cuts is known to provoke a pH drop, since hydrogen chloride is produced when calcium crosslinks the pectin (Hughes et

al., 1975; Andersson et al., 1994). It can thus be questioned whether the acrylamide mitigating effect in potatoes is not merely attributable to a decrease in pH. De Vleeshouwer et al. (2006) found that an increasing citrate and phosphate buffer concentration seemed to reduce the final acrylamide content, at constant pH. This effect was ascribed to the sterical hindrance caused by the buffer ions. The acrylamide-lowering effect of citric acid could thus possibly be attributed to both a pH drop and a sterical hindrance. The addition of citric acid, NaCl or $CaCl_2$ might also change the oil uptake (Bunger et al., 2003; Rimac-Brncic et al., 2004; Kolek et al., 2006a; Kolek et al., 2006b; Pedreschi et al., 2007c). Therefore this could be an additional factor, possibly influencing the formation of acrylamide in fried foodstuffs.

Unfortunately above-mentioned treatments may also have an impact on the sensorial product quality. Low pH for example also suppresses the Maillard reaction, responsible for the generation of desirable flavours and colours. Acidification may moreover result in a sour product taste (Jung et al., 2003; Kita et al., 2004; Franke et al., 2005). This effect however depends upon the applied soaking or blanching treatment and the type and concentration of the acid used. It was suggested that acetic acid would be a better acidulant for the pre-treatment of potato crisps compared to citric acid, due to the less appearing sourness (Kita et al., 2004). Addition of (sulphur containing) amino acids may also generate unpleasant off-flavours upon heating, which should be taken into account as well (Claeys et al., 2005a). Calcium chloride might improve product texture, but on the other hand can cause a bitter aftertaste (Varela et al., 2007). Consequently, these pre-treatments may also cause unwanted sensorial defects. To date few investigations have been published specifically concerning the effect on the integral sensorial properties of the final product, when mitigating acrylamide formation. To our knowledge, studies performing profound sensorial testing using taste panels have even not been reported yet in this context.

Therefore, the main purpose of this investigation was to determine the acrylamide-lowering impact of several pre-treatments and to find out whether this mitigation is merely due to pH related effects or to other factors. In addition, the impact on the sensorial quality of the most efficient treatments was evaluated on potato crisps by means of a surface colour measurement and a sensory panel.

7.3 Materials and methods

7.3.1 Reagents and chemicals

HCl (25% w/w) and petroleum ether (bp 40-60°C, Chem-Lab, Belgium) were used for the determination of the oil content. K_2CrO_4 and $AgNO_3$ (Merck, Germany) were applied for the salt content determination. NaOH (> 99% w/w) and HNO_3 (65% w/w) were used to adjust the pH in the potato powder model system. Furthermore NaCl, $MgCl_2.6H_2O$ and K_2HPO_4 were applied, all with a purity > 99% (w/w) and delivered by Chem-Lab (Belgium). $CaCl_2$ (36% w/v) and citric acid (> 99.8% w/w) were supplied by Brenntag (Belgium), while L-lactic acid (50% w/v) and calcium-L-lactate.$5H_2O$ (> 99.9% w/w) were provided by Purac Biochem (The Netherlands). In addition, two products containing calcium-L-lactate and calcium chloride were provided by Purac Biochem. The first one, named *Ca100*, contained 24.5% calcium (w/w), while the second, named *Ca200*, contained 20.8% calcium (w/w). Acetic acid was bought in retail as vinegar (7% v/v). Glycine, L-glutamine, L-lysine and L-cysteine (> 99.8% w/w) were supplied by Sigma Aldrich (Belgium). Sodium acid pyrophosphate ($Na_2H_2P_2O_7$ > 95% w/w) was delivered by Sibeco (Belgium). All products used during sensory analysis were of food grade quality. All reagents and chemicals used for the acrylamide analysis were similar as described under section 2.3.1.

7.3.2 Preparation of potato powder mixtures in tubular reactor

The artificial mixtures were prepared as described under section 3.3.3, using the same dried and sieved potato powder (Table 2.1). In short, the potato powder was mixed with water, containing different dissolved compounds, and oil in order to obtain a homogeneous mixture with a final composition of 41% potato powder, 38% water and 21% oil. Then the mixture was heated in the closed tubular reactor for 6 min at 170°C (± 1°C). With these heating conditions, acrylamide could be quantified over the whole range of pH values investigated. After heating, a quick cooling was established in an ice bath. All reported acrylamide levels are the average of at least two heating experiments.

7.3.3 Preparation of potato crisps

Potatoes (*Solanum tuberosum* L.), var. Bintje, were cut in slices of 1.5 mm thickness and washed five times under distilled water. In order to perform each frying experiment in a repeatable manner, a large homogeneous batch of raw potato slices with similar size was prepared at the beginning of each series of experiments. The slices were subsequently blanched in distilled water or in solutions containing different compounds in various concentrations for exactly 5 min at 65°C. The potato/solution ratio was 0.1 (w/w) and was deliberately kept constant. After superficially drying on a paper towel, the slices (± 50 g) were fried for 3 min at 170°C in the same deep-fryer as described under section 2.3.5. Directly after frying, the crisps were cooled on an absorbing paper and the surface colour was measured. A part of the batch was thoroughly homogenized for acrylamide analysis, while another part was used for sensory analysis.

7.3.4 pH measurement

The pH of the potato powder mixtures was evaluated by placing the pH electrode (Schott, Germany) directly into the homogenized mixture. The pH of the blanched potato slices was determined, adding 90 mL of distilled carbon dioxide free water to 10 g of homogenized potato. After 10 min incubation, the pH of the slurry was measured with the pH electrode.

7.3.5 Determination of oil content

The oil content was determined as described under section 3.3.6.

7.3.6 Determination of salt content

The salt content was measured by means of a precipitation titration with silver nitrate. Accordingly, the amount of chlorides was determined in an aqueous extract, which is correlated with the amount of sodium present (Paul and Southgate, 1978).

7.3.7 Acrylamide analysis

Acrylamide analyses were performed as described under section 2.3.2.

7.3.8 Sensory analysis

The potato crisps were subjected to a quantitative descriptive analysis (QDA), performed according to ISO 6658 (2005). Although most of the assessors were already acquainted with similar sensory analyses and were trained and selected in previous studies (Mestdagh et al., 2005; Vermeiren et al., 2006; Ragaert et al., 2006), a preliminary set of training sessions was carried out. Accordingly, 21 assessors were familiarized with the scoring system, the foodstuff to be evaluated and the sensory vocabulary. By means of potato crisps, blanched in solutions containing several amounts of acids, amino acids or salts, the relevance of each descriptor was discussed and the most important descriptive terms were retained. The degree of sourness was trained using crisps blanched in citric and acetic acid solutions. For the saltiness and bitterness, crisps blanched in respectively NaCl and $CaCl_2$ solutions were used, while for the popcorn-like taste, L-lysine was applied at several concentrations. Water-blanched crisps were used as the reference potato crisp. During each session, with about 18 assessors, this reference product was openly included as a sort of calibration standard for the assessors and as a remainder of the meaning of the previously agreed scores for each descriptor (Carpenter et al., 2000). Therefore, these scores were also indicated on the rating scales. However, to check the assessor's performance, this reference product was also included as a blind control during each session (Carpenter et al., 2000).

Product texture and taste were evaluated under IR-light in a special room with individual booths. Snap was defined as the textural perception at the first bite, while crispness was perceived upon subsequent chewing. Concerning the taste, the product sourness, saltiness and bitterness was evaluated, besides popcorn-like flavour and taste of fried potato. Each of these descriptors was evaluated using a continuous line scale with five anchor points, being 0 (absent), 2.5 (slightly present), 5 (moderately present), 7.5 (strongly present) and 10 (very strongly present). The distance between the origin and the point, indicated by the panellist was measured and standardized to scores between 0 and 10. The product acceptability was also evaluated under IR light, based on the taste only ("taste appraisal") and based on both product texture and taste ("overall appraisal"). For this hedonic evaluation, a similar five

anchor line scale was used, ranging from zero (dislike very much), over 5 (neither like nor dislike) to 10 (like very much). Consequently, a product with a score above 5 could be considered as acceptable. Water was provided to cleanse the palate in between two tests.

7.3.9 Colour evaluation

Crisp surface colour was measured using a Konica Minolta spectrophotometer CM-2500d (Konica Minolta, Japan), operating in the CIE L*a*b* colour space. Immediately after cooling down, several potato crisps were placed on a white paper. The readings were carried out in fifteen fold placing the instrument measuring port (8 mm aperture) at both sides of the crisps. In such a way, the reflectance of a representative part of the surface was measured. The measurement conditions were similar as under section 6.3.6. Crisp colour was expressed as the change in surface colour (ΔE) compared to the raw potato. ΔE was calculated by following formula $\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2}$. The $L_0^* a_0^* b_0^*$ values correspond to the raw potato slices while the L*a*b* values correspond to the measurements of the different fried products.

7.3.10 Statistical analysis

The statistical package *R* was used to perform the regression and to calculate the 95% prediction and confidence intervals, shown in Figure 7.1 (Gentleman and Ihaka, 1997). In order to properly assess the 95% confidence intervals on the acrylamide reduction factors in the potato model system, a bootstrap method was applied. The repeatability of the deep-frying procedure was evaluated in chapter 2, yielding a variation coefficient of 15%, which was used to calculate the 95% confidence intervals for the acrylamide contents in the crisps. The sensory analyses were statistically evaluated using analysis of variance (One-way ANOVA) using SPSS 12.0 (SPSS Inc., Chicago, IL, US). Significant differences among means of treatments were evaluated by the post hoc multiple comparison Tukey test with statistical significance established at $P \leq 0.05$ (O'Mahony, 1986). Pearson correlation analysis was also performed to determine linear relationships between sensory attributes.

7.4 Results and discussion

7.4.1 Influence of pH on acrylamide formation in the potato model system

The formation of acrylamide was investigated as a function of pH, heating homogeneous potato powder mixtures with different initial pH in a closed tubular reactor. The model system approached the chemical composition of French fries and produced a fairly constant pH over the whole matrix. Moreover, this closed heating methodology eliminated some variable physical and chemical factors normally occurring during the frying process, such as surface deformation, heat flux, water evaporation and oil ingress into the food, as discussed in chapters 2 to 5. The pH of the potato model system was adjusted by addition of NaOH or HNO_3 in a pH range between 3.5 and 8.

The acrylamide contents of the mixtures, heated at 170°C for 6 min, are shown in Figure 7.1. A third order mathematical model was built which correlates the pH of the mixtures to the final acrylamide content. Also the 95% confidence intervals are plotted in Figure 7.1. Acrylamide formation showed an optimum between pH 7 and 7.5, which was slightly lower compared to previous studies where an optimum pH around 8 was obtained (Rydberg et al., 2003). This small difference can possibly be explained by a distinct buffering capacity of the used potato matrices. Furthermore, a decrease in acrylamide content was noted upon acidification of the potato powder matrix, as observed earlier (Jung et al., 2003; Rydberg et al., 2003; Kita et al., 2004; Pedreschi et al., 2004).

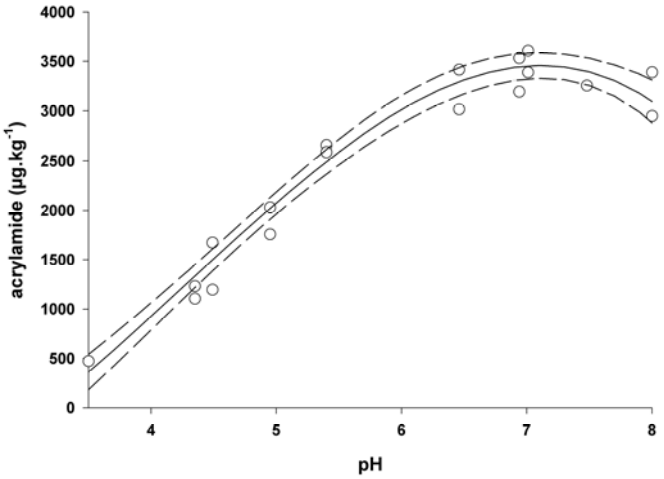

Figure 7.1. Acrylamide formation as a function of pH in the potato model system, heated at 170°C for 6 min. o: measurements; —: fitted equation; ---: 95% confidence interval on the equation

7.4.2 Influence of additives on acrylamide formation in potato model system

Subsequently, the capability of several components to reduce the final acrylamide content was investigated, upon addition to the potato powder at a level of 50, 100 or 200 µmol/g mixture. These molar levels are in the same order of magnitude as the acrylamide precursor asparagine, present in the potato powder. The compounds were added separately or in combination with other additives, as shown in Table 7.1. Again, the potato model system proved its fit-for-purpose since the added components could be mixed homogeneously throughout the entire matrix, enabling a better evaluation of their chemical impact on acrylamide formation. The pH of the mixtures was determined just before heating. In Table 7.1, the acrylamide content in each heated mixture was compared with the acrylamide level in the heated potato powder mixture to which no additives were added. This control mixture had a pH of 5.4 and an acrylamide contamination of 2492 $\mu g.kg^{-1}$. Accordingly, the impact of pH on the final acrylamide content could be evaluated. Furthermore, the acrylamide content in each mixture was compared with the acrylamide level, obtained from the mathematical model, shown in Figure 7.1, at the same pH level of the experimental mixture evaluated. Accordingly, possible additional mitigating or promoting effects, apart from the pH effect, could be determined as well (last column of Table 7.1).

