KINETIC MODELING OF ACRYLAMIDE FORMATION IN AQUEOUS REACTION SYSTEMS AND POTATO CRISPS

Jeroen J. Knol

TEXT

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Kinetic modeling of acrylamide formation
in aqueous reaction systems and potato crisps

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ABSTRACT

Acrylamide, a potential carcinogen, can be formed in heat-processed foods, such as French fries, potato crisps, coffee and bread. Although there is still no clarity about the risks associated with the current intake levels of dietary acrylamide, mitigation of acrylamide in foods is strongly advised from the principle of precaution.

The aim of this work was to derive mathematical models that describe the formation of acrylamide as a function of product and processing parameters. In three parts, the necessary steps are taken to achieve the aim and objectives.

In the **first** part, some background information is given around the subject of this thesis: **I** information about acrylamide and its associated health risks, **II** information about the Maillard reaction and the formation routes for acrylamide, **III** information about the production process of potato crisps and strategies for acrylamide reduction and **IV** information about modeling.

The **second** part deals with the development of mechanistic models. The main mechanism of acrylamide formation is the Maillard reaction between reducing sugars and asparagine. The technique of multiresponse modeling was used to unravel the reaction networks of the formation of acrylamide in the Maillard reaction using aqueous reaction systems. With the kinetic models, the fate of reactants, intermediates and main products could be estimated, giving more insight into the reaction network for the formation of acrylamide in a quantitative way. Furthermore, these models have shown that acrylamide is an intermediate in the Maillard reaction and that at high temperatures the Maillard reaction rapidly goes into the advanced stages, forming high amounts of organic acids and high molecular weight melanoidins.

In the **third** part, the use of empirical models was tested to describe and predict the formation of acrylamide in real foods, *i.e.* potato crisps. Empirical models, with the Logistic-Exponential model as the best performing model, can model the formation of acrylamide in potato crisps. By improving the precision of the estimated parameters these models can be used to make predictions for acrylamide formation based on precursor knowledge and can therefore be a promising tool for the crisps industry to mitigate acrylamide formation.

In conclusion, the work described in this thesis has contributed to insights in modeling the kinetics of acrylamide formation and contributes to the development of strategies to lower acrylamide formation in food.

CONTENTS

Chapter 1
General introduction

1.1 INTRODUCTION

The human diet is considered to play an important role in the global variation in human cancer rates (1). Many epidemiological studies have been carried out to study the relationship between diet and cancer. Although *correlations* between diet and cancer are relatively easy to establish, it is much more difficult to find *causal relationships* (2). The results of such epidemiological studies have, therefore, not been consistent (3). The reason for this inconsistency may be the fact that certain components within the diet are responsible for positive or negative health effects of the diet and the variability of the content of these active components and the actual exposure is not always taken into consideration in epidemiological studies (3-5).

With the advancements in modern food science, more and more knowledge has been gained about the nutritional and anti-nutritional factors of foods and the influence of preservation and processing on the variability and exposure of these components. The discovery of several food-borne toxicants, *i.e.*, potential carcinogens that are not naturally present in foods, but that may be developed during preservation or cooking may contribute to the understanding of the causal relationship between diet and cancer. This is especially so when predictive models are used to correct the level of these components for the influence of processing and preparation to enhance the sensitivity of nutritional epidemiological studies (5). Well-known examples of these food-borne toxicants are charcoal grilled meat and fish and traditionally smoked foods, which may contain heterocyclic amines, nitrosamines and polycyclic aromatic hydrocarbons (6-8).

In 2002, another food-borne toxicant was added to this list when the Swedish National Food Administration announced that heat-treated foods, such as French fries, crisps, coffee and bread, contained relatively high levels of acrylamide (9). Acrylamide is formed in foods at temperatures above 120°C in the Maillard reaction between reducing sugars and asparagine (10, 11). Acrylamide has been shown to be neurotoxic to humans and is classified by the International Agency for Research on Cancer as possible carcinogenic (12). The potential carcinogenicity of acrylamide and the relatively high amounts (up to mg/kg) that could be formed in processed foods, led to great concern. Extensive research activities were started by national authorities, food industry and research institutes to find ways of controlling and minimizing acrylamide formation and to assess the potential health risks of dietary acrylamide.

This PhD study was part of the HEATOX project, a European Union-funded project on the identification, characterization and risk minimization of heat-generated food toxicants with particular emphasis on acrylamide (13).

1.2 AIM AND OBJECTIVES

The main objectives of the HEATOX project were to assess health risks that may be associated with hazardous compounds in heat-treated food and to find methods of minimizing the formation of these compounds, thereby providing safe, nutritious and high-quality foods. This PhD study focused on the reaction mechanisms and kinetics of the formation of acrylamide. The aim of the present study is:

- To derive mathematical models that describe quantitatively the formation of acrylamide as a function of several parameters

In order to achieve this aim, the following five objectives were put forward:

- Identify and quantify the main intermediates and end products in the Maillard reaction of reducing sugars and asparagine
- Establish the reaction pathways and propose a mechanistic model from a quantitative point of view
- Establish how processing conditions interfere with the reaction routes and influence the final composition of reaction mixture
- Elucidate the kinetics of acrylamide formation in a real food system by mathematical models
- Develop predictive models for the formation of acrylamide in real food systems

1.3 OUTLINE

This thesis is divided into three parts; **part I** (CHAPTER 2) gives background information on the subject of this thesis, **part II** (CHAPTERS 3-4) is devoted to the development of mechanistic mathematical models (OBJECTIVES 1-3) and **part III** (CHAPTERS 5-6) is devoted to the use of empirical models to describe and predict the formation of acrylamide in a real food system (OBJECTIVES 4-5).

Chapter 2 gives background information about the chemical properties of acrylamide, its use in industry, its occurrence in food and estimations about intake. Furthermore, the metabolism and toxicity of acrylamide and the potential health risks of dietary acrylamide are discussed. This chapter also gives an overview of the Maillard reaction, the main reaction in food responsible for the formation of acrylamide and the different reaction routes for the formation of acrylamide. Finally, the production process of potato crisps and mitigation strategies for acrylamide formation are discussed as well as the use of mechanistic and empirical models.

Chapter 3 describes the development of a multiresponse mechanistic model for the formation of acrylamide in the Maillard reaction between glucose and asparagine at pH 6.8. The identification and quantification of the reactants, main intermediates and end products is described. Building on the work of Brands (14) and Martins (15), who derived comprehensive models for the Maillard reaction of sugars and casein, and glucose and glycine, respectively in the temperature range between 80-120°C, a reaction network was set-up for the formation of acrylamide for the development of a multiresponse kinetic model.

In **Chapter 4** the kinetic model of Chapter 3 is tested at pH 5.5 and with another reducing sugar, namely fructose. Fructose was used, as this reducing sugar is more reactive in the formation of acrylamide. The pH of the systems was lowered to pH 5.5, which is the pH that can be found in potato tissue. The model was further extended with the inclusion of organic acids.

In **Chapter 5** the use of empirical models to predict the formation of acrylamide in potato crisps is studied. The formation of acrylamide was followed in time during the frying of potato slices in oil under the same experimental conditions. Special attention was given to the temperature development in the oil and crisps to give more insight in the frying process and to make future comparisons between studies possible. The formation patterns of acrylamide were fitted with three empirical models and the performances of these models were statistically evaluated.

In **Chapter 6** the performance of the empirical models was further investigated and attempts were made to improve the precision of parameters. Furthermore, the parameters of the empirical models were tested for correlation with the precursor content of the raw potato slices. The correlation that was found was used for the development of a predictive model. The predictive capacity was tested with the results from another study.