As show in Table 7.1, three organic acids were evaluated, which are already used in the food industry as acidity regulator, flavouring or preservative. Addition of these acids significantly reduced the pH, in comparison with the control mixture to which only water was added. At a concentration of 100 µmol/g mixture, citric acid was the most efficient acidifier, followed by L-lactic and acetic acid, as can be explained by the acid dissociation constants. The acrylamide content followed the same trend. Comparing the acrylamide levels with the control mixture at the same pH, no significant additional decrease or increase could be detected. The additional acrylamide-lowering effect of citric acid, as suggested earlier (De Vleeschouwer et al., 2006), appeared not to be significant and could thus not be confirmed. Similarly, also the other organic acids mitigated acrylamide formation only due to their impact on the product pH, at the applied concentration levels.

Table 7.1. Impact of addition of several components at different concentrations on the pH and acrylamide content in the potato model system, heated at 170°C for 6 min

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

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

Subsequently, the impact on acrylamide formation of four free amino acids was investigated. L-lysine and glycine belong to the group causing the most intense Maillard browning. L-glutamine, structurally resembling L-asparagine, belongs to the amino acid group producing intermediate browning, while the sulphur-containing L-cysteine is known to generate the lowest browning (Ashoor and Zent, 1984; Ajandouz and Puigserver, 1999). The nucleophilic sulphur atom of L-cysteine and the amino groups of the other amino acids might readily give

rise to Michael type addition reactions with acrylamide (Stadler et al., 2004; Fennell et al., 2005). These amino acids might also reduce acrylamide formation by competing with asparagine to react with reducing sugars in the Maillard reaction. As shown in Table 7.1, these components only marginally changed the pH of the mixtures, compared to the control mixture. There was however a significant impact on the acrylamide formation. L-cysteine appeared to reduce the acrylamide content in the most effective way, with a reduction of about 92%, followed by L-lysine (39%) and glycine (24%). These results confirm previous investigations and above-mentioned hypotheses, although there were some differences in degree of reduction between different studies (Rydberg et al., 2003; Bråthen et al., 2005; Kim et al., 2005). Rydberg et al. (2003) for instance found a more efficient acrylamide reduction of glycine compared to L-lysine in a potato model system. On the other hand, Kim et al. (2005) showed quite similar reductions of L-lysine and glycine in blanched potato crisps. In a dough system, L-cysteine was even observed to be the least efficient acrylamide-lowering agent of the three. When this sulphur-containing amino acid was however applied in current experiments, unpleasant off-flavours were detected upon opening the tubular reactor, making its application in real foodstuffs intolerable and unacceptable to consumers. Therefore, this amino acid was excluded in subsequent experiments. Interestingly, L-glutamine significantly promoted the formation of acrylamide with 50% (Table 7.1). In literature, conflicting results were found for this amino acid. Several studies showed a significant decrease upon addition of free L-glutamine (Rydberg et al., 2003; Bråthen et al., 2005; Hanley et al., 2005). Similar to our results, Claeys et al. (2005a) observed an increase, although this was not attributed to a direct acrylamide formation from the amino acid. These results indicate that the efficiency of free amino acids to lower the acrylamide content may depend on the experimental set-up, such as the heating conditions or the way of applying these components to the foodstuff (e.g. by soaking or blanching or homogeneous mixing).

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

pronounced reductions in the final acrylamide content compared to the former. Comparing the acrylamide content with the control at the same pH level, it became clear that the supplementary lowering effect due to the presence of amino acids was quite similar as for the addition of pure amino acids to the model system (about 25% for glycine and 35% for L-lysine), indicating that the synergistic (citric acid) or antagonistic effects (acetic acid) were quite restricted.

Besides, the effect of several salts was evaluated in the potato model system (Table 7.1). NaCl did not appear to significantly reduce the acrylamide content, even at a concentration of 200 µmol/g mixture, which corresponds to about 1% (w/w). This is in contrast to previous investigations, where significant acrylamide reductions were obtained at this concentration level. These earlier reported experiments were however performed in an aqueous asparagine/glucose model system (Kolek et al., 2006a; Kolek et al., 2006b). Yet, in real foodstuffs, NaCl appeared to have more acrylamide-lowering properties compared to model systems (Franke et al., 2005; Gökmen and Senyuva, 2007), as further investigated below. In addition, the effect of sodium acid pyrophosphate ($Na_2H_2P_2O_7$) was evaluated. Pyrophosphate is already used in the potato processing industry to reduce darkening of the blanched potato cuts, caused by a reaction between Fe^{3+} and chlorogenic acid present in the potato cuts (Mazza and Qi, 1991). This additive lowered the pH of the mixtures and accordingly the acrylamide content with 25% (Table 7.1), but no significant additional reduction, apart from the lower pH, was assessed. In a previous study (Pedreschi et al., 2007b), no acrylamide-lowering effect at all was found in French fries, blanched in water containing sodium pyrophosphate. Yet, the pH was not monitored. Also for K_2HPO_4, no supplementary acrylamide inhibiting effect was found at the investigated concentration level (Table 7.1). In contrast, the acrylamide content increased with 40%, compared to the control mixture to which no additives were added. This was due to the increased pH, since no significant difference was assessed with the control mixture at the same pH. In a previous study, it was suggested that phosphate ions exerted an inhibiting effect on acrylamide formation, apart from the pH effect (De Vleeschouwer et al., 2006). This could however not be confirmed in the current model system at the applied concentration level.

Finally, $CaCl_2$ and $MgCl_2$ were investigated. $MgCl_2$ is already used as a coagulant and $CaCl_2$ as a firming agent during fruit and vegetable processing (Andersson et al., 1994; Khalil, 1999; Carbonell et al., 2006; Varela et al., 2007). These compounds did significantly lower the final

acrylamide content in the model system, although this effect was more distinct for $CaCl_2$ (Table 7.1). Also the pH of the model system clearly dropped, probably as a result of the ion exchange with pectic substances, being present in the potato powder (Andersson et al., 1994). However, the acrylamide-lowering effect was much more than merely a pH effect. Compared to the control mixture at the same pH, a reduction of 70% and 34% was found for the addition of $CaCl_2$ and $MgCl_2$ respectively, at 100 µmol/g mixture. In order to further clarify this, the pH drop in the potato powder was counteracted by addition of NaOH to the $CaCl_2$ containing mixture prior to heating. Still a significant difference (58%) with the control mixture at the same pH was observed, confirming the efficient acrylamide reducing effect of calcium, next to its pH-lowering effect, as was previously postulated (Park et al., 2005; Lindsay and Jang, 2005a; Lindsay and Jang, 2005b; Gökmen and Senyuva, 2007). Since calcium appeared to efficiently lower acrylamide formation, calcium-L-lactate was also investigated (Table 7.1). Similar reductions were obtained, compared to $CaCl_2$, for the 50 µmol/g mixture. In contrast to $CaCl_2$, the acrylamide content and pH did however not further drop at a calcium-L-lactate concentration of 100 µmol/g mixture.

From the experiments with the potato powder model system, it could be concluded that the addition of free glycine and L-lysine gave significant acrylamide reductions, while keeping the pH of the mixture at its original level. Addition of organic acids and sodium acid pyrophosphate also significantly mitigated the final acrylamide content, but merely due to the lower pH. Ca^{2+} and Mg^{2+} ions combined a reduction in pH with an additional acrylamide-lowering effect, previously ascribed to the binding of divalent ions to asparagine in order to prevent the Schiff base formation (Lindsay and Jang, 2005a; Lindsay and Jang, 2005b; Gökmen and Senyuva, 2007), which is a key step in acrylamide formation as discussed in chapter 1.

7.4.3 Influence of additives in blanching water on acrylamide formation in potato crisps

In a following step, the most effective acrylamide-lowering agents were evaluated on potato crisps. Prior to frying, different components were diffused into the potato slices by means of a blanching step at 65°C. From literature, it appeared that the large product surface and the applied blanching temperatures caused a more efficient acrylamide reduction compared to soaking treatments performed at lower temperatures, due to a higher diffusion of acrylamide

precursors from the potato tissue and due to a more efficient diffusion of additives into the slices (Kita et al., 2004; Pedreschi et al., 2004; Pedreschi et al., 2007b). For each treatment, the acrylamide lowering effect was determined, comparing the acrylamide content with the content in potato crisps, blanched in distilled water prior to frying (Table 7.2). The measured pH of the crisps, blanched in distilled water, was 6.7. The difference with the reference pH of the model system (5.4) could be explained by the different degree of dilution of measured sample.

In contrast to the previous experiments with the model system, these trials did not only investigate the chemical interaction of the added components with the acrylamide formation mechanism. Also other factors, continuously changing upon frying, were taken into account, such as the alteration of texture and composition. As discussed in the introduction, some of the investigated additives may also have an impact on the oil uptake, which might subsequently influence acrylamide formation. Therefore, experiments with NaCl were performed, although no acrylamide-lowering effect was observed in the potato model system (Table 7.1).

Table 7.2 shows the acrylamide-lowering effect of the blanching treatments, as well as the final oil content, the pH of the blanching solutions and of the blanched potato slices. Components were added to the blanching solutions at concentrations of 0.025, 0.05 or 0.1 M. Similar to Table 7.1, three organic acids were evaluated, being citric, acetic and L-lactic acid. At a concentration of 0.1 M, all three acids completely eliminated the formation of acrylamide. At 0.05 M, only acetic acid did not totally reduce acrylamide, due to the less pronounced pH-lowering capacity of this organic acid, as compared to the other two, as discussed above. The different pH of the potato slices also determined the acrylamide mitigating capacity at the lowest concentration level investigated in the blanching water, being 0.025 M. Moreover, the final oil content decreased about 25-30% in comparison with the control, blanched in water.

Table 7.2. Impact of several additives on the pH of blanched potato slices, on the oil content and on the acrylamide reduction, in terms of percentage compared to water-blanched crisps

components added to blanching water	concentration ($mol.L^{-1}$)	pH blanching water	pH blanched potato slices	oil content (%)	acrylamide reduction (%)[a]
water (control)		7.1	6.7	40	0
citric acid	0.1	-	-	-	100 (± 16)
	0.05	2.3	3.4	-	100 (± 16)
	0.025	2.5	4.1	25	98 (± 15)
acetic acid	0.1	-	-	-	100 (± 16)
	0.05	3.7	4.6	-	79 (± 13)
	0.025	3.8	4.8	29	80 (± 13)
L-lactic acid	0.1	-	-	-	100 (± 16)
	0.05	2.9	3.9	-	100 (± 16)
	0.025	3.2	4.8	29	89 (± 14)
glycine	0.1	-	-	-	68 (± 11)
	0.05	7.5	6.6	35	63 (± 10)
	0.025	-	-	-	58 (± 9)
L-lysine	0.1	-	-	-	85 (± 13)
	0.05	7.1	6.6	38	63 (± 10)
	0.025	-	-	-	73 (± 12)
citric acid + glycine	0.05 + 0.05	-	-	-	97 (± 15)
citric acid + L-lysine	0.05 + 0.05	-	-	-	99 (± 16)
acetic acid + glycine	0.05 + 0.05	-	-	-	81 (± 13)
	0.025 + 0.05	-	-	-	71 (± 11)
acetic acid + L-lysine	0.05 + 0.05	-	-	-	89 (± 14)
	0.025 + 0.05	-	-	-	75 (± 12)
NaCl	0.1	7.8	6.7	27	43 (± 7)
	0.05	-	-	-	28 (± 4)
$Na_2H_2P_2O_7$	0.1	-	-	-	90 (± 14)
	0.05	4.7	6.0	29	83 (± 13)
$CaCl_2$	0.1	7.2	6.5	-	93 (± 15)
	0.05	7.5	6.1	24	64 (± 10)
	0.025	-	-	-	17 (± 3)
Ca100	0.07[b]	7.7	6.5	-	82 (± 13)
	0.04[b]	7.6	6.3	23	50 (± 8)
Ca200	0.06[b]	7.7	6.7	-	72 (± 11)
	0.03[b]	7.6	6.3	23	45 (± 7)

[a] compared to potato crisps, blanched in water (control). 95% confidence intervals are mentioned between brackets

[b] molar concentration, calculated for the calcium ions

-: measurements not performed

Furthermore, the acrylamide-lowering effect was investigated in potato crisps upon addition of free glycine and L-lysine to the blanching water, at different concentration levels (Table 7.2). These additives did not markedly change the final oil content. Also the pH of the blanched slices did not noticeably differ from the control. The pH and oil content were therefore only evaluated at the 0.05 M concentration level. Similar to the experiments with the potato model system (Table 7.1), L-lysine appeared to reduce more efficiently the formation of acrylamide, although the differences were less pronounced at the 0.05 and 0.025 M concentration levels. This confirms the above-mentioned hypothesis that the efficiency of free amino acids to reduce acrylamide depends upon the experimental set-up, in this case the applied concentration level.

Similar to the experiments performed in the potato model system, several combinations of amino acids with organic acids were evaluated (Table 7.2). The oil content and pH of the blanching solutions and blanched potato slices was not measured since similar results were expected as for the addition of organic acids only. Since a total acrylamide reduction could already be obtained using only citric acid, the effect of additional amino acids was not observed. Similar to the experiments with the model system (Table 7.1), a rather antagonistic effect was observed upon addition of glycine and L-lysine to acetic acid-containing blanching water. The blanching treatment with glycine and L-lysine in combination with 0.025 M acetic acid revealed an insignificantly lower acrylamide reduction compared to the crisps, treated only with 0.025 M acetic acid (Table 7.2). Yet, the mitigation appeared to be higher in the crisps treated with acid and L-lysine, compared to the crisps blanched in acid-glycine solutions. Although no significant synergistic acrylamide reductions were realized, these combined treatments could improve product appearance, by promoting Maillard browning (Low et al., 2006), as evaluated further on.

In contrast to the experiments with the potato model system and in accordance with previous reports (Franke et al., 2005; Kolek et al., 2006a; Kolek et al., 2006b; Pedreschi et al., 2007a), NaCl significantly decreased the acrylamide content (Table 7.2). Moreover, at the highest concentration level, applied in the blanching water (0.1 M), the final salt content in the crisps (3.5 $g.kg^{-1}$ crisp) still appeared to be only half of the amount being present in salted crisps, sold in retail. Also the oil content was significantly reduced (27%) by NaCl addition to the blanching water. Furthermore, in chapter 3 and 5 it was demonstrated that acrylamide formation decreased upon lowering the oil content of the potato model system, probably due

to a lower heat transfer from the oil to the system. Therefore, a decreased oil uptake seems a plausible mechanism behind acrylamide reduction in the NaCl treated. $CaCl_2$ confirmed its acrylamide mitigating effect, although only a marginal decrease was observed at the lowest concentration level (0.025 M). Moreover, a similar decrease in oil content was observed as for the NaCl treated crisps. Also sodium acid pyrophosphate ($Na_2H_2P_2O_7$) appeared to be effective in reducing acrylamide, due to a mild reduction in pH of the raw potato slices. Also a lower oil content was observed compared to the control, blanched in water. The currently applied concentration of sodium acid pyrophosphate in industry is however only about 2.5 mM, probably leading to less pronounced reductions of the pH and acrylamide content.

Furthermore, two combinations of $CaCl_2$ and calcium-L-lactate were investigated, named *Ca100* and *Ca200*. As shown in Table 7.2, the applied molar concentrations, calculated for the calcium ions, were different compared to $CaCl_2$ since the exact composition was not known at the time of research. *Ca100* however appeared to contain 24.5% calcium (w/w), while the second one only contained 20.8% calcium (w/w). This difference in calcium dosage was also visible in the acrylamide-lowering effect. A linear correlation was even observed (R^2 = 0.86) between the percentage acrylamide reduction and the molar concentration of calcium ions, applied in the blanching solutions. The oil contents were however similar between the different calcium treatments. Since there was less calcium present in the *Ca200* product, acrylamide was less efficiently reduced compared to the *Ca100* product, although the differences were not significant. In contrast to the potato model system (Table 7.1), no clear reduction in pH was observed for the blanched potato slices (Table 7.2).

A reduced oil absorption was thus assessed for the crisps treated with acids, NaCl or calcium containing components, as was reported previously (Khalil, 1999; Bunger et al., 2003; Rimac-Brncic et al., 2004; Kolek et al., 2006a). From earlier studies (Aguilar et al., 1997), low-temperature blanching also appeared to lower the final oil content of deep-fried products. These processes or additives may change important textural characteristics such as the product surface roughness and crust porosity, leading to a different oil uptake (Aguilar et al., 1997; Khalil, 1999) and eventually also to different acrylamide contents, as clearly shown for NaCl. Furthermore, it is known that the oil is mostly absorbed at the end of the frying process (Ufheil and Escher, 1996; Bouchon et al., 2003; Miranda and Aguilera, 2006). Interestingly, this is also the stage during which significant acrylamide formation takes place, as the surface is drier, allowing the temperature to rise far above 100°C (Grob et al., 2003; Jackson and Al-

Taher, 2005). Consequently, besides chemical interactions with the acrylamide formation mechanisms, some additives may also change structural properties of the potato tissue, which may lead to a different oil uptake and concomitantly altered heat transfer and acrylamide formation in the foodstuff.

7.4.4 Brief sensorial appraisal of potato crisps

In order to evaluate the sensorial acceptability of the potato crisps and before serving these snacks to a sensory panel, the authors tasted the crisps mentioned in Table 7.2. Sodium acid pyrophosphate was not of food grade quality and consequently not evaluated. This brief appraisal learned that the crisps, blanched in water, were quite tasty. Other potato crisps were however of unacceptable quality. Yet, for each component, it was the objective to combine significant acrylamide mitigation with acceptable sensorial quality.

The organic acids and especially citric acid rendered the product quite sour at the highest concentrations applied. Since acrylamide was also very efficiently reduced at the lowest concentrations (0.025 M), this concentration level was selected for a more extensive evaluation by the sensory panel. This concentration was also chosen for a combined treatment of acetic acid with the amino acids glycine and L-lysine.

Potato crisps with added glycine and L-lysine were of acceptable quality. However, at a concentration of 0.1 M, an unusual popcorn-like taste was perceived for the L-lysine-treated crisps. Since this taste is not associated with potato crisps by the consumer, the crisps at a lower concentration level of 0.05 M glycine and L-lysine were retained for further sensory evaluation, providing still a significant acrylamide reduction of 63% compared to the water-blanched control (Table 7.2).

For NaCl, the highest concentration level was selected for further analysis, since the salty taste is product-familiar. It moreover led to a significant acrylamide reduction (Table 7.2). At 0.1 M, a bitter aftertaste was however perceived in the $CaCl_2$ treated products, which was much less pronounced at 0.05 M. This concentration level was consequently retained for more elaborate sensory analysis, as discussed below. Similarly, *Ca100* and *Ca200* were also included at concentrations of 0.04 and 0.03 M calcium.

7.4.5 Extensive sensory evaluation of potato crisps by taste panel

A sensory panel evaluated the selected potato crisps, based on several descriptors linked with product texture and taste (Table 7.3). Water-blanched crisps were provided and indicated as the reference product. These so-called control crisps were also included as a blind control during each session. The scores attributed by the panellists to this blind control were not significantly different from the previously agreed scores for this product, indicating the consistent use of the rating scales (Carpenter et al., 2000). The overall appraisal of these crisps was 5.5, which is above the limit of acceptability, being 5. However, it has to be mentioned that the sensory quality of these crisps could not directly be compared with commercial crisps, since no flavourings were added to the evaluated products, as is usually done.

Concerning the addition of acids, the sensory panel detected a sour taste only for the crisps, blanched in 0.025 M citric acid (Table 7.3). Apparently, this sourness also led to a significantly lower perception of the fried potato taste. Consequently these crisps were rejected, as can be observed from the low taste and low general appraisal scores, which were significantly lower compared to the control. Yet, the crisps treated with acetic and lactic acid were not significantly different from the control, as was the case for the crisps blanched in 0.05 M glycine or L-lysine. These products scored even higher on taste and overall appraisal, although not significantly compared to the control. Addition of NaCl led to a more salty product taste, but this did not change the appraisal scores, compared to the control.

For the potato crisps, blanched in solutions containing both acetic acid and glycine or L-lysine, no sour taste was perceived, similar to crisps blanched in acetic acid only (Table 7.3). The crisps treated with acetic acid and glycine were not significantly different compared to the control. However, for crisps blanched in water containing acetic acid and L-lysine, an unpleasant popcorn-like taste was perceived, which is unfamiliar for potato crisps. Moreover, this taste suppressed the fried potato taste. Therefore, this product was clearly rejected by the panellists.

Table 7.3. Mean sensory scores (evaluated by taste panel) and surface colour (evaluated with spectrophotometer) of potato crisps, having experienced blanching treatments in water to which several components were added

components added to blanching water	concentration ($mol.L^{-1}$)	texture		taste					taste appraisal	overall appraisal	surface colour (ΔE)
		snap	crispness	potato	sour	salt	bitter	popcorn			
water (control)		6.7^{a}	6.8^{a}	6.6^{bc}	0.0^{a}	0.2^{a}	0.0^{a}	0.1^{a}	5.7^{bc}	5.5^{bcd}	7.9^{bc}
citric acid	0.025	7.6^{abc}	7.5^{abc}	2.6^{a}	5.5^{b}	0.0^{a}	0.1^{a}	0.3^{a}	1.7^{a}	1.8^{a}	6.5^{c}
acetic acid	0.025	8.1^{abc}	8.3^{abc}	4.6^{ab}	0.2^{a}	0.0^{a}	0.5^{a}	0.0^{a}	5.6^{bc}	5.2^{bc}	7.4^{c}
lactic acid	0.025	8.3^{abc}	8.5^{abc}	6.3^{bc}	0.3^{a}	0.0^{a}	0.1^{a}	0.0^{a}	5.3^{bc}	5.3^{bc}	11.4^{ab}
glycine	0.05	7.7^{abc}	7.6^{abc}	6.3^{bc}	0.0^{a}	0.5^{a}	0.5^{a}	0.0^{a}	5.9^{bc}	6.0^{bcd}	12.0^{ab}
L-lysine	0.05	7.9^{abc}	8.0^{abc}	6.7^{bc}	0.0^{a}	0.2^{a}	0.3^{a}	0.2^{a}	6.6^{c}	6.6^{cd}	14.4^{a}
acetic acid + glycine	0.025 + 0.05	7.8^{abc}	7.9^{abc}	6.8^{bc}	0.0^{a}	0.2^{a}	0.4^{a}	0.1^{a}	6.5^{c}	6.8^{cd}	8.3^{bc}
acetic acid + L-lysine	0.025 + 0.05	8.3^{abc}	8.4^{abc}	3.5^{a}	0.1^{a}	0.2^{a}	0.1^{a}	6.1^{b}	4.2^{b}	4.3^{b}	11.8^{ab}
NaCl	0.1	7.3^{ab}	7.3^{ab}	6.3^{bc}	0.3^{a}	3.8^{b}	0.1^{a}	0.3^{a}	5.6^{bc}	5.7^{bcd}	9.9^{abc}
$CaCl_2$	0.05	9.1^{c}	9.1^{c}	7.0^{c}	0.0^{a}	0.6^{a}	0.5^{a}	0.1^{a}	7.3^{c}	7.5^{d}	8.3^{bc}
Ca100	0.04	9.1^{c}	8.9^{bc}	7.3^{c}	0.2^{a}	0.6^{a}	0.2^{a}	0.0^{a}	7.0^{c}	7.0^{cd}	9.8^{abc}
Ca200	0.03	8.7^{bc}	9.0^{bc}	6.5^{bc}	0.0^{a}	0.7^{a}	0.0^{a}	0.1^{a}	7.1^{c}	7.2^{cd}	8.0^{bc}

Different letters in the same column indicate significant difference ($P < 0.05$) by Tukey test

The addition of calcium clearly provoked a more crispy texture, as could be concluded from the significantly higher snap and crispness scores for $CaCl_2$, *Ca100* and *Ca200*, compared to the control crisps (Table 7.3). This was appreciated by the panellists as could be observed from the higher appraisal scores, around 7. The bitter aftertaste, which was perceived by the authors during preliminary sensory evaluation of crisps treated with 0.1 M $CaCl_2$, was not detected by the panellists at the lower molar concentration.