In **Chapter 7** the main achievements of this thesis are summarized and put in perspective and it is discussed to what extent the objectives and aim of this study were met. The achievements of the HEATOX project are also briefly discussed of which this PhD work was a part. Finally, the role of kinetic modeling, a tool to unravel and predict the formation of acrylamide, is discussed in a wider perspective.

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Chapter 2

Acrylamide, the Maillard reaction, potato crisps and modeling

2.1 INTRODUCTION

This chapter provides background information about the subject of this thesis: modeling the kinetics of acrylamide formation in aqueous reaction systems and potato crisps. A brief overview is given on the chemical properties of acrylamide, the occurrence and intake of acrylamide in food, the toxicity of acrylamide and the health risks of dietary acrylamide. Next, the Maillard reaction, the main mechanism of acrylamide formation, and the different formation routes of acrylamide are discussed. Furthermore, an overview is given about the production process of potato crisps, the factors influencing the formation of acrylamide during this process and the possible strategies for mitigation of acrylamide formation. Finally, the use of multiresponse modeling in unraveling the mechanism of acrylamide formation in the Maillard reaction and the use of empirical modeling to describe and predict the formation of acrylamide in a real food system is discussed briefly as an introduction to the topic.

2.2 ACRYLAMIDE

2.2.1 Chemical properties

Acrylamide is a reactive molecule and highly soluble in water and polar solvents such as acetone, methanol and ethanol. It is not soluble in non-polar solvents. It is an odorless, white crystalline solid with a melting point of 84.5°C and a molecular mass of 71.08. At temperatures above 84.5°C spontaneous polymerization can occur (1). The chemical structure of acrylamide is shown in **Figure 2.1**.

Acrylamide is synthesized as monomer for the production of polyacrylamide by hydration of acrylonitrile. Polyacrylamide has numerous industrial applications such as in wastewater treatment, in paper and pulp production, and in mining (1). Polyacrylamide gels are also used to separate proteins and other components by electrophoresis in research facilities. Polyacrylamide always contains a certain percentage of non-polymerized acrylamide as the result of incomplete polymerization (2), but degradation of polyacrylamide to release free monomeric acrylamide is reported to be unlikely (3).

2.2.2 Toxicity of acrylamide

The toxicity of acrylamide has been investigated extensively. The major findings of these studies indicate that acrylamide is neurotoxic in animals and humans, and it has been shown to be a reproductive toxicant, germ-cell mutagen, and carcinogen in rodents (2-4).

Metabolism
After absorption in the body, acrylamide can be oxidized into the epoxide glycidamide (2,3-epoxypropionamide). This reaction is catalyzed by cytochrome P450 2E1 (1). The epoxide

group can be cleaved further by an epoxide hydrolase to form 2,3-dihydroxypropionamide (5). Both acrylamide and glycidamide can react in vivo with hemoglobin, serum albumin, DNA and enzymes (2, 6). Acrylamide and glycidamide may also conjugate with glutathione (GSH); the GSH-conjugates are converted to mercapturic acids (5, 7-11). **Figure 2.2** shows the major metabolic pathways of acrylamide. The hemoglobin adducts of acrylamide or glycidamide in blood and the mercapturic acids of acrylamide or glycidamide in the urine can serve as biomarker for the exposure of acrylamide (12, 13).

Neurotoxicity

The neurotoxicity of acrylamide is characterized by ataxia and skeletal muscle weakness and is well-known from occupational exposures (14). The No Observable Effect Level (NOEL) for neurotoxic effects in mouse and rat studies is in the range of 0.2–10 mg/kg bodyweight/day (14), which is far above dietary exposure. The neurotoxicity of acrylamide might be cumulative, which means that the comparatively low dietary exposure of acrylamide might not be negligible (15).

Carcinogenicity

Acrylamide has been classified by the International Agency for Research on Cancer as probably carcinogenic to humans (4). This classification was based on positive results with bioassays in rodents and was supported by evidence that acrylamide is metabolized in mammalian tissues to glycidamide (**Figure 2.2**), which is a more reactive genotoxic metabolite. Acrylamide and especially glycidamide are reactive towards DNA (genotoxic) and can form several DNA adducts, which is considered to be the key process in the carcinogenicity of acrylamide (16). There is also speculation about a neuroendocrine-mediated mechanism for the carcinogenicity of acrylamide (14). Rat and mice experiments have shown that acrylamide is a multi-organ carcinogen, causing tumors at multiple sites such as lung, skin, brain, uterus, thyroid and mammary gland (16).

2.2.3 Occurrence and intake

In the past, the incomplete polymerization of acrylamide has led to contaminations in the environment by its industrial use (17-19). The use of polyacrylamide coagulants for water purification has therefore been restricted and strict guidelines have been setup. The World Health Organization (WHO) proposed a strict maximum guideline of 0.5 µg/L for drinking water (20). Acrylamide has also been regulated in EU countries by the EU 98/83 Drinking Water Directive that stated a minimum quality requirement of 0.1 µg/L for water intended for human consumption (21). Tobacco smoke may also contain acrylamide in amount of 1-2 µg per cigarette, although the presence of acrylamide is not seen as the major problem of tobacco smoking (1, 22).

The environmental contamination of the groundwater with acrylamide in the southwest of Sweden (Hallandsås) and the following exposure studies led to the discovery of the presence of acrylamide in fried foods (23, 24). After the publication of the first findings of acrylamide

in heat-treated foods (25), considerable research activities were started to gain knowledge about the occurrence of acrylamide in food products and its intake. The two most important enumerations of acrylamide occurrences in foods are the 'Acrylamide Monitor Database' of IRMM/JRC in collaboration with DG SANCO and CIAA (26) and the 'Acrylamide Analytical Database' operated by JIFSAN/JECFA (27). The highest levels of acrylamide are generally found in heat-treated (>120°C) plant-derived foods such as French fries, potato crisps, bread, breakfast cereals and coffee. Heat-treated animal derived foods such as meat and fish products contain generally low levels of acrylamide (25, 28). **Table 2.1** shows the results from the IRMM/JRC 'Acrylamide Monitor Database' for the acrylamide levels in food (26). There is large variability not only for the amounts of acrylamide reported for the different food groups, but also within groups. The variability is probably due to differences in processing conditions, precursors content and food composition (29).

Table 2.1

Acrylamide levels (µg/kg) in some commercial or laboratorial prepared foods (26).

	Number	Min	1. Quartile	Median	3. Quartile	Max
Potato chips	1377	5	85	186	363	4653
Potato crisps	831	5	314	528	938	4215
Potato fritter	121	10	172	352	713	3072
Fine bakery ware	1056	4	53	145	350	3324
Gingerbread	1003	5	138	303	668	7834
Crisp bread	411	5	79	244	505	2838
Infant food	157	5	35	79	166	910
Diabetics cakes + biscuits	402	5	102	230	620	3044
Breakfast cereals	333	5	20	60	132	1649
Coffee roasted	221	79	223	286	373	975
Coffee substitutes	108	116	497	773	1226	2955

Number: Number of individual data that were considered in the respective food category. **Min**: Minimum value; A minimum value below 30 indicates acrylamide levels that were reported as 'below LOD' or below 'LOQ'. These data were considered in the statistical evaluations as half of the respective LOD or LOQ. **1. Quartile**: The acrylamide content of 25% of the considered samples were found below that numeric value. **Median**: The acrylamide content of 50% of the considered samples were reported below that value. **3. Quartile**: It is similar to the first quartile, but covers 75% of the data. **Max**: Maximum acrylamide level that was reported for that food category. **Potato chips**: fried potato sticks, also known as French fries, **Potato crisps**: fried thin potato slices, **Potato fritter**: grated potatoes fried into a pancake

Intake of dietary acrylamide varies between populations as a result of different factors, such as cultural differences, gender and age. Furthermore, the set-up of the study (grouping of food items, recording of food intake) can have an influence on the estimation of the acrylamide intake. The range of mean intake of dietary acrylamide is 0.2-1.4 µg/kg body weight per day (30). The contributions of different food groups to the intake of dietary

acrylamide vary between countries. For the Netherlands the contributions of the most important food groups are presented in **Figure 2.3**. The biggest contribution in acrylamide exposure comes from fried potato products, crisps and coffee.