Table 7.4 shows the correlation between the descriptors, used by the panellists to evaluate the crisps. A significant correlation was found between both textural descriptors, snap and crispness, as well as between the taste and general appraisal. From this table, it can be concluded that the product crispness, snap and fried potato taste were positively correlated with the taste and overall appraisal. Sourness showed a negative correlation with the product appreciation parameters, as well as with the fried potato taste. The latter was also the case for bitterness and popcorn-like flavours. Apparently, these foreign flavours induced the suppression of the regular taste of fried potatoes, leading to unacceptable final product quality.

Table 7.4. Correlation coefficients between potato crisp descriptors

	snap	**crispness**	**potato**	**sour**	**salty**	**bitter**	**popcorn**	**taste appraisal**
crispness	0.82**	1						
potato	0.11	0.12	1					
sour	-0.08	-0.11	-0.45**	1				
salty	-0.02	-0.04	0.02	-0.10	1			
bitter	0.06	-0.03	-0.05	-0.05	-0.12	1		
popcorn	0.07	-0.07	-0.27**	-0.03	-0.05	-0.06	1	
taste appraisal	0.16*	0.19*	0.56**	-0.58**	0.09	-0.03	-0.23**	1
overall appraisal	0.26**	0.27**	0.59**	-0.57**	0.10	-0.04	-0.20**	0.92**

* significant correlation at the 0.05 level
** significant correlation at the 0.01 level

7.4.6 Colour evaluation of potato crisps

The sensory analyses were performed under IR light, in order to exclude bias due to product colour. The surface colour was measured by means of a spectrophotometer. The results, also presented in Table 7.3, are expressed as the difference in colour compared to raw potato tissue (ΔE). Accordingly, a higher ΔE value corresponded to a darker and browner product.

The crisps, blanched in water containing citric or acetic acid appeared to be brighter compared to the control, although the difference was statistically not significant (Table 7.3). Interestingly however, crisps treated with L-lactic acid were significantly browner compared to the crisps treated with acetic or citric acid. Apparently, the L-lactic acid did not counteract but even promoted to some extent the browning. This seems unusual since the pH of the blanched potato slices was lowered to 4.8 by addition of L-lactic acid, as compared to the slices blanched in water (Table 7.2). Consequently, a reduced Maillard browning was expected. Yet, the difference in colour between the control crisps, blanched in water was not significant.

When free glycine or L-lysine were added to the blanching water, a darker coloured product was obtained upon frying, compared to the control (Table 7.3). This difference was mostly pronounced for L-lysine. This is not surprising since L-lysine is known as a very reactive amino acid in the Maillard reaction, due to the α- and ε-amino groups (Ajandouz and Puigserver, 1999; Becalski et al., 2003). Also in combination with acetic acid, L-lysine appeared to induce more browning compared to glycine. Moreover, at the applied concentrations, both amino acids could counteract the inhibition of browning, caused by addition of acetic acid.

NaCl promoted to some extent the surface browning, although not significantly compared to the control (Table 7.3). Also the addition of $CaCl_2$, eventually in combination with calcium-L-lactate, did not significantly change the surface colour, compared to the control. Previously, it was also shown that addition of NaCl and $CaCl_2$ to potato strips did not change the surface colour upon subsequent frying (Bunger et al., 2003; Franke et al., 2005; Gökmen and Senyuva, 2007), although other studies found a reduced browning upon addition of NaCl (Santis et al., 2007; Pedreschi et al., 2007a; Pedreschi et al., 2007c).

From the colour measurements, it could thus be concluded that the addition of citric and acetic acid produced brighter coloured crisps, in contrast to L-lactic acid. Addition of glycine or L-lysine gave rise to more browning upon frying. This could counteract the browning-inhibiting effect of acetic acid. $CaCl_2$, calcium-L-lactate or NaCl did not significantly change product colour, compared to the control blanched in distilled water. However, it has to be mentioned that it is not always the goal to produce crisps with a darker colour. Therefore, the addition of free amino acids could be more useful in the production of French fries, where a too pale colour is generally not wanted.

Furthermore, no correlation was found between browning (ΔE) and the acrylamide content in potato crisps. The L-lysine or glycine treated crisps were quite dark coloured, but had a lower acrylamide contamination compared to e.g. crisps, blanched in NaCl. This discrepancy was already discussed in previous studies upon applying several additives to fried potato products (Low et al., 2006). Yet, when no additives were used, it was shown in previous chapter that the reducing sugar content and type of sugar also play a major role in the relationship between Maillard browning and acrylamide formation.

7.5 Conclusion

The challenge of this study was to find strategies to minimize acrylamide formation while maintaining the expected product quality for the consumer. The results revealed that both aspects are closely linked to each other. An integrated approach is thus required, in order to combine the knowledge how to reduce acrylamide with the corresponding product-specific quality aspects, such as colour, texture and taste.

Several additives efficiently lowered the final acrylamide content, following different mechanisms. The amino acids L-lysine and glycine, and calcium ions appeared to chemically interfere with the acrylamide precursors, limiting the formation of acrylamide both in the model system and upon crisp preparation. Besides, the acids, NaCl and calcium-containing additives exerted a lowering impact on oil absorption, which may have led to a lower heat transfer and acrylamide contamination in the final product. This was specifically observed for the experiments with NaCl. No reduced acrylamide formation was observed in the potato model system, where the oil content was kept constant. However, NaCl reduced the oil content in the crisps, and concomitantly the final acrylamide content. Consequently, a reduced oil uptake in snack products would in parallel lead to lower acrylamide formation. This would perfectly fit within the ongoing consumer trend to move towards healthier and low-fat products in order to counteract obesity and coronary heart diseases (Mellema, 2003; Minihane and Harland, 2007).

Organic acids reduced acrylamide formation by lowering the pH of the foodstuff. Citric acid appeared to render the product more sour and thus less acceptable as compared to L-lactic and acetic acid, at the applied concentrations. Addition of citric and acetic acid also seemed to inhibit Maillard browning, which could be counteracted by the addition of amino acids. Here, care should however be taken in order not to produce product-foreign flavours.

Although not evaluated in this study, possible off-flavours, off-tastes or too pale crisps, occurring as a result of acrylamide-lowering additives such as citric acid, could in some cases be covered up using flavourings. Except for specially marketed low sodium crisps, salt is added at about 1-2% on final weight just before packaging. In order to make the product more appealing to consumers, other flavourings, such as *paprika*, *barbecue* or *onion* are also applied (Mottur, 1989; Reyes and Barringer, 2005). These crisp flavours usually are specific mixtures of natural spices and natural or artificial flavours and could thus (further) improve sensorial product quality.

However, not only the acrylamide-lowering capacity of above-mentioned pre-treatments should be considered. Besides, other reactions in the fried product might be stimulated, which may negatively influence product safety. The addition of salts such as NaCl and $CaCl_2$ prior to the frying step appeared to increase the rate of oil degradation upon subsequent frying (Mehta and Swinburn, 2001; Padilla, 2005). In a previous study, blanching of potato strips in calcium acetate also appeared to negatively influence the oil quality upon prolonged frying (Mazza and Qi, 1992). This would lead to a more rapid oil turnover rate in the industrial frying installation, causing extra production costs. Although the final salt content in the crisps, treated with NaCl, was still half of the amount found in crisps sold in retail, addition of extra salt (NaCl) during processing may increase the salt consumption, which has been linked to increased development cardiovascular diseases (O'Shaughnessy and Karet, 2006). Furthermore, washing and blanching causes important losses of ascorbic acid due to thermal degradation and diffusion into the blanching water (Haase and Weber, 2003). Blanching in water, recycled from the production process, could however increase the ascorbic acid retention to some extent (Arroqui et al., 2002).

After the evaluation of several factors, possibly mitigating the formation of acrylamide in potato products, the last chapter investigates the dietary intake of this probable human carcinogen. To decrease the exposure to acrylamide it is namely important to identify the major sources, contributing to the daily intake. In this context, the role of out of home eating is also studied. Furthermore, it is important to evaluate the impact of acrylamide-lowering strategies against the overall exposure. Finally, the impact of creative alternatives, such as increased availability of fruit and vegetables, to lower the intake is investigated as well.

Chapter 8

Importance of a Belgian canteen lunch on the dietary intake of acrylamide

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Chapter 8 Importance of a Belgian canteen lunch on the dietary intake of acrylamide

8.1 Abstract

A food and drink intake survey was carried out among university students and staff members. Consumption data were collected on days when the participants took a hot lunch in a Belgian university canteen. The dietary acrylamide exposure was calculated through a probabilistic approach and revealed a median intake of 0.40 μg.kg $bw^{-1}.day^{-1}$ [90% confidence interval: 0.36 – 0.44], which is in accordance with previous exposure calculations. Biscuits (35%), French fries (30%), bread (24%) and chocolate (11%) were identified to be the main sources of dietary acrylamide. Foodstuffs consumed in between the three main meals of the day (so called snack type foods) contributed the most to the intake (42%). The exposure was lower in an intervention group which received free portions of fruit and vegetables, indicating that a nutritionally balanced diet may contribute to a decreased acrylamide intake. French fries had a significant impact on the acrylamide intake, due to their frequent consumption in the canteen. This demonstrates the important responsibility of caterers and canteen kitchens in the mitigation of acrylamide exposure through reduction of acrylamide in their prepared products, in particular in French fries, which are regularly consumed in Belgium.

Keywords: acrylamide; canteen food; balanced diet; dietary intake

8.2 Introduction

In April 2002, acrylamide was identified as a heat-induced process contaminant, present in fried, baked, grilled or toasted carbohydrate-rich foodstuffs (Tareke et al., 2002). Based on previous acrylamide intake studies and current toxicological data, the genotoxic and carcinogenic risks of dietary acrylamide might not be negligible for humans (FAO/WHO, 2005). Up till now, no single study could however provide conclusive evidence on the human health effects (Wilson et al., 2006). Consequently, it was recommended to reduce acrylamide concentrations in foodstuffs as much as possible (FAO/WHO, 2005).

On the other hand, acrylamide-containing foodstuffs contribute significantly to the total micro and macro nutrient composition of the diet, as well as to the total daily energy intake (Petersen and Tran, 2005). Given its ubiquity in the diet, totally removing the dietary exposure of acrylamide is thus impossible. Therefore, it is interesting to know which food categories contribute the most to the daily intake in order to consider practical measures to reduce the acrylamide content in these products.

Furthermore, out of home eating has considerably increased the last decennia and has gained an important place in the habitual diet (Guthrie et al., 2002). Consequently, the catering sector has become a strategic partner to promote a balanced diet in Europe (Lachat et al., 2005). However, public health and toxicological issues regarding this sector have so far received little attention in nutrition research.

The main purpose of this investigation was to determine the importance of canteen food on the dietary acrylamide exposure. More specifically, this study has four objectives: 1/ to assess acrylamide exposure in the habitual diet of young professionals and students through a probabilistic approach; 2/ to identify the food groups that contribute most to acrylamide intake; 3/ to investigate the relative contribution of a Belgian university canteen meal on acrylamide intake, and 4/ to document the effect of an increased accessibility to fruit and vegetables on acrylamide exposure. This trial was part of a larger intervention study that investigated the effect of a lunch with increased accessibility to fruits and vegetables on the daily food intake pattern.

8.3 Materials and methods

8.3.1 Study area

A food intake study was carried out in the canteen of the Faculty of Bioscience Engineering at Ghent University (Belgium). This restaurant is representative to other canteens of the university in the sense that the same suppliers cater for all kitchens. Preparation methods and menus are standardized and the meals offered are largely the same in all canteens. The faculty canteen serves about 250 hot meals *per* day. The meals contain a protein component (meat, fish or vegetarian), with a choice of vegetables and a carbohydrate source (rice, French fries, mashed or boiled potato).

8.3.2 Study design

Food intake data were obtained from a three-day record of food and drinks. Participants were asked to record all food and drinks on days during which they took a hot lunch in the canteen. Those days could be chosen freely in the first two weeks of December 2005. Portion sizes of the canteen meals were measured from purchased samples, while other foodstuffs were quantified using a standardized reference manual for Belgian food products (Bellemans and De Maeyer, 2005). Information on cooking practices was also recorded to separate boiled and deep-fried potato products. Weight, height, date of birth and gender were self-reported.

A convenience sample of 160 university students and faculty staff members (60 men and 100 women) aged between 18 and 35 years provided valid and complete data. Since this survey was part of a larger study, investigating the effect of an improved lunch on the daily food intake pattern, the participants were randomly assigned into an intervention and a control group. After drop-outs and excluding non-residents, 85 persons in the intervention group and 75 persons in the control group were retained for analysis. In this way 480 daily consumption patterns were obtained.

The intervention group was offered two portions of fruit, with one portion being one pear, one apple or two mandarins. One pre-packed fresh salad was offered as vegetable portion. Salads offered were tomato (200 g portion), cucumber (150 g) or seasonal salad (150 g) containing a mix of cabbage, cucumber lettuce and carrots. The portions of fruit and vegetables were offered free of charge. The control group received no extra food. At the end, all volunteers were rewarded with 2 cinema tickets.

8.3.3 Acrylamide contamination data

The acrylamide levels used for the exposure calculations were obtained from several sources. The Institute for Reference Materials and Measurements (IRMM, 2006) composed a European Monitoring Database containing more than 7000 validated acrylamide levels originating from different European Member States and from the European food industry. Samples were analysed between July 2002 and June 2006. Before publication on the IRMM website, the levels were evaluated for reliability based on laboratory and method performance/quality criteria, such as e.g. proficiency testing results, limits of detection (LOD) and limits of quantification (LOQ) (Lineback et al., 2005). In addition to these IRMM data, about 500 acrylamide concentration levels were used, originating from food products on the Belgian market and provided by the Belgian Federal Agency for the Safety of the Food Chain. The majority of these data was not yet included into the European Monitoring Database at the time of study. Finally, also the acrylamide levels of 10 ready-to-eat French fry portions, sampled in the canteen during the food intake survey, were determined in our laboratory using the LC-MS/MS analysis method, as described under section 2.3.2. For the intake estimation, the left censored data, being the data below LOD or LOQ, were respectively replaced by the corresponding LOD divided by 2 or LOQ divided by 2 (Tressou et al., 2004; WHO, 2006).

8.3.4 Grouping of food items

The food items were classified into 14 different food categories. The food groups contributing the most to the acrylamide exposure are mentioned in Table 8.1. The classification was based on the divergent acrylamide levels between the categorized foodstuffs and was in agreement with the categorization in previous acrylamide exposure assessments (Konings et al., 2003; Boon et al., 2005; Dybing et al., 2005; Matthys et al., 2005). Food groups such as onion bread and food for diabetics, included in the IRMM database, were not consumed and consequently not included as a food group. For the food group ‘coffee drink’, data for liquid coffee and data originating from (roasted and ground) powder were used. The latter were recalculated to the drink using a previously proposed conversion factor of 0.046 (Dooren et al., 1995).

8.3.5 Probabilistic exposure assessment

The exposure assessment was focused on the acrylamide-containing foodstuffs. Acrylamide intake was modelled multiplying consumption data with contamination levels through a probabilistic approach. The exposure was expressed as µg acrylamide *per* kg body weight (bw) *per* day. The variability of the consumption and contamination levels was characterized by a non-parametric, discrete, uniform distribution. In this approach, the collected data points themselves are considered to form a discrete uniform distribution, meaning that all collected data points have the same probability of occurrence (Vose, 2000).

In addition, the characterization of uncertainty was performed using non-parametric Bootstrap. The Bootstrap theory assumes that the true distribution F (of e.g. acrylamide concentration levels in French fries) can be reasonably approximated by the distribution F' of *N* observed values. Obviously, this is a more reasonable assumption when more data are collected. For a sufficiently large number of times, *N* random samples (with replacement) are taken from the distribution F'. Each time, a statistic of interest is calculated from that sample. In such a way, a distribution of uncertainty about a parameter is obtained (Vose, 2000).

Propagation of variability and uncertainty was performed by second order Monte Carlo simulation using @RISK (@RISK 4.5 risk analysis software for Excel, Palisade, UK), randomly combining the consumption distribution with the contamination distribution. This simulation technique consists of two Monte Carlo loops, the one nested inside the other. The inner loop deals with the variability, while the outer loop deals with the uncertainty of the input variables. Latin Hypercube sampling was used (Baert et al., 2007). The variability was described performing one thousand iterations. One thousand Bootstrap simulations were executed to estimate the confidence intervals (CI).

The exposure distributions were calculated for each food group separately and for all food groups together. In addition, the exposure distributions were calculated independently for the three main meals of the day (breakfast, lunch and dinner) and for the food and drinks consumed in between (defined as snack type food). Breakfast was arbitrarily defined as the first meal of the day, between getting up and 10.00 am. Lunch was consumed between 11.30 am and 2.00 pm in the university canteen. Dinner was defined as the meal consumed roughly

between 6.00 pm and 8.00 pm. Because of this separate calculation, the total intake at a certain percentile is each time different from the sum of the different food items (or meals) at that percentile (Matthys et al., 2005). Since the acrylamide content of French fries consumed inside the canteen was measured, a distinction could be made between the contamination level of French fries, served in the university canteen and French fries consumed elsewhere. It was assumed that French fries, consumed outside the canteen, had contamination levels comparable to the values of the Belgian and European databases. With this distinction, it was moreover possible to evaluate the impact of the canteen French fries on the total acrylamide exposure.

8.4 Results

8.4.1 Evaluation of consumption and contamination levels

Food consumption and acrylamide contamination levels of the most important food groups are presented in Table 8.1. The acrylamide levels vary considerably between single food items within food groups, as reported previously (Wilson et al., 2006; Dybing et al., 2005). From Table 8.1, it is clear that bread is consumed the most on median daily basis, followed by canteen French fries, biscuits and chocolate. Men have a higher intake of food *per* kg bodyweight, compared to women.

Table 8.1. Descriptive statistics of acrylamide contamination (µg.kg^{-1}) and food consumption (mg.kg bw^{-1}.day^{-1}) for the most important food groups

	contamination (µg.kg foodstuff^{-1})*			**consumption (mg.kg bw^{-1}.day^{-1}) - P 50 - P 95** (mean)**		
	N	**mean**	**P 50 (P 0 – P 100)**	**total (*N* = 160)**	**male (*N* = 60)**	**female (*N* = 100)**
biscuits	1130	276	142 (< 5 – 6798)	404 – 1727 (589)	375 – 1801 (576)	483 – 1658 (598)
bread	119	27	15 (< 7 – 150)	1639 – 3708 (1768)	1868 – 4083 (2063)	1589 – 3168 (1592)
breakfast cereals	380	125	70 (< 5 – 1649)	0 – 1294 (291)	0 – 1313 (267)	0 – 1204 (305)
chocolate	43	190	130 (< 8 – 826)	142 – 618 (215)	88 – 618 (196)	163 – 624 (226)
coffee drink	262	14	12 (< 0.5 – 59)	0 – 6362 (1381)	0 – 4768 (1107)	0 – 6632 (1546)
crisp bread	557	329	182 (< 5 – 2838)	0 – 523 (88)	0 – 312 (46)	0 – 576 (113)
French fries (outside canteen)	538	377	220 (< 5 – 3300)	0 – 1127 (125)	0 – 1134 (123)	0 – 1113 (126)
French fries (canteen data)	10	58	50 (32 – 116)	814 – 2349 (813)	892 – 2362 (980)	629 – 2286 (713)
potato crisps	925	707	522 (< 5 – 4215)	0 – 547 (98)	0 – 599 (137)	0 – 521 (75)
gingerbread	1025	556	308 (< 5 – 7834)	0 – 206 (24)	0 – 217 (35)	0 – 5 (17)
sweet spiced biscuit	47	353	277 (< 15 – 1234)	0 – 399 (75)	0 – 406 (76)	0 – 295 (74)

* for values below the limit of detection (LOD) and limit of quantification (LOQ), LOD/2 and LOQ/2 were respectively used
** 50th and 95th percentile
N: number of observed values

8.4.2 Acrylamide intake estimation

Table 8.2 shows the characteristics of the dietary acrylamide intake. The median intake is estimated to be 0.398 µg.kg $bw^{-1}.day^{-1}$, with a 90% CI between 0.359 and 0.442. The 5^{th} percentile of intake is 0.103 µg.kg $bw^{-1}.day^{-1}$ while the 95^{th} percentile is 1.481 µg.kg $bw^{-1}.day^{-1}$. All percentiles are higher for men compared to women (results not shown). More specific, the median intake for respectively women and men is 0.393 and 0.408 µg.kg $bw^{-1}.day^{-1}$. This difference is not significant.

Table 8.2. Variability and uncertainty of the dietary acrylamide intake (µg.kg $bw^{-1}.day^{-1}$) (best estimation [90% confidence interval])

percentile	acrylamide exposure
5	0.103 [0.087 - 0.118]
10	0.140 [0.124 - 0.160]
25	0.232 [0.207 - 2.260]
50	0.398 [0.359 - 0.442]
75	0.681 [0.615 - 0.755]
90	1.105 [0.997 - 1.227]
95	1.481 [1.315 - 1.663]
99	2.591 [2.170 - 3.112]
99.9	4.521 [3.265 - 7.370]
mean	0.537 [0.457 - 0.699]

8.4.3 Importance of each food group

The importance of each food group to the acrylamide intake is presented in Table 8.3. These results show that the median daily acrylamide intake (P 50) can be attributed to biscuits (35.4%), canteen French fries (29.9%), bread (23.5%) and chocolate (11.2%). These are also the most frequently consumed foodstuffs, as shown in Table 8.1. Bread is the most important contributor to dietary acrylamide for the lower percentiles (up to the 40^{th} percentile). For percentiles higher than 40, biscuits represent the main source of acrylamide. Above the 95^{th} percentile, biscuits, French fries, potato crisps, breakfast cereals, chocolate, bread, crisp bread, sweet spiced biscuit, coffee drink and gingerbread are (in decreasing order of importance) the predominant sources of acrylamide. Other food categories such as baby's biscuits, choco-spread, coffee substitutes drink and popcorn contribute little to the total acrylamide intake and are therefore not mentioned in Table 8.3.

Table 8.3. Contribution of the most important food groups to the estimated intake (µg.kg bw^{-1}.day^{-1}) of acrylamide (best estimation [% on total exposure])

percentile	**5**	**10**	**25**	**50**	**75**	**90**	**95**	**99**	**99.9**	**Mean**
biscuits	0 [0]	0 [0]	0.004 [18.6]	**0.042 [35.4]**	0.160 [36.3]	0.416 [31.8]	0.693 [27.7]	1.598 [24.7]	3.533 [24.8]	0.161 [29.4]
bread	0.003 [100]	0.007 [100]	0.015 [77.3]	**0.028 [23.5]**	0.056 [12.6]	0.110 [8.4]	0.162 [6.5]	0.312 [4.8]	0.521 [3.7]	0.048 [8.8]
breakfast cereals	0 [0]	0 [0]	0 [0]	**0 [0]**	0.023 [5.3]	0.100 [7.7]	0.189 [7.5]	0.492 [7.6]	1.089 [7.6]	0.037 [6.7]
chocolate	0 [0]	0 [0]	0.001 [4.1]	**0.013 [11.2]**	0.046 [10.5]	0.106 [8.1]	0.165 [6.6]	0.367 [5.7]	0.677 [4.7]	0.040 [7.3]
coffee drink	0 [0]	0 [0]	0 [0]	**0 [0]**	0.024 [5.5]	0.059 [4.5]	0.088 [3.5]	0.165 [2.5]	0.297 [2.1]	0.019 [3.5]
crisp bread	0 [0]	0 [0]	0 [0]	**0 [0]**	0 [0]	0.054 [4.1]	0.158 [6.3]	0.560 [8.7]	1.300 [9.1]	0.029 [5.2]
French fries (outside canteen)	0 [0]	0 [0]	0 [0]	**0 [0]**	0 [0]	0.026 [2.0]	0.267 [10.7]	0.996 [15.4]	2.510 [17.6]	0.046 [8.5]
French fries (canteen)	0 [0]	0 [0]	0 [0]	**0.036 [29.9]**	0.072 [16.3]	0.116 [8.8]	0.145 [5.8]	0.226 [3.5]	0.344 [2.4]	0.047 [8.5]
potato crisps	0 [0]	0 [0]	0 [0]	**0 [0]**	0.031 [7.0]	0.219 [16.7]	0.404 [16.1]	0.907 [14.0]	1.667 [11.7]	0.068 [12.5]
gingerbread	0 [0]	0 [0]	0 [0]	**0 [0]**	0 [0]	0 [0]	0.034 [1.4]	0.316 [4.9]	1.045 [7.3]	0.012 [2.3]
sweet spiced biscuit	0 [0]	0 [0]	0 [0]	**0 [0]**	0.020 [4.6]	0.068 [5.2]	0.135 [5.4]	0.355 [5.5]	0.760 [5.3]	0.026 [4.8]

8.4.4 Importance of each meal

The distribution of acrylamide intake over the different meals and snacks of the day is presented in Table 8.4, in terms of percentage for the 50th and 95th percentile. Within each meal, the most important contributing food categories are calculated, also in terms of percentage on the meal. From Table 8.4, it is clear that the snacks contribute the most to the acrylamide intake, both at the median and at higher percentiles. Biscuits are the main source of intake in between the three meals of the day. At higher percentiles, the contribution of potato crisps, chocolate, sweet spiced biscuit and coffee becomes more important. The overall share of the dinner is more distinct for the upper percentiles, coinciding with an increasing contribution of French fries. The canteen French fries are by far the most important source of acrylamide intake during lunchtime, while bread is the major acrylamide source during breakfast and dinner, at the 50th percentile. For the higher percentiles, other food categories contribute to the acrylamide intake, which were not contributing at the median intake level, as already observed in Table 8.3.