2.2.4 Health risks of dietary acrylamide

The toxic effects of acrylamide that have been mentioned in **section 2.2.2** are the results from animal studies. Extrapolating these results to humans should be done carefully; metabolisms vary between species and the doses used in the experiment are much higher than the dietary exposure of acrylamide (30, 31).

To assess the effect of dietary acrylamide epidemiological studies have been performed. These studies have contradictory outcomes about the risks associated with the current intake levels of dietary acrylamide, although recent studies by Hogervorst *et al*. have given more indications about a positive relation between high acrylamide intake and various types of cancer (32-37). However, these epidemiological studies have not included all the dietary sources of acrylamide and were mostly based on the use of food frequency questionnaires and were not designed to measure actual acrylamide exposure. In a recent study by Olesen *et al*., in which biomarkers were used for the actual acrylamide exposure, a positive association was found between the risk for breast cancer and increased acrylamide-hemoglobin adducts in red blood cells (38).

Another way to assess the risk of a certain component is the 'margin of exposure' (MOE). The MOE is defined as the ratio between a dose leading to tumors in animal experiments (BMDL10) and the human intake. The BMDL10 corresponds to the lower limits of a one-sided 95% confidence interval on the BMD10, which is defined as the daily benchmark dose (BMD) at which 10% of the animal population develops a tumor above the control. A lower value for MOE represents a higher risk. The MOE value can be used in risk management for setting the priority list. The MOE for acrylamide was calculated between 50-2,000 (14). The MOE of other carcinogenic components such as polycyclic aromatic hydrocarbons is in the range of 10,000–25,0000. This indicates that from the viewpoint of 'margins of exposure' acrylamide can be considered to be a concern for human health (14).

Overall, the evidence of acrylamide posing a cancer risk for humans has been strengthened, but the real impact of dietary acrylamide on human health is still unclear and further research is needed to clearly establish the impact of the presence of acrylamide in foods on the health of consumers. However, from the principle of precaution, the Joint FAO/WHO Expert Committee of Food Additives advises to continue appropriate efforts to reduce acrylamide concentrations in food in the meantime. In that respect, the present study aims to provide tools for that. Since acrylamide is formed in the Maillard reaction, an overview of the Maillard reaction is given in the next section to provide the reader with a basic understanding, necessary for modeling of the Maillard reaction in the chapters to follow.

2.3 THE MAILLARD REACTION

Aroma, taste and color of foods change when they are heated. Well-known examples are the brown crust and aromas of freshly baked bread, the color and bitter flavors of roasted coffee and the golden brown color of fried chips (39, 40). Responsible for these changes is the reaction between sugars and amino acids, reported almost a century ago by Louise Camille Maillard (41). Next to its influence on the appearance and taste of foods, the Maillard reaction also has an influence on the nutritional value of foods. The digestibility can be decreased, toxic and mutagenic components may be formed, but also antioxidants can be formed (42).

2.3.1 Chemistry of the Maillard reaction

After the discovery of Louise Camille Maillard (41), it took almost 40 years before Hodge (43) proposed the first comprehensive reaction scheme of the Maillard reaction (**Figure 2.4**). In the first stage of the reaction an aldose sugar, such as glucose, condenses with an amino compound. This amino compound is a free amino group of an amino acid or in proteins the ε-amino group of lysine or the α-amino group of terminal amino acids. The condensation product that is formed is N-substituted glycosylamine; this product undergoes a rearrangement to form the Amadori rearrangement product (ARP). The subsequent degradation of ARP is dependent on the pH of the system. At pH 7 or lower, the ARP undergoes mainly 1,2-enolisation with the formation of hydroxymethylfurfural (HMF) or furfural. The degradation of ARP involves mainly 2,3-enolisation when the pH is higher than 7, with the formation of reductones and fission products. All these compounds are highly reactive and will react further. Carbonyl groups condense with free amino groups. Dicarbonyl groups react with amino acids to form aldehydes and α-aminoketones in the so-called Strecker degradation. In the advanced stage of the Maillard reaction a range of reactions can take place, which in the final stage will lead to the formation of brown nitrogenous polymers, the melanoidins. Since the work of Hodge other pathways have been proposed and the Maillard reaction has been further unraveled (44-47). In the reaction between ketoses, such as fructose, and amino groups ketosylamines are formed which after the Heyns rearrangement form 2-amino-2-deoxyaldoses (48). More recently, a mechanism involving not the Amadori, but the deoxyhexosuloses (3-deoxyaldoketose, 1-deoxy-2,3-diketose and 4-deoxy-2,3-diketose) as key intermediates was proposed (49). The reaction of sugar via the 3-deoxyaldoketose route is favored at lower pH (<5) and leads to formation of brown colored compounds. At higher pH (>7) the 1-deoxy-2,3-diketose and 4-deoxy-2,3-diketose reaction routes are favored, which leads to flavor formation. However, still a great deal of the Maillard reaction has not been unraveled. Discoveries of food-borne toxicants as acrylamide, HMF and furan have contributed to renewed interest in the Maillard reaction.

2.3.2 The formation of acrylamide in the Maillard reaction

Shortly after the discovery of the presence of acrylamide in foods (25), it was discovered that acrylamide is formed in the Maillard reaction with asparagine as main precursor (50-52). Although there are other reaction mechanisms that can lead to acrylamide formation, which will be discussed briefly in the next section, the Maillard reaction is considered to be the main reaction route for the formation of acrylamide in foods.

Thermal decomposition of asparagine in the absence of a carbonyl source can generate acrylamide by decarboxylation and deamination (52, 53). The presence of reducing sugars increases the yield of acrylamide from asparagine considerably (50-52). This explains the relatively high amount of acrylamide in heat-treated potato and cereal products, which contain reducing sugars and are particularly rich in free asparagine (50-52).