Table 8.4. Distribution of acrylamide intake over the different meals and most important foodstuffs within each meal, in terms of percentage

	50th percentile				**95th percentile**			
	breakfast	**snack**	**lunch**	**dinner**	**breakfast**	**snack**	**lunch**	**dinner**
total	**23.2**	**42.2**	**15.5**	**19.1**	**20.6**	**39.7**	**8.7**	**31.0**
biscuits		96.3			24.6	42.1		
bread	100			100	12.1	0.5	6.1	14.3
breakfast cereals					32.1			
chocolate		3.7			8.9	9.4		7.0
coffee drink					7.4	4.9		1.0
crisp bread					1.6			11.2
French fries (outside canteen)								37.4
French fries (canteen data)			100				93.9	
potato crisps						35.5		
sweet spiced biscuit					7.1	5.7		5.3

8.4.5 Impact of extra fruit and vegetables

A comparison of the acrylamide intake between the control group and the intervention group, receiving free of charge fruit and vegetables during lunch, is shown in Table 8.5. It can be observed that the acrylamide intake levels are lower in the intervention group, with a reduction of 10.2% at the 50th percentile, compared to the control group. However, this difference is not significant. A decreased intake can be observed for lunch, dinner and snacks, but not for breakfast.

Table 8.5. Median acrylamide intake (µg.kg bw^{-1}.day^{-1}) in the intervention and the control group (best estimation [90% confidence interval])

		50th percentile	% difference with respect to the control group
control group (non vegetable & fruit group)	**total**	**0.422 [0.368 - 0.473]**	
	breakfast	0.057 [0.046 - 0.072]	
	snack	0.116 [0.093 - 0.142]	
	lunch	0.044 [0.030 - 0.057]	
	dinner	0.059 [0.046 - 0.079]	
intervention group (vegetable & fruit group)	**total**	**0.379 [0.335 - 0.427]**	**- 10.2**
	breakfast	0.065 [0.053 - 0.079]	+14.0
	snack	0.103 [0.081 - 0.129]	-11.2
	lunch	0.038 [0.028 - 0.053]	-13.6
	dinner	0.042 [0.031 - 0.054]	-28.8

8.5 Discussion

8.5.1 Acrylamide intake assessment

A solid probabilistic exposure assessment was carried out combining the food intake data with a large amount of European and Belgian acrylamide contamination levels. The acrylamide intake, mentioned in Table 8.2, corresponds well with the range of previous calculations performed in Belgium and in other European countries (Konings et al., 2003; Svensson et al., 2003; Boon et al., 2005; Dybing et al., 2005; Matthys et al., 2005). In addition, the estimation is in agreement with the long-term dietary acrylamide exposure in developed countries, as calculated by the FAO/WHO, which is between 0.3 and 0.8 µg.kg bw^{-1}.day^{-1} (FAO/WHO, 2002). The joint FAO/WHO expert committee on food additives recently

estimated the average intake in the general population between 0.3 and 2.0 µg.kg $bw^{-1}.day^{-1}$. However, for high percentile consumers (90^{th} to 97.5^{th}) the estimates ranged from 0.6 to 3.5 µg.kg $bw^{-1}.day^{-1}$ (FAO/WHO, 2005). In the present study, the 95^{th} percentiles are also almost four times higher than the median intake levels. It is thus clear that a significant part of the population is subject to a much higher acrylamide exposure.

The higher acrylamide intake of men compared to women can be explained by the fact that men have a higher intake of food than women (Dybing and Sanner, 2003; Matthys et al., 2005). From Table 8.1, it is also clear that men consumed more French fries than women.

Apart from the canteen French fries and the data obtained from the Belgian federal food agency, the majority of the acrylamide contamination data used for the above-mentioned calculations originated from the European Monitoring Database, containing mostly German data. To quantify whether the origin of the contamination data significantly affected the outcome, the acrylamide exposure was calculated using only the Belgian data, and only the European data, revealing however no significant difference between the calculations (results not shown).

8.5.2 Importance of food group and meal to the acrylamide intake

In agreement with our findings (Table 8.3 and 8.4), other studies (Dybing and Sanner, 2003; Svensson et al., 2003; Matthys et al., 2005; Petersen and Tran, 2005) confirm that bread considerably contributes to the acrylamide intake, mainly because it is consumed in a relatively large amount (Table 8.1). The substantial contribution of snack type products, such as biscuits and potato crisps was specifically attributed to this age category (18-35 years) (Svensson et al., 2003; Boon et al., 2005; Matthys et al., 2005). On the other hand, the contribution of French fries in this study is lower compared to previous simulations (Boon et al., 2005; Matthys et al., 2005). The relatively low contamination levels, measured in the university canteen and used for the exposure calculation, are probably the reason for this, as discussed further on. It is plausible that the consumption of chocolate and sweet spiced biscuit is to some extent overestimated due to St. Nicholas day (6^{th} of December), a local saint's day traditionally accompanied by an increased consumption of these food products. This bias towards a higher consumption of specialty food during a specific period of time (e.g. Pre-

Christmas or Eastern) has previously already been demonstrated (Dybing et al., 2005). However, it should be stated that this possible bias in this study is only restricted to food groups which appear to be less important to the overall acrylamide intake, as compared to e.g. bread and French fries. Consequently, this will not have a major impact on the findings of this study.

Different dietary patterns can also be observed between the median and 95th percentile of the distribution (Table 8.3 and 8.4). Indeed, some food categories are consumed at the higher percentiles, which are not consumed at lower percentiles (Dybing et al., 2005; Matthys et al., 2005). These figures may represent different dietary patterns, according to the level of exposure. However, it needs to be mentioned that the dietary patterns observed at the highest percentiles may not concern the long-term high consumers, but only participants who reported having consumed these foodstuffs on the recalled days (Dybing et al., 2005). Consequently, the number of individuals participating in this survey may be too small in order to attribute this "tail" phenomenon to consumers who occasionally have a day with high exposures *versus* consumers who may always be at the high end of exposure (due to the consumption of certain foods that contain high levels of acrylamide).

8.5.3 Scenario study addressing the effect of canteen food on the total acrylamide intake

As shown in Table 8.4, the acrylamide exposure during lunch can mainly be attributed to the consumption of canteen French fries. It was also observed that the share of French fries in the total acrylamide intake was rather low compared to previous acrylamide exposure assessments, due to the low degree of acrylamide contamination in the analysed canteen French fries. Therefore, the total acrylamide intake was recalculated applying the European contamination data for the French fries, consumed during lunch, instead of the acrylamide data of the French fries sampled in the university canteen. A median acrylamide intake of 0.573 [90% CI: 0.516 – 0.631] $\mu g.kg\ bw^{-1}.day^{-1}$ was obtained accordingly. The share of French fries increased from 29.9% (Table 8.3) to 47.9%, which is more in agreement with previous studies (Svensson et al., 2003; Boon et al., 2005; Matthys et al., 2005). The lunch meal also became more important with respect to the other meals. This shows the important responsibility of the caterers and canteen kitchens in the mitigation of acrylamide exposure

through the reduction of acrylamide in their prepared products, in particular in French fries. Because of their particularly high consumption within the Belgian diet in general and within the currently investigated canteen lunch in particular, French fries can have a significant contribution to the acrylamide intake, especially if not sufficient care is taken in order to reduce the acrylamide formation during frying. From previous research (Amrein et al., 2003; Becalski et al., 2004; De Wilde et al., 2005; De Wilde et al., 2006a; De Wilde et al., 2006b), it is known that the reducing sugar content of potatoes plays a key role in the mitigation of acrylamide in French fries, as well as the calibre size and seasonal factors. Consequently, caterers should pay specific attention to the selection of raw material in order to mitigate as much as possible the formation of acrylamide in the prepared French fries.

From the low acrylamide contents, measured in the canteen French fries, it can moreover be concluded that the acrylamide formation is well under control in the canteen kitchen. This can be ascribed to an optimised deep-frying process, during which the temperature is not allowed to exceed 170°C. Moreover, the frying time is fully standardized, meaning that the fried product is automatically removed from the oil bath after a fixed period of time.

8.5.4 Impact of extra fruit and vegetables

Many epidemiological studies have shown health benefits resulting from eating sufficient fruit and vegetables (FAO/WHO, 2004). Intervention studies where fruit and vegetables were made highly accessible have mainly looked at the consumption of fruit and vegetables, but less to the secondary effects on other foods. The present results show that, besides the health promoting effect of fruit and vegetables, an increased availability of these foodstuffs to canteen customers might also have an impact on the acrylamide intake. As shown in Table 8.5, the acrylamide intake on total basis is lower in the intervention group, which received extra fruit and vegetables during lunch, however not significantly lower. Yet, it is remarkable that a decrease can also be observed for the lunch, dinner and snacks, but not for the breakfast. This different trend can be explained by the fact that the vegetable and fruit portions were only distributed during lunch. The results presented here may be a first indication that increased availability of fruit and vegetables, containing no acrylamide, may indeed lead to a decreased intake of acrylamide-containing foodstuffs. This would be an extra health benefit besides the known health promoting effects of some constituents in fruits and vegetables (FAO/WHO,

2004). A more profound study on the energy balance in general and on the consumption of fruit and vegetables in particular is planned in order to further investigate this hypothesis.

8.6 Conclusion

Snack type products contributed the most to the acrylamide intake. However, the acrylamide intake during lunch could become more important when the degree of French fries contamination increases. This is of particular importance within the currently investigated Belgian diet, generally characterized by a regular consumption of these foodstuffs. Therefore, caterers should pay specific attention to the selection of raw material, more specifically potato tubers low in reducing sugars, in order to mitigate as much as possible the formation of acrylamide in the prepared French fries. Too long heating times and too high frying temperatures should also be avoided since this also increases the acrylamide level in the final product. In combination with a nutritionally balanced meal, composed of foods from many sources and rich in fruits and vegetables, a significant reduction in acrylamide exposure to a large population of out of home eating consumers can be achieved. In addition, a moderate consumption of fried and fatty foods should be encouraged (Slayne and Lineback, 2005). Previous research has proven that worksite canteens are an ideal working space to promote a genuine healthy diet (Roos et al., 2004; Lassen et al., 2004). This exemplary role may be specifically attributed to (school) canteens which prepare foodstuffs for younger children and adolescents, a population group which can still easily pick up good eating habits and which is considered to be more exposed to acrylamide compared to older age groups (Dybing et al., 2005).

General conclusions,

recommendations and perspectives

General discussion and conclusions

Deep-fat frying is an ancient but complex process, which consists of immersion of foodstuffs in hot oil. This induces a dynamic and continuously changing heat and mass transfer. Water evaporates from the food surface as steam, while the frying oil is absorbed. Moreover, the foodstuff undergoes major structural and physicochemical transformations, including starch gelatinisation, protein denaturation and crust formation. These changes determine, in combination with the ongoing Maillard reaction, the final physical and sensorial attributes of the fried foodstuff (Vitrac et al., 2000; Miranda and Aguilera, 2006).

For decennia, the nonenzymatic browning reaction (Maillard reaction) has been subjected to numerous scientific investigations worldwide, due to its importance on the nutritional and sensorial quality and safety of heated foodstuffs. Because of its complexity, further investigations are however required in order to gain deeper insights into the reactions we wish to manipulate (Ames, 1990; Finot, 2005). The recent discovery of acrylamide in fried foodstuffs in 2002 (Tareke et al., 2002; Rosén and Hellenäs, 2002) stimulated the research on the related Maillard reaction and forms the point of departure of the present work. The first objective was to evaluate process-related factors which may influence acrylamide formation, in order to find solutions to lower the final acrylamide content in fried potato products. Doing so, the product quality had to be guaranteed for the consumer. Accordingly, acrylamide formation or mitigation was linked with Maillard browning and other sensorial aspects such as texture and taste. The second objective focused on the exposure of acrylamide and on factors which may both lower the dietary intake and improve the overall nutritional quality of the diet.

Because of the constantly changing deep-frying environment as well as the variability in raw potato material composition, a closed potato model system was first developed, with a constant and homogeneous composition, approaching the real composition of fried potatoes. Accordingly, the heat and mass transfer were stabilized, which allowed to study the chemical impact on acrylamide formation of specific factors, keeping other physical variables more constant. Where possible, the results obtained from this model system were evaluated against real frying experiments. A thorough screening and monitoring of factors which may influence the applied heating methodologies and acrylamide analysis technique appeared to be crucial in order to obtain a reliable experimental outcome, as elaborated in chapter 2.

Influence of the frying medium on acrylamide formation

As discussed in the first chapter, several formation pathways of acrylamide are proposed, with reducing sugars and asparagine as the most important precursors. Moreover, some oil degradation products were suggested to be potential acrylamide precursors. Yet, within the pragmatic experimental concentration levels and heating conditions applied in chapter 4, only acrolein appeared to significantly increase acrylamide formation in the presence of asparagine. The importance of this α,β-unsaturated aldehyde as a precursor however appeared to be negligible compared to reducing sugars. In fried potato products, it can thus be concluded that the investigated oil degradation products are not significantly contributing to the overall acrylamide formation.

In addition, the difference in composition between fresh and (partially) degraded frying oils or between different types of frying oil might result in a distinct polarity of the frying medium (Dobarganes et al., 2000). This could lead to a different transfer of heat and oil to the foodstuff being fried. In chapter 3 and 4, no evidence was found that the oil or heat transfer were changed to such an extent that acrylamide formation during preparation of French fries was significantly influenced. In literature, there is however no consensus on whether the oil type or quality would considerably influence oil uptake in fried potato products (Moreira et al., 1997; Mehta and Swinburn, 2001; Mellema, 2003). The reason for this could be the fact that many other, possibly more important factors have been reported to significantly affect oil uptake, such as initial moisture content of the raw material, surface-to-volume ratio, frying temperature and time, way of potato cutting and use of coatings or batters (Mehta and Swinburn, 2001; Mellema, 2003). It is furthermore known that several pre-treatments influence the surface microstructure of raw potato cuts and consequently the oil absorption upon subsequent frying (Moyano and Berna, 2002; Rimac-Brncic et al., 2004). This was confirmed in chapter 7, where acrylamide reduction occurred in parallel with a reduced oil uptake upon frying, caused by several components added to the blanching water of potato crisps. This would suggest that textural and compositional product changes may indeed considerably influence the heat transfer and consequently affect acrylamide formation. This was also confirmed in the model system, where the oil content clearly influenced the final acrylamide content, as shown in chapter 3 and 5.

The parallelism between a reduced oil uptake and lower acrylamide formation fits within the ongoing trend towards healthier foodstuffs, low in fat. This awareness originates from several studies, associating the consumption of oxidized or saturated fats with several diseases such as diabetes, hypertension, coronary heart disease and cancer (Billek, 2000; Saguy and Dana, 2003; Mellema, 2003; Frankel, 2005). Although the oil type and degradation did not seem to significantly influence acrylamide formation, a permanent control of the deep-frying oil quality as well as a judicious selection of oil type remains important. In combination with measures lowering the final oil content, healthier and safer fried foodstuffs can be produced.

Influence of raw material on acrylamide formation

Besides oil as a heating medium, water is another important constituent in fried foodstuffs. Water, evaporating from the frying foodstuff as steam, leaves voids for the oil to enter later in the frying process. This is suggested to be the reason why fat uptake is largely determined by the initial moisture content of the raw potato. Consequently, pre-drying of the raw material prior to frying leads to final products with a lower fat content (Mehta and Swinburn, 2001; Krokida et al., 2001c). However, because of this, the acrylamide precursors are also concentrated in the liquid phase of the partially dried raw material. To investigate the impact of this concentration effect on acrylamide formation, potato powder mixtures with different initial moisture content were heated in the closed model system (chapter 5). Accordingly, it became clear that the yields of acrylamide formation, expressed relatively to the amount of asparagine, increased upon lowering the moisture content of the raw material to reach an optimum. Moreover, the moisture content corresponding to this optimum appeared to be dependent upon the initial oil content of the potato powder mixture, indicating that both oil and water content interfere with the formation of acrylamide upon heating. In addition, a lower thermal input had a clear decreasing impact on the final acrylamide yield.

Consequently, pre-drying leads to a lower fat uptake upon frying, but may on the other hand increase the acrylamide yield due to a concentration effect of the precursors. However, due to pre-drying, the desired dry matter content of the final product is reached sooner during frying, which may allow to shorten the period of frying, again lowering the acrylamide formation in the fried product. In this way, the final acrylamide content could possibly be mitigated. Of course, product quality characteristics, which were not considered in chapter 5, should also be

safeguarded. Nevertheless, it was previously stated that the quality of the final product could be controlled, optimizing the drying and frying process (Krokida et al., 2001c).

Besides the moisture content, the concentration of acrylamide precursors in the raw material, and more specifically the reducing sugar content, also has a major impact on the final acrylamide content. In chapter 1, a range of agricultural factors was discussed with significant influence on the reducing sugar content. Due to unfavourable and sometimes unpredictable conditions, this parameter cannot systematically be controlled. A rapid technique to assess the acrylamide contamination in fried potato products would however be very useful for the consumer and processing industry. As investigated in chapter 6, a link exists between surface colour (Maillard browning) of French fries and their final acrylamide content. Unfortunately, this relationship is disturbed by a difference in the glucose/fructose ratio in the raw material, which may again be caused by several agricultural factors. Consequently, the quantitative and qualitative determination of the reducing sugar content in the raw potato remains a powerful tool to predict acrylamide formation.

To extract the acrylamide precursors form the potato cuts, several raw material pre-treatments exist, such as soaking or blanching in water or in acidic solutions. As discussed in chapter 7, lowering the pH as well as addition of divalent ions or free amino acids different from asparagine appeared to efficiently lower the acrylamide content, both in the model system and upon preparation of potato crisps. Sensory product evaluation was essential in order to combine efficient acrylamide mitigation with acceptable product quality. Possible negative side phenomena caused by addition of these acrylamide-lowering additives, such as increased deep-frying oil hydrolysis or oxidation or corrosive effects on the equipment, may however not be negligible and need to be further investigated before implementation in industry. The cost of these additional treatments and food grade additives should also be considered.

Exposure to acrylamide and health risks

The risks associated with the carcinogenicity of acrylamide intake in humans, mentioned in chapter 1, are still uncertain. Nevertheless, the margin of exposure (MOE) approach clearly indicates acrylamide as a human health concern. To reduce the intake of this probable human carcinogen, the current research provides additional insight into the formation of acrylamide and shows acrylamide mitigation strategies in potato products, which are considered as a major source of acrylamide in the human diet. Besides a lower degree of contamination, a diverse dietary pattern composed of foods from many sources and rich in fruits and vegetables could also contribute to a lower exposure, as demonstrated in chapter 8. Due to a considerable raise in out of home eating, the role of caterers and canteen kitchens has increased the last decennia. Consequently, these food suppliers have an important responsibility in the mitigation of acrylamide exposure through reduction of acrylamide in their served products, in particular French fries.

In order to stimulate and help industry, caterers and consumers to lower acrylamide formation in their potato products, the most important recommendations are suggested underneath:

Recommendations to agriculture and industry

The selection of raw material, low in reducing sugars, is maybe the most important factor that should be considered. This can be done by choosing the appropriate cultivar. Current techniques, applied for the selection of cultivars, are mostly based on Maillard browning. As discussed in chapter 6, the determination of the reducing sugar content is more reliable.

Elevated nitrogen fertilization lowers the final reducing sugar content in the tuber. Here, the legal fertilization limits should of course also be considered. The harvest is preferentially performed at full maturity of the tuber, if permitted by the climatological conditions. Large tubers generally have a lower reducing sugar content compared to smaller ones. The latter should thus preferentially be used for other purposes besides baking and frying. Storage of the tubers should be at about 8°C in the dark and certainly not below 4°C.

An additional washing or blanching step promotes the extraction of acrylamide precursors. Depending on the raw material, the treatment should be optimized towards higher temperatures (circa 80°C) and shorter times. A repeated refreshment of the water is recommended in order to increase extraction efficiencies. Eventually, additives such as organic acids, amino acids, calcium or NaCl, can be applied in the blanching water, as studied in chapter 7.

Since there is a link between acrylamide content and final product colour, the elimination of dark coloured potato crisps by means of in-line optical sorting would contribute to a lower degree of overall acrylamide contamination in the packed crisps.

Recommendations to catering and consumer

The potato tubers, used for frying, should be low in reducing sugars. Larger tubers tend to contain less sugar. The catering industry and consumer should store their potatoes at 8°C in the dark. In case the potatoes were exposed to lower temperatures, the potatoes should be stored at 15-20°C for about two weeks before frying, enabling a reconditioning of the tubers.

A thorough rinsing in warm water prior to frying extracts the acrylamide precursors from the surface of the potato cuts and lowers acrylamide formation upon subsequent frying. Moreover, it contributes to a more homogeneous colour of the final product surface. For optimal product quality, French fries should be fried in two steps. The par-frying step occurs at about 160-165°C for a few minutes (depending on the variety). Do not overload your frying basket. If too much food is immersed in the oil, the oil temperature will drop too much. The product will take longer to cook and will become greasy. A general rule is to fry one part of food in six parts of oil (Mehta and Swinburn, 2001). The crucial step for acrylamide formation is however the second frying step. This operation should be performed at temperatures not exceeding 170-175°C. In addition, excessive browning of the fried potato products should be avoided at all times. The final colour should be gold-yellow and certainly not brownish. Above-mentioned cooking clues enable the catering and consumer to prepare fried potato products with a reduced degree of acrylamide contamination, without losing desired product quality.

Furthermore, the general advice is to keep your diet as complete as possible, with a lot of diversity. Do not forget to eat a lot of fruits and vegetables. That is, of course, not only because of acrylamide but also because of the high content of fat in fried products.

Perspectives for further research

Considering the results and conclusions of this work, several suggestions for further research can be given. To decrease acrylamide exposure, the investigations should cover all stages of the production process, starting from the agricultural sector, over the food-processing industry to the catering and consumer. The suggestions below are again focused on potato products. It is however clear that further scientific research in other food categories, prone to acrylamide formation, such as cereal products, chocolate or coffee, should also continue. For products such as coffee, it appears even a greater challenge to mitigate acrylamide formation upon roasting. These specific technological processes are hard to adjust without inducing major losses in product-specific characteristics and quality.

Due to global warming, extreme weather conditions become more important (Dorey, 2005; Redner and Petersen, 2006). In the summers of 2004 and 2006, Western Europe suffered from a relatively long period of drought and heat, followed by a month of extreme rainfall (De Meulenaer et al., 2007). This is known to induce a second growth of the potato tubers. If late in the season, this contributes among other things to a large amount of glassy potatoes, characterized by a lower dry matter and higher reducing sugar content, boosting acrylamide formation upon frying. In addition, important losses upon storage occur due to rotting phenomena (Burton, 1989a). Investigating the response of several potato cultivars towards this seasonal variability could bring important insights into their resistance against more extreme climatological conditions. It would not only be of importance for lowering their potential to form acrylamide, but also to prevent important economical losses in the primary sector.

These studies should be accompanied with breeding programmes, yielding new cultivars with improved growth and compositional characteristics. Since these studies are rather time-consuming, the importance of genetically modified cultivars should certainly not be ignored, as discussed in a recent publication (Rommens et al., 2006).

As demonstrated in chapter 6 and 7, acrylamide mitigation should be combined with measurements of final sensorial product quality. In this context, the link between product texture, oil content and acrylamide formation should be further investigated in order to produce healthier and tastier foodstuffs low in oil and acrylamide. The formation of acrylamide should furthermore be considered in a broader context of the Maillard reaction, which may also produce other suspected carcinogenic or anti-oxidative components. Even after several decennia of research, the overall effect on human health of this vast spectrum of Maillard reaction products remains a challenge (Ames, 1990; Somoza, 2005).

Each acrylamide-lowering measure should furthermore be placed against other health risks. It makes no sense to mitigate acrylamide, while creating other food safety risks, e.g. by producing foodstuffs with a high sodium (salt) content. There are moreover indications that addition of salts to blanching water might increase oil degradation in the subsequent deep-frying step (Mehta and Swinburn, 2001; Padilla, 2005). Also the possible health consequences of the use of asparaginase, also applied in the treatment of leukaemia (Holcenberg, 2004; Kamen, 2004) should be carefully investigated. Potential residual activity after heat treatment should be evaluated.