Figure 2.5 shows an overview of the pathways in the Maillard reaction leading to the formation of acrylamide. In the initial stage of the Maillard reaction asparagine condensates with the carbonyl group of a reducing sugar to form a *N*-glycosyl conjugate which is in equilibrium with its Schiff base. From the Schiff base, the Maillard reaction would preferably proceed via the Amadori compound (pathway **I**), leading to the formation of flavor and color compounds. The low yield of acrylamide (1-5%) and the high yield of melanoidins confirm this (50-52, 54-57). The Amadori compound is not a favored intermediate in the formation of acrylamide (58). The acrylamide formation follows other pathways in the Maillard reaction. Several studies provided more evidence about these possible formation pathways (53, 58, 59). The Schiff base may decarboxylate to the intermediate azomethine ylide (pathway **II**), which can lead to the decarboxylated Amadori compound (53,59). The decarboxylation of the Schiff base may proceed via the zwitterionic form (pathway **IIa**) or via intramolecular cyclization to oxazolidin-5-one intermediate (pathway **IIb**) (53,59). The azomethine ylide may react to imine **I**, assumed by the higher tendency of the carbonyl group in β-position to the nitrogen atom to delocalize the negative charge (pathway **IIIa**). Hydrolysis of imine **I** leads to the Strecker aldehyde (3-oxopropanamide). This aldehyde, however, did not release high amounts of acrylamide (58, 60). Alternatively, the azomethine ylide intermediate may react to imine **II** (pathway **IIIc**), which preferably hydrolyzes (pathway **IVa**) to the decarboxylated amino acid 3-aminopropionamide (58). Granvogl and Schieberle have shown that acrylamide can be formed efficiently from 3-aminopropionamide by elimination of ammonia (61). According to Wedzicha *et al.* (62) imine **II** can also form acrylamide after protonation and β-elimination (pathway **IVb**). Tautomerization of azomethine ylide, which leads to a decarboxylated Amadori product (pathway **IIIb**), followed by breaking of the carbon-nitrogen covalent bond as a consequence of a β-elimination reaction releases acrylamide with an aminoketone (58). Next to the α-hydroxycarbonyls, such as reducing sugars, α-dicarbonyls or any other carbonyl may act as carbonyl source in the formation reaction of acrylamide from asparagine (50, 59, 61, 63-65). The α-hydroxycarbonyls generate, however, much higher amounts of acrylamide compared to α-dicarbonyls or aldehydes (58, 60).

2.3.3 Alternative routes for the formation of acrylamide

As stated earlier, the main mechanism for the formation of acrylamide in foods is the reaction of asparagine and reducing sugars in the Maillard reaction. There are, however, other reaction routes for the formation of acrylamide. These reactions play a minor role in the overall acrylamide generation and are, therefore, described only briefly.

Acrolein
Acrolein is an unsaturated aldehyde and can be produced from lipids (triglycerides) by strong heat treatment (66). Small amounts of acrolein can be found in some foods, such as fried foods, cooking oils and roasted coffee (67). Yasuhara *et al.* have shown that acrylamide can be formed from acrolein and these authors proposed two pathways (68). In the first pathway, acrylic acid produced from acrolein reacts with ammonia (produced from α-amino acids via Strecker degradation in the presence of carbonyl compounds) to produce acrylamide. The second pathway involves an acrylic radical formed from hemolytic fusion of acrolein that absorbs an amine radical formed from an amino acid to yield acrylamide (68).

Acrylic acid
Acrylic acid, which plays a role in the formation of acrylamide from acrolein (68), can be formed by thermal decomposition of aspartic acid, carnosine and β-alanine (69-71). Acrylic acid can also be generated indirectly from serine and cysteine via the formation of pyruvic acid (72). Acrylic acid, as mentioned in the 'acrolein pathway', reacts with available ammonia to form acrylamide. The formation of acrylamide from acrylic acid is limited by the availability of free ammonia in foods (72).

3-aminopropionamide
3-aminopropionamide, which can be an intermediate in the Maillard reaction between asparagine and reducing sugars (**Figure 2.5**), can also be formed from enzymatic decarboxylation of asparagine (73, 74). It was demonstrated that 3-aminopropionamide is a very effective precursor of acrylamide in the absence of further 'catalysts' such as carbonyls (73, 74).

2.4 PRODUCTION OF POTATO CRISPS AND THE FORMATION OF ACRYLAMIDE

Although acrylamide is only formed during the frying step in the production process of fried potato products, there are different factors in the other steps in the process that can have an influence to what extent acrylamide is formed. **Figure 2.6** illustrates the complexity of all the factors that can influence the final acrylamide content in fried potato products and how this also influences the quality of the products (75). In the following sections an overview of the production process of potato crisps is given and the main factors influencing the formation of acrylamide in the various steps of the process are discussed.

2.4.1 Potato crisps production process

Potato crisps (*English*) or chips (*North-America and Europe*) are thin slices, around 0.8–1.7 mm thick, which are deep-fried in oil until they are dry and brittle (76). It is said that crisps originated in the United States in the mid-nineteenth century in Saratoga and were known under the name of Saratoga chips (77). **Figure 2.7** gives a general overview of the production process of potato crisps. The process involves different steps. The potatoes are inspected, washed and peeled and cut into slices. The slices are again washed to remove starch and sugars from the surface. Next, the slices are sorted and checked before blanching, and partially dried afterwards to remove excessive water from the surface. The partially dried slices are immersed in oil having a starting temperature of around 180-190°C and an end temperature of around 150-175°C. After frying, they are salted and flavored. The crisps are once again inspected for their quality (burned or underdone crisps) and cooled down before packaging.

2.4.2 Mitigation strategies

Since the discovery of acrylamide, industry and research institutes have studied the possibilities to reduce the formation of acrylamide in foods. The Confederation of the Food and Drink Industries (CIAA) has developed a toolbox for the mitigation of acrylamide (78). This toolbox gives a summary and brief description of the different strategies industry can apply to reduce the formation of acrylamide in their products and can maintain the product quality. Recently, the ILSI Europe Task Force on Process-related Compounds published a report on the risk-benefit considerations of mitigation measures on acrylamide (79). The main strategies in the reduction of acrylamide in the production process of potato crisps will be discussed here by means of the most influential steps in the production process presented in **Figure 2.7**.

Potato selection

Dry matter, starch and reducing sugar content play an important role in the overall quality of potato crisps. The composition of potatoes depends among others on potato variety, growing temperature, soil moisture and maturity stage (80). Reducing sugar concentration and the amino acid profile of the potato play also a role in the extent of the acrylamide formation. Various studies have shown that there is a good correlation between acrylamide formation and the amount of reducing sugars (81-86). The effect of the amount of asparagine on acrylamide formation seems to be negligible relative to the reducing sugar concentration (87), although a study by Viklund *et al.* has shown that low asparagine concentrations can have an influence on acrylamide formation (88). By selecting varieties low in sugar and asparagine, manufacturers of potato crisps could lower the acrylamide concentration in their products. Furthermore, plant breeders have traditionally focused their attention on developing potato varieties for the industry with low amounts of sugar; by developing potato cultivars with lower amounts of asparagine they could contribute to the lowering of acrylamide formation (89, 90). Another factor that can contribute to the potential of acrylamide formation is the amount of fertilization. The free asparagine content in potatoes increases with an increased

use of nitrogen fertilizer (91). Decreased use of nitrogen fertilizer can, however, lead to increased reducing sugar content, so an appropriate balance should be found to lower the potential of acrylamide formation (91).

Potato storage

Potatoes are stored at low temperatures to minimize losses from spoilage and shrinkage. Too low temperatures (4-5˚C) or too high (35˚C) temperatures can result in crisp discoloration (80). Too low temperatures also result in a process called cold-induced sweetening (CIS); in this process starch is being converted into reducing sugars, which has an effect on acrylamide formation (92, 93). Too high temperatures can result in breaking dormancy of the tubers leading to sprouting (89). The sprouting can, however, be controlled by the use of chloropropham (CIPC). The use of sprouting inhibitors seems to have no significant influence of the potential of acrylamide formation (92, 94). Before the stored potatoes are processed, they are reconditioned; this process involves a short time (days to weeks) in which the potatoes are stored at higher temperatures (12-20˚C). Reconditioning lowers the reducing sugar levels and therefore has its effect on acrylamide formation. By selecting the right storage and recondition settings, manufacturers can lower acrylamide concentrations in their products.

Slicing and washing

The washed and peeled potatoes are sliced in thin slices of about 0.7–1.8 mm. The optimal thickness is 1–1.2 mm (80). The slice thickness determines the surface-to-volume ratio. A lower surface-to-volume ratio could have a lowering effect on the formation of acrylamide. It must be stated that the organoleptic properties of the crisps are also affected by the surface-to-volume ratio, and frying times can be prolonged. The longer frying time, however, can have an opposite effect, resulting in a subtle difference (89). Washing or soaking is used to rinse off starch, sugars and other substances present on the surface of the slices. The removal of starch and sugars improves the quality of the crisps. Reducing the sugar on the surface also reduces color and acrylamide formation (89).

Blanching

In some potato processing plants blanching is used prior to frying potato slices in order to improve the color of crisps. Blanching processes are performed either in water or in solutions such as sodium bisulfite, phosphoric acid, sodium citrate, citric acid, calcium chloride or magnesium chloride (89). The blanching solution is heated to 65–95˚C, blanching takes one minute or longer. The high temperatures make the cell membranes of the potatoes more permeable, increasing the extraction of sugars and asparagine (95). Blanching can reduce acrylamide formation in potato crisps significantly (85, 89, 96-98). However, blanching also impacts on the texture and flavor of the crisps, increases the oil content and leaches out vitamin C (78, 99). The use of vinegar, acetic acid or citric acid lowers the pH of the blanching solution, which can have a significant effect in lowering acrylamide formation in crisps (96, 100-103). The use of other additions, such as calcium chloride and different amino acids has also been tested, resulting mostly in lower acrylamide concentrations (103-105).

Frying

Frying is the crucial step in the formation of acrylamide in crisps. The high temperature (150–190°C) of the oil and the presence of asparagine and reducing sugars create the ideal situation for acrylamide formation. The process steps mentioned before were all focused on precursor control; in the frying process the parameters to control acrylamide formation are temperature and time (86). Choosing the right temperature and time combination, low concentrations of reducing sugars and asparagine can reduce the formation of acrylamide significantly. Lowering the thermal input by lower frying temperatures or shorter frying times results usually in lower acrylamide concentrations (101, 106, 107). However, lower temperatures may result in longer frying times to obtain the same quality, which can have finally overall no effect on lowering acrylamide formation (30). Acrylamide can be degraded at very high temperatures (>180°C), high-temperature, short time frying could potentially lead to lower acrylamide levels (30, 50, 108). We noticed a slightly decreasing acrylamide content in the crisps in time, which could be caused by the degradation reaction of acrylamide as will be discussed in **Chapter 5** and **6**.

2.4.3 Critical Quality Points

Food quality can be interpreted as an extension of food safety (109). Acrylamide levels in crisps can, therefore, been seen as a negative quality attribute. For instance in Germany efforts are made by industry and governmental authorities to minimize acrylamide levels in food (110). The principle of Critical Quality Points (CQP) is to assure final quality of the product by controlling those points in the production process that are critical for the final quality attribute as discussed in the previous sections. Using the principle of CQP, one could make an extensive analysis of the important stages in ones own production process to control acrylamide levels and maintain the acrylamide level under predefined upper levels.

2.5 MODELING

The complexity of the Maillard reaction and the formation routes of acrylamide have been illustrated above. There are different ways to gain insight in the Maillard reaction or in the formation of acrylamide. One way to get insight in how reactions occur is the use of labeled reactants or intermediates; this is an ideal way to establish the reaction pathways (46). However, these studies often do not quantify the main intermediates and end products, and therefore do not give insight to what extent the reactions occur and how temperature, time or pH can influence the reactions. Knowledge of the kinetic parameters, rate constants and activation energies, can be valuable to predict the extent of the reactions and can contribute in for instance reducing the formation pathway of acrylamide in the Maillard reaction. With simple kinetic models (zero, first and second order reaction models) one individual reaction pathway is fitted (111). The technique of multiresponse modeling takes the kinetic modeling to a higher level; reactants, intermediates and end products are studied at the

same time and simultaneously fitted to a complex reaction system of different pathways, including temperature dependence for the rate constants by the use of an Arrhenius relationship. By applying this multiresponse technique in the mechanistic studies, one could obtain more insight in reaction pathways and gain more information as degradation reactions of reactants and intermediates is simultaneously analyzed with the formation of intermediates and end products.

Most mechanistic studies to unravel the reaction networks of the formation of acrylamide use aqueous or dry model systems. However, these mechanistic studies in model systems are not easily applied to real food systems, as most foods are complex mixtures of a number of compounds. Because of the complex food matrices, reactants cannot meet each other readily and their activity will be different from model experiments; furthermore, generally there are temperature and concentration gradients within the food during heating. Hence, mechanistic models have to be combined with mass and heat transfer models, and adapted or modified to account for the physical changes in the food product, such as cell shrinkage and damage, cell separation and starch gelatinization that occur in potato products during processing (112). Using models that only give a mathematical description of the formation or degradation of acrylamide in food bypasses the problem of considering all the mechanisms that occur during processing of foods. Corradini and Peleg demonstrated that the formation and degradation of acrylamide could be successfully modeled by mathematical functions, where confounding effects of heat and mass transfer effects are taken up in empirical constants (113). These empirical models can be quite useful in describing and predicting the formation of acrylamide in food and can be used in the development of predictive models. In **Chapter 5** the use of these empirical model to describe the formation of acrylamide in potato crisps is studied. The performance of these models was further investigated in **Chapter 6** and the predictive capacity of the best performing empirical model was tested. These predictive models seem to be a promising tool to be applied in CQP to control acrylamide levels.

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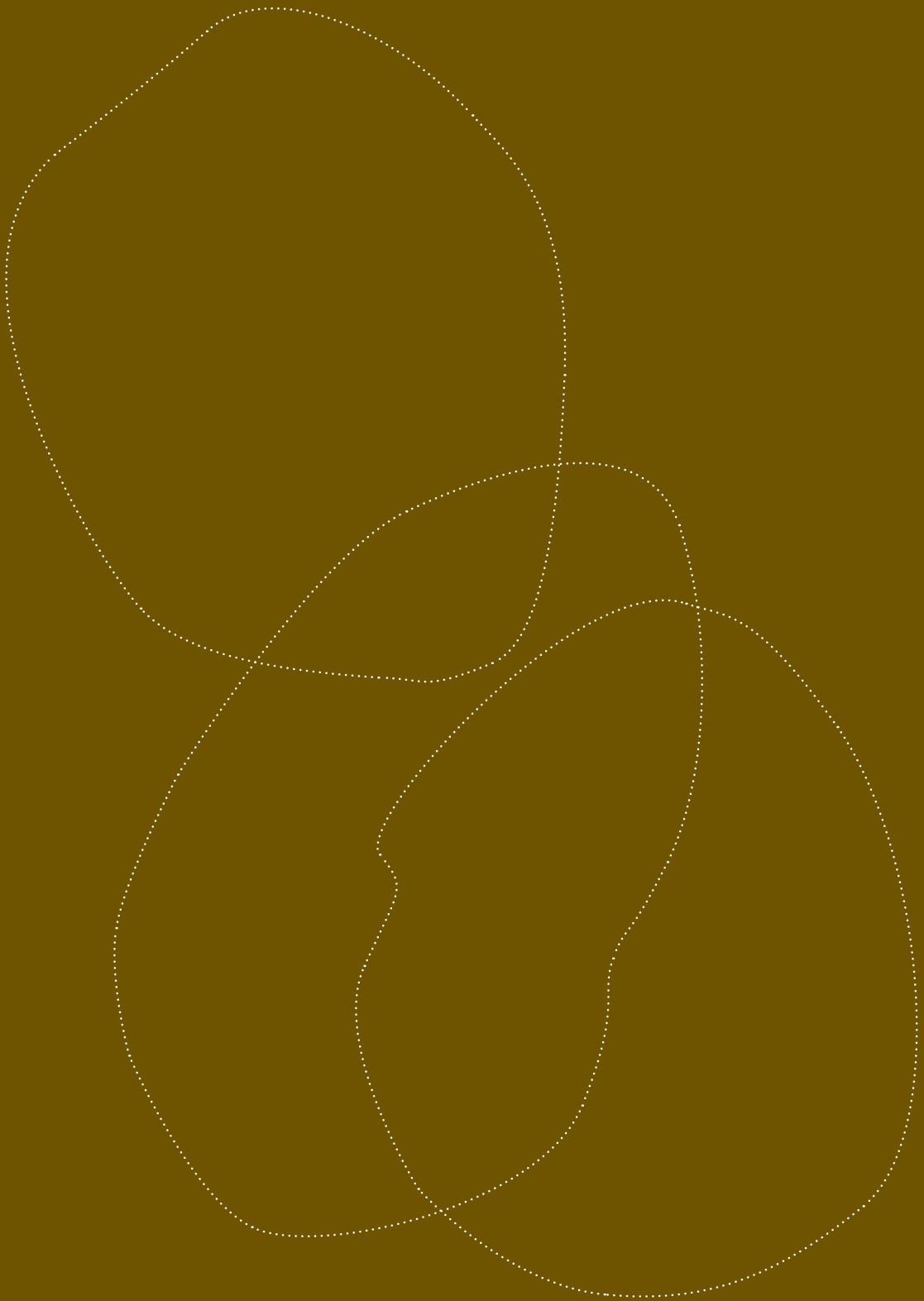
Chapter 3

Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system



A kinetic model for the formation of acrylamide in a glucose-asparagine reaction system is proposed. Equimolar solutions (0.2 M) of glucose and asparagine were heated at different temperatures (120-200 °C) at pH 6.8. Besides the reactants, acrylamide, fructose, and melanoidins were quantified after predetermined heating times (0-45 min). Multiresponse modeling by use of nonlinear regression with the determinant criterion was used to estimate model parameters. The proposed model resulted in a reasonable estimation for the formation of acrylamide in an aqueous model system, although the fate of glucose, fructose, and asparagine was slightly underestimated. The formation of acrylamide reached its maximum when the concentration of sugars was reduced to about 0. This supported previous research, showing that a carbonyl source is needed for the formation of acrylamide from asparagine. Furthermore, it is observed that acrylamide is an intermediate of the Maillard reaction rather than an end product, which implies that it is also subject to a degradation reaction.

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3.1 INTRODUCTION

In April 2002, Tareke *et al.* (1) reported that several heat-processed foods contain relatively high amounts of acrylamide. These results were confirmed by other research groups: fried and baked potato products were found to contain the highest amounts of acrylamide, followed by cereal products, whereas only low amounts were detected in meat products (2-4). A high amount in foods is of major concern as acrylamide is known to be a neurotoxic, genotoxic, and carcinogenic compound in animals (5), which is classified by the IARC (6) as a probable human carcinogen.

About half a year later the suggestion was published that acrylamide is formed in the Maillard reaction and that asparagine is the precursor (7-9). Further elucidation of the pathway by use of ^{13}C-labeled isotopes showed that asparagine needs a carbonyl source to form acrylamide (10, 11). According to Friedman (5), the proposed mechanisms predict that acrylamide may result from the general reaction of asparagine with any aldehyde or ketone. Simultaneously, many researchers focused on optimization of methods for determination of acrylamide. A review about this subject was published by Wenzl *et al.* (12). Evaluation of the health risk resulted in the recommendation that intake levels of acrylamide should be reduced to protect health, although there is no direct risk on cancer with the current intake of about 35 μg day^{-1} person^{-1} (13, 14). Possible ways to reduce the formation of acrylamide include reducing the temperature, reducing precursors during processing, and influencing the pathway of formation by changing the pH (15-17). More information on research, analysis, formation, and control of acrylamide is available in an extensive review that was published recently (18).

The Maillard reaction is highly complex and cannot be described with simple reaction kinetics. To better understand mechanisms behind the formation of acrylamide, an advanced approach is necessary (19). Kinetic modeling, using multiresponse models, has proven to be a useful tool to enable further unraveling of reaction mechanisms in the Maillard reaction (20, 21). Knowledge of kinetics provides the tool to predict the effect of certain time-temperature combinations in a quantitative way.

Model systems of asparagine and a carbonyl such as glucose, both dry and in aqueous solutions, have been used mainly to study the pathway of acrylamide formation (3, 22). The yield of acrylamide was about 0.1% of asparagine, and a wide range of other compounds (*e.g.*, melanoidins) was found. Furthermore, an effect of time and temperature on both formation and degradation of acrylamide has been found. Recently, a kinetic model for the formation of acrylamide in potato, wheat, and rye model systems was published by Wedzicha *et al.* (23). However, using food model systems to gain kinetic insights into reactions can cause disturbances by the presence of food matrix components. Therefore, the aim of this study was to attempt to model the formation of acrylamide in a glucose-asparagine reaction system at different time-temperature combinations. Once information about the kinetics of formation of acrylamide is obtained, one can move on to more realistic systems to describe the effect of complicating factors as present in real foods.

3.2 MATERIAL AND METHODS

3.2.1 Chemicals

The following compounds were obtained commercially: D-glucose, D-fructose, Na_2HPO_4, and KH_2PO_4 (Merck, Darmstadt, Germany) and L-asparagine (Fluka, Buchs, Switzerland). All chemicals used in this study were of analytical grade.

3.2.2 Preparation of reaction mixtures

Equimolar solutions of glucose and asparagine (0.2 M) were prepared in phosphate buffer (0.1 M, pH 6.8). Samples (10 mL) were heated in hermetically closed screw-capped glass tubes (Schott, 16 × 160 mm) at 120, 140, 160, 180, and 200 °C in an oil bath. The tubes were immersed in the oil up to the cap. At predetermined heating times (0, 1, 2, 4, 9, 15, 30, and 45 min), samples were taken and immediately cooled in ice and stored at -20 °C prior to analysis. Experiments were carried out in triplicate.

3.2.3 Analysis of acrylamide

The analysis of acrylamide was based on the HPLC method described by Barber *et al.* (24). Prior to analysis, samples were diluted in Millipore water (1:10) and centrifuged for 5 min at 16000*g*. The HPLC system used for the analysis consisted of a P4000 pump, an AS3000 autosampler, and a UV3000 detector (Thermo Separation Products, San Jose, CA). A Synergi 4 μm hydro reversed-phase C18 column (80 Å, 250 × 2.00 mm) with AJO-4286 guard column (Phenomenex, Aschaffenburg, Germany) was used for the analysis (25). Samples (20 μL) were eluted isocratically at 20 °C with a solution containing 1% methanol and 99% 5 mM heptanesulfonic acid in Millipore water at a flow rate of 0.2 mL/min. Acrylamide (t_r = 6.2 min) was detected by its absorbance at 200 nm with a UV detector and quantified by the external standard procedure with a calibration curve. The limit of detection obtained (10 $\mu g\ kg^{-1}$) was similar to what Barber *et al.* (24) found.

3.2.4 Analysis of sugars

The diluted samples (1:10) were analyzed for sugars by HPLC (see **section 3.2.3**) by use of the method of Martins *et al.* (26). An ion-exchange column (ION-300, Interaction Chromatography Inc., San Jose, CA) with guard column was used for the analysis at 85 °C. Sulfuric acid (2.5 mM) in Millipore water was used as the eluent with a flow rate of 0.4 mL/min. Sugars were detected by monitoring the refractive index and quantified by the external standard procedure with a calibration curve. The formation of sugar isomers in the Maillard reaction has been confirmed by HPLC and electrochemical detection recently (27, 28).