Concerning the industrial production process of fried potato products, blanching remains an important step to lower the acrylamide content upon subsequent frying, through extraction of the reducing sugars from the potato cuts. The extraction efficiency is however hindered due to the enrichment of the blanching water with sugars and other soluble components. A continuous replacement of the blanching water with fresh water is however not feasible, both from environmental and economical point of view. Yet, a selective removal of the rate-limiting acrylamide precursors (reducing sugars) from the warm blanching water could be realized, through enzymatic oxidation with e.g. glucose oxidase, enabling a closed loop circuit of warm blanching water. This could save energy and water and reduce the environmental impact. In addition, it could increase the retention of other valuable components in the potato cuts, such as amino acids and ascorbic acid (Arroqui et al., 2002), safeguarding the sensorial and nutritional product quality. The feasibility of this kind of treatment in combination with a high-speed production line remains to be investigated.

In this work, a canteen survey was used to investigate the acrylamide intake in a specific canteen environment and age category. In 2004, a national Belgian food intake survey was performed, providing an enormous amount of food consumption patterns (3178 respondents). In combination with an enlarged amount of contamination data, the importance to the overall acrylamide intake of several mitigating strategies could be evaluated, as well as the contribution of specific foodstuffs. Nevertheless, there is still a clear lack of knowledge concerning the intake of contaminants in general and acrylamide in particular in the third-world countries, due to a lack of available consumption or contamination data. Extrapolation of acrylamide contamination data of developed countries would not be correct due to different raw material composition or food preparation techniques. The costly analysis techniques are probably the main barrier, besides a lack of knowledge about acrylamide and food safety in general. Consequently, accurate, quick and cheap acrylamide analysis techniques should be developed, as well as educational programmes informing the public how to cultivate, store and prepare safe food.

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Curriculum vitae

Curriculum vitae

Frédéric Mestdagh, geboren te Gent op 19 september 1980, behaalde in 2003 met grote onderscheiding het diploma van bio-ingenieur in de scheikunde aan de Universiteit Gent. Tijdens zijn studies verbleef hij een semester in Montpellier (Frankrijk) in het kader van het Europese uitwisselingsprogramma *Erasmus*.

Sinds oktober 2003 is hij werkzaam als doctoraatsbursaal aan het Laboratorium voor Bromatologie (Faculteit Farmaceutische Wetenschappen) en de Onderzoeksgroep Levensmiddelenchemie en Humane Voeding (Faculteit Bio-ingenieurswetenschappen), beide aan de Universiteit Gent. Hij heeft er wetenschappelijk onderzoek verricht onder het promotorschap van Prof. dr. apr. Carlos Van Peteghem en Prof. dr. ir. Bruno De Meulenaer. Voor dit onderzoeksproject werd hem een beurs toegekend van het Bijzonder OnderzoeksFonds (BOF) van de Universiteit Gent. De resultaten van dit onderzoek en andere onderzoeksprojecten leidden tot meerdere publicaties in peer-reviewed wetenschappelijke tijdschriften. Daarnaast verzorgde hij diverse voordrachten en postervoorstellingen op nationale en internationale workshops en symposia.

Gedurende deze periode stond Frédéric eveneens in voor de organisatie en begeleiding van de praktische oefeningen van de opleidingsonderdelen Levensmiddelenchemie, Bioproducttechnologie, Food Chemistry en Food Chemistry and Analysis. Daarnaast nam hij als lesgever deel aan diverse intensieve studieprogramma's en begeleidde 7 thesisstudenten bij het uitvoeren van hun Master scriptie. Verder was hij betrokken bij andere onderzoeksprojecten en bij de validatie van de acrylamide analyse binnen het door Beltest® geaccrediteerde Laboratorium voor Bromatologie. Hij droeg eveneens bij tot het basisonderhoud en de ondersteuning van de computer hard- en software infrastructuur binnen de onderzoeksgroep.

Curriculum vitae

Frédéric Mestdagh, born in Ghent on September 19th 1980, graduated in 2003 with great distinction as bio-engineer chemistry at Ghent University. During his studies he staid for a semester in Montpellier (France) in the framework of the European exchange program *Erasmus*.

Since October 2003, he works as doctoral researcher at the Laboratory of Food Analysis (Faculty of Pharmaceutical Sciences) and the Research Group Food Chemistry and Human Nutrition (Faculty of Bioscience Engineering), both at Ghent University. There he performed scientific research, with Prof. dr. apr. Carlos Van Peteghem and Prof. dr. ir. Bruno De Meulenaer as promotors. For this research project, he received a scholarship from the Special Research Fund of Ghent University. The results of this and other research projects were published in a number of peer-reviewed scientific journals. Besides, he presented the results during several national and international workshops and symposia.

During this period, Frédéric was also responsible for the organization of the practical exercises of Food Chemistry, Food Chemistry and Analysis and Bio-product Technology. Moreover, he gave several lectures during a number of intensive courses and he supervised 7 students during the fulfilment of their Master thesis. Furthermore, he was involved with other research projects and with the validation of the acrylamide analysis within the Beltest® accredited Laboratory of Food Analysis. He also contributed to the basic maintenance of the computer hard- and software infrastructure of the research group.

Publications in A1 peer-reviewed journals

- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J.M. & Verhé, R. (2005). Influence of storage practices on acrylamide formation during potato frying. Journal of Agricultural and Food Chemistry, 53, 6550-6557.
- Mestdagh, F., De Meulenaer, B., De Clippeleer, J., Devlieghere, F. & Huyghebaert, A. (2005). Protective influence of several packaging materials on light-oxidation of milk. Journal of Dairy Science, 88, 499-510.
- Mestdagh, F., De Meulenaer, B., Van Poucke, C., Detavernier, C., Cromphout, C. & Van Peteghem, C. (2005). Influence of oil type on the amounts of acrylamide generated in a model system and in French fries. Journal of Agricultural and Food Chemistry, 53, 6170-6174.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J.M. & Verhé, R. (2006). Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003. Journal of Agricultural and Food Chemistry, 54, 404-408.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J.M. & Verhé, R. (2006). Selection criteria for potato tubers to minimize acrylamide formation during frying. Journal of Agricultural and Food Chemistry, 54, 2199-2205.
- Mestdagh, F., De Meulenaer, B., Cucu, T. & Van Peteghem, C. (2006). Role of water upon the formation of acrylamide in a potato model system. Journal of Agricultural and Food Chemistry, 54, 9092-9098.
- Mestdagh, F., De Meulenaer, B. & Van Peteghem, C. (2007). Influence of oil degradation on acrylamide formation in a model system and in French fries. Food Chemistry, 100, 1153-1159.
- Mestdagh, F., Lachat, C., Baert, K., Moons, E., Kolsteren, P., Van Peteghem, C. & De Meulenaer, B. (2007). Importance of a canteen lunch on the daily intake of acrylamide. Molecular Nutrition and Food Research, 51, 509-516.
- Mestdagh, F., De Wilde, T., Castelein, P., Németh, O., Van Peteghem, C. & De Meulenaer, B. (2007). Impact of the glucose/fructose ratio on the relationship between acrylamide and Maillard browning in French fries. European Food Research and Technology, submitted for publication.

- De Meulenaer, B., De Wilde, T., Mestdagh, F., Govaert, Y., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J.M. & Verhé, R. (2007). Comparison of potato varieties between seasons and their potential for acrylamide formation. Journal of the Science of Food and Agriculture, submitted for publication.
- Mestdagh, F., Maertens, J., Cucu, T., Delporte, K., Van Peteghem, C. & De Meulenaer, B. (2007). Impact of additives on acrylamide formation in a potato model system. Food Chemistry, submitted for publication.
- Mestdagh, F., De Wilde, T., Delporte, K., Van Peteghem, C. & De Meulenaer, B. (2007). Impact of chemical pre-treatments on the acrylamide formation and sensorial quality of potato crisps. Food Chemistry, submitted for publication.
- Mestdagh, F., De Meulenaer, B., De Wilde, T., Govaert, Y., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J.M. & Verhé, R. (2007). Optimization of the blanching process to reduce acrylamide in fried potato products. LWT - Food Science and Technology, submitted for publication.

Publications in journals without peer-review

- Mestdagh, F., Van Peteghem, C., De Meulenaer, B., Cauwenbergh, D. & Dewettinck, K. (2004). Acrylamide in levensmiddelen. Schadelijk voor de gezondheid? Food Industry Magazine, nr. February, 28-29.

Chapters in books

- Van Camp, J., De Henauw, S. & De Meulenaer, B. (2006). Voeding en gezondheid (Flanders' Food). Chapter 3, Carbohydrates (Mestdagh, F.), Lannoo Campus, Leuven, 27-34.

Extended abstracts for symposia en workshops

- De Meulenaer, B., Mestdagh, F., De Clippeleer, J., Devlieghere, F. & Huyghebaert, A. (2003). Protective influence of several packaging materials on the light-oxidation of milk. *In* “Presummit symposium on innovative research in dairy science and technology, book of abstracts”, Technologisch Instituut, Antwerp, ISBN 90-76019-22-3, p. 59-60.

- De Wilde, T., De Meulenaer, B., Mestdagh, F., Verhé, R., Govaert, Y., Fraselle, S., Degroodt, J.M., Vandeburie, S., Demeulemeester, K., Calus, A., Ooghe, W. & Van Peteghem, C. (2004). Acrylamide formation during frying of potatoes: thorough investigation on the influence of crop and process variables. Communications in Agricultural and Applied Biological Sciences, 69, 109-112.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Verhé, R., Govaert, Y., Fraselle, S., Degroodt, J.M., Vandeburie, S., Demeulemeester, K., Calus, A., Ooghe, W. & Van Peteghem, C. (2004). Acrylamide formation during frying of potatoes: thorough investigation on the influence of crop and process variables. Czech Journal of Food Sciences, 22, 15-18.
- Mestdagh, F., De Meulenaer, B., Van Peteghem, C., Cromphout, C. & Thas, O. (2004). Towards a better understanding in acrylamide formation, degradation and reduction in model systems (and foodstuffs). Czech Journal of Food Sciences, 22, 11-14.
- Mestdagh, F., De Meulenaer, B. & Van Peteghem, C. (2005). A model system to define the impact of specific factors influencing acrylamide formation in foods. Proceedings of COST 927 "Thermally Processed Foods: Possible Health Implications", Working Group and Management Committee Meeting, Larnaca, Cyprus, p. 15-16.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Verhé, R., Govaert, Y., Fraselle, S., Degroodt, J.M., Vandeburie, S., Demeulemeester, K., Calus, A., Ooghe, W. & Van Peteghem, C. (2005). The influence of inter- and intraspecies variability of potatoes on the formation of acrylamide during frying. Proceedings of Food Chemistry in Flanders V, p. 105-106.
- Mestdagh, F., De Meulenaer, B. & Van Peteghem, C. (2005). Influence of oil type and oil quality on acrylamide formation of French fries. Proceedings of Food Chemistry in Flanders V, p. 117-118.
- Mestdagh, F., De Meulenaer, B., Cucu, T. & Van Peteghem, C. (2006). The role of water on the formation of acrylamide in a potato model system. Communications in Agricultural and Applied Biological Sciences, 71, 217-221.
- Mestdagh, F., Lachat, C., De Meulenaer, B., Baert, K., Moons, E., Kolsteren, P. & Van Peteghem, C. (2006). Probabilistic exposure assessment of dietary acrylamide for students at Ghent University, Belgium. Proceedings of AOAC Europe Symposium on Contaminants and Risk Management, Limassol, Cyprus, p. 57-58.

Participation at symposia and workshops

- 7-9 September 2003: IDF World Dairy Pre-Summit Symposium, Brugge, Belgium
- 16 October 2003: PhD symposium, Leuven, Belgium
- 17 November 2003: EFSA Workshop about the formation of acrylamide in foodstuffs, Brussels, Belgium
- 9 December 2003: Workshop acrylamide (organized by the Scientific Institute of Public Health), Brussels, Belgium
- 29 September - 1 October 2004: Chemical Reactions in Foods, Prague, Czech Republic (oral presentation)
- 1-2 October 2004: Workshop Cost Action 927: Thermally Processed Foods: Possible Health Implications, Prague, Czech Republic
- 15-16 April 2005: Workshop Cost Action 927: Thermally Processed Foods: Possible Health Implications, Larnaca, Cyprus (oral presentation)
- 26 May 2005: KVCV Symposium Food Chemistry in Flanders V, Trends in the Analysis of Foodstuffs, Gent, Belgium (poster presentation)
- 21 September 2006: PhD symposium, Gent, Belgium (poster presentation)
- 6-7 November 2006: AOAC Workshop: Foods to Dye for – Contaminants: sampling, analysis, legal limits', Limassol, Cyprus (oral presentation)
- Several workshops about chromatographic apparatus and detectors (organized by Waters, Agilent, Thermo)