3.2.5 Analysis of asparagine

Samples were diluted 1:1000 with Millipore water and the EZ:faast amino acid analysis kit (Phenomenex, Aschaffenburg, Germany) was used for determination of asparagine (29). Norvaline was added as an internal standard (20 nmol) before sample preparation. After sample preparation, samples were analyzed on a Carlo Erba GC5300 system (Interscience BV, Breda, The Netherlands) on a Zebron amino acid column (10 m × 0.25 mm) that was included in the kit. Samples (2.5 µL) were injected onto the column at 250 °C (split ratio 1:15). The oven temperature started at 110 °C and was programmed to heat to 250 °C at a rate of 20 °C/min, followed by a 1 min isothermal hold. The external standard procedure was used for quantification.

3.2.6 Analysis of melanoidins

Quantification of melanoidins was performed by measuring the absorbance at 470 nm on a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). When necessary, the samples were diluted with Millipore water. The concentration of melanoidins was calculated from the Lambert-Beer equation with an extinction coefficient of 282 L/mol·cm (30), a value derived for melanoidins formed from glucose and asparagine. The concentration of melanoidins is thus expressed as moles of sugar incorporated in the brown polymers. Although melanoidins are of course complex mixtures of various molecules, several studies have shown that they can be quantified in this way (20, 31, 32).

3.2.7 Kinetic modeling

A kinetic model was derived from the proposed reaction network taken from Stadler *et al.* (33) (shown in **Figure 3.1**). Our arguments for using the reaction network published by Stadler *et al.* (33) and the steps taken to derive a kinetic model will be discussed further on. For each reaction step, a differential equation was set up, and these were translated into a mathematical model. The differential equations were solved by numerical integration. For both numerical integration and parameter estimation, the software package Athena Visual Studio v. 10.0 (34) was used. The parameters of the model, that is, the rate constants and activation energies, were estimated by nonlinear regression by use of the determinant criterion (20). For modeling purposes, the individually measured concentrations were used (measured in triplicate). For the visualization of the experimental results (**Figures 3.2 - 3.6**), the average values with their standard deviation (represented by the error bars) were used.

3.3 RESULTS AND DISCUSSION

3.3.1 Identification and quantification of reactants and main products

During heating of the glucose-asparagine reaction mixtures, the concentrations of the reactants decreased over time. As expected, the rate of degradation of glucose and asparagine increased with temperature. At 180 and 200 °C, a complete loss of glucose was observed after 9 and 5 min, respectively (**Figure 3.2**). The loss of asparagine (**Figure 3.3**) was slower compared to the loss of glucose. A complete loss of asparagine was measured at 180 and 200 °C after 30 and 9 min, respectively. The loss of sugar being faster than the loss of amino acid is in line with what has been found in a glucose-glycine reaction system by Martins and Van Boekel (20). The slower loss can possibly be explained by the regeneration of asparagine from the initial condensation products such as the Amadori rearrangement product and the possible formation of diglucosyl-amine (20).

As fructose and mannose are known to be formed from glucose by isomerization (26), some formation of these compounds was expected. Analysis showed no formation of mannose, but fructose was indeed formed (**Figure 3.4**). This corresponds with findings of Brands and Van Boekel (35) and Martins and Van Boekel (20), where only fructose was found as an isomerization product of glucose. There was a clear maximum at 160, 180, and 200 °C in the formation of fructose at 9, 4, and 2 min, respectively. For the reaction mixtures heated at 180 and 200 °C, these maximum values were followed by a complete loss after 30 and 9 min, respectively. The decrease in fructose concentration can be explained by the reversible isomerization reaction between glucose and fructose so that fructose re-forms back into glucose, which is then consumed. However, it is also quite likely that fructose participates in the formation of acrylamide (36-38).

The initial rate of formation of acrylamide increased with temperature (**Figure 3.5**). At 140 and 160 °C, the increase in acrylamide concentration was followed by a steady state in which the formation of acrylamide was probably in equilibrium with its degradation. At 180 and 200 °C, the increase was followed by a fast decrease. The maximal acrylamide formation at 180 and 200 °C corresponded with the time at which the concentration of glucose and fructose reached zero (**Figures 3.2, 3.4**). This is in line with the findings of Yaylayan *et al.* (10), who showed that asparagine needs a carbonyl source to form acrylamide. The decrease in acrylamide can be explained by its reactive nature. The increase and subsequent decrease of the acrylamide concentration at 180 and 200 °C is a typical behavior for an intermediate (20). Acrylamide may have polymerized or reacted further in Michael-type addition reactions (33). Wedzicha *et al. (23)* attributed the loss of acrylamide after prolonged heating at 180 °C to the reactivity of acrylamide with food components. These pathways have not been fully elucidated, and further research in this area is necessary to expand the reaction network of the Maillard reaction between asparagine and glucose.

Melanoidins are known as the main end products of the Maillard reaction. The structure of these brown nitrogenous polymers and copolymers is largely unknown, which makes quantification difficult. Previous studies have shown that it is possible to relate the absorbance to the number of sugar molecules incorporated in the melanoidins in aqueous model systems (20, 30, 32). At 160, 180, and 200 °C, we observed after some time the formation of insoluble particles, which are probably high molecular weight melanoidins. The absorbance measurements were hindered by the scattering effect of these particles. Moreover, the values for absorbance decreased during formation of these particles, causing an underestimation of the melanoidins concentration. Because of this, we excluded the results for the reaction mixtures where these solid compounds were found. This was the case for the reaction mixtures heated for 45 min at 160, 180, and 200 °C, for 30 min at 180 and 200 °C, and for 15 min at 200 °C. Considerable browning was observed in samples during heating. The induction time, during which no browning was detected, decreased with increasing temperature (**Figure 3.6**). Furthermore, the formation of acrylamide could not be associated directly with the degree of coloring of the reaction mixtures at 160, 180, and 200 °C after 15, 9, and 4 min, respectively. This is due to their difference in status: acrylamide is an intermediate, whereas melanoidins are the main end products of the Maillard reaction. As our proposed kinetic model applies only to an aqueous model system, this relationship cannot be used yet directly in food systems. The relationship between acrylamide and color formation in foods, for instance the one found in yeast-leavened wheat bread by Surdyk *et al.* (37), is induced by effects such as concentration gradients, diffusion and evaporation, and the presence of other compounds such as other amino acids and sugars and buffering agents. Therefore, more research is needed with model systems that can take these effects into consideration.

3.3.2 Proposal of a kinetic model based on a reaction network

By analyzing the reactant degradation and the formation of the main products in the Maillard reaction between glucose and asparagine, a kinetic model could be proposed. The mathematical model derived from the kinetic model was then confronted with the experimental data to test the hypothesized model. Several reaction networks for the formation of acrylamide in foods have been proposed. These publications have revealed the Maillard reaction as the major reaction pathway involved (3, 7-9). The hypothesis proposed by Mottram and co-workers (7, 23) emphasizes the pathway of Strecker degradation. The hypothesis proposed by Stadler *et al.* (8), however, pointed toward glycoconjugates such as N-glycosides formed in the early Maillard reaction as key intermediates. A recent publication by Stadler *et al.* (33) supports their hypothesis as well as the work published by Zyzak *et al.* (11) and Yaylayan *et al.* (10). Furthermore, Stadler *et al.* (33) suggest that the mechanism proposed by Mottram *et al.* (7) is not a main one. We did not pursue the identification and quantification of all these suggested intermediates in this study and, therefore, we are not able to shed more light on this matter. However, we chose the reaction network proposed by Stadler *et al.* (8), shown in **Figure 3.1A**, as initial basis for the proposal of a kinetic model, as their proposed reaction network has been more elaborately described in recent literature (33). To fit the model to the experimental data, this reaction network was first adapted to reduce

the number of parameters. Because the intermediates (*e.g.*, *N*-glycosyl conjugate, azomethine ylide) were not analyzed and quantified in our experiments, the number of parameters was relatively high in comparison to the number of measured responses. Second, the new reaction network proposed in **Figure 3.1B** also takes the measured responses for fructose and melanoidins and the observed loss of acrylamide into account. The formation of fructose was suggested by including the reversible isomerization reaction between glucose and fructose. Furthermore, the reaction between asparagine and fructose was added, since fructose is also a reactant in the formation of acrylamide (36-38). The formation of melanoidins was suggested to result from further reaction of Schiff base, and the loss of acrylamide was accounted for by the putative formation of hitherto unknown product(s).

3.3.3 Test of the hypothesized mechanism

The proposed reaction model presented in **Figure 3.1B** was translated into a mathematical model. For each reaction step, a differential equation was set up by use of the law of mass action, and the obtained differential equations were solved by numerical integration. The temperature dependence, which plays a major role in the Maillard reaction, was also taken into account by including an Arrhenius relationship between the rate constants (k) of the different reactions. The Arrhenius equation

$$k = k_o \cdot \exp\left(\frac{-E_a}{R \cdot T}\right) \tag{3.1}$$

where k_o is the so-called frequency factor, R is the gas constant [8.314 J/(mol·K)], E_a is the activation energy (J/mol), and T is the absolute temperature (Kelvin), was reparametrized to avoid statistical problems in estimation (39) as follows:

$$k = X \cdot \exp\left(-Y \cdot E_a\right) \tag{3.2}$$

where

$$X = k_o \cdot \exp\left(\frac{-E_a}{R \cdot T_{av}}\right) \tag{3.3}$$

$$Y = \frac{1}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{av}}\right) \tag{3.4}$$

$$T_{av} = \frac{\Sigma T}{n} \tag{3.5}$$

The model was fitted to the data obtained at 120, 140, 160, 180, and 200 °C at pH 6.8 simultaneously by substituting **Eq. 3.2** for the rate constants.

At the lower reaction temperatures (120 and 140 °C), quite a good fit was observed for all compounds (**Figures 3.7 – 3.11**). For the acrylamide and melanoidins concentrations, the model fits the data well at all temperatures (**Figures 3.10, 3.11**). However, the good fit for the melanoidins concentration may have been partially caused by the lack of data for the higher temperatures. For the estimation of the acrylamide concentration, the model was not restrained by experimental data for the products formed in the degradation reaction from acrylamide, and therefore, the model was able to fit the loss of acrylamide (k_6) to the experimental observations for acrylamide. More knowledge about the reaction products from acrylamide would validate the estimated rate constants for the formation and loss of acrylamide.

The observed lack of fit for the individual responses was rather high, especially for fructose and at the higher temperatures. The model underestimated the decrease in glucose concentration, especially during the first 10 min (**Figure 3.7**). The increase in fructose was also underestimated, but the model predicted the tendencies as found in the experimental observations (**Figure 3.9**). The same goes for asparagine, where there was an underestimation for the decrease (**Figure 3.8**). These underestimations are undoubtedly caused by the limitations of the present model. Reactions of glucose and fructose are limited to the isomerization reaction and the reaction with asparagine to form the Schiff base. The model does not take into account the degradation of sugars to carbohydrate fragments and the formation of compounds via the Strecker degradation route. Therefore, this model is currently under further investigation, but nevertheless the results already show some remarkable phenomena that are worth discussing.

The estimated parameters, that is, rate constants and activation energies, are shown in **Table 3.1**. The rate constants for the different reaction temperatures have been derived from the estimated values found for X. The estimated values of X are the same as the derived values for the rate constants at 160 °C, as T_{av} in this case is 160 °C. The temperature dependence is consistent for all six reactions, but the precision of estimation for the parameters is low. The average 95% highest posterior density interval is about ±27% for the rate constants and ±11% for the activation energies. The activation energy for the formation of the Schiff base from fructose and asparagine is about 2 times higher than the activation energy for the same formation reaction with glucose and asparagine as reactants, suggesting that the contribution from fructose becomes more important at higher temperatures. The rate of the Schiff base formation reaction from fructose and asparagine (k_3) increases, therefore, much faster with increasing temperature than the rate constant of reaction 1 (k_1). At 120 °C, k_3 is 35% lower than k_1, but at 180 °C k_3 is 2-3 times higher than k_1. This is consistent with the observations by Robert *et al.* (36) that fructose is more efficient in the formation of acrylamide than glucose by a factor of about 3 at 180 °C. The higher reactivity of fructose with asparagine in the formation of the Schiff base could make the reversible isomerization step of fructose to glucose insignificant. Therefore, model discrimination was performed for our proposed model with or without the reversible isomerization reaction from fructose to glucose.

Table 3.1

Estimates of rate constants[a] and activation energies[a] as found by kinetic modeling for the proposed kinetic model.

k	120°C	140°C	160°C	180°C	200°C	E_a (kJ/mol)
k_1 *	0.131 ± 0.025	0.308 ± 0.049	0.668 ± 0.13	1.35 ± 0.36	2.58 ± 0.89	57.6 ± 8.0
k_2 **	4.98 ± 1.2	16.7 ± 3.0	50.1 ± 9.6	136 ± 35	341 ± 114	81.7 ± 8.6
k_3 *	0.0819 ± 0.045	0.369 ± 0.14	1.45 ± 0.44	5.04 ± 1.6	15.8 ± 6.4	102 ± 14
k_4 **	0.176 ± 0.081	0.712 ± 0.23	2.53 ± 0.51	8.05 ± 1.2	23.2 ± 4.3	94.4 ± 11
k_5 **	15.7 ± 3.5	28.4 ± 4.6	48.7 ± 5.9	79.6 ± 8.9	125 ± 16	40.1 ± 5.0
k_6 **	7.96 ± 5.1	28.1 ± 13	88.1 ± 25	250 ± 45	650 ± 136	85.1 ± 14

[a] ± 95% highest posterior density (HPD) interval; * 10^{-3} l·mmol·min^{-1}; ** 10^{-3} min^{-1}.

Model discrimination can be used to provide information about the most plausible model (20). In this case we used the Akaike criterion (the model with the lowest value is preferred) and the posterior probability (the model with the highest posterior probability is preferred) to assess the most plausible model (40, 41). The results from our test, shown in **Table 3.2**, support the model without the reversible isomerization reaction.

Table 3.2

Model discrimination results for our proposed model with (**A**) or without (**B**) the reversible isomerization reaction from fructose to glucose[a].

model	*p*	SS	*n*	AIC_c	ΔAIC_c	PPB	PPS
A	16	$1.32 \cdot 10^5$	600	6505.2	27.3	-89.83	0.234
B	14	$1.29 \cdot 10^5$	600	6477.9	0.0	-89.33	0.757

[a] *p*, number or parameters; SS, residual sum of squares; *n*, number of data points including the replicates; AIC_c, Akaike information criterion; ΔAIC_c, AIC_c difference with the smallest value taken as reference; PPB, log10 of posterior probability; PPS, normalized posterior probability share.

3.4 CONCLUDING REMARKS

The aim of this study was to work toward a kinetic model for the formation of acrylamide in the Maillard reaction of glucose and asparagine at an initial pH of 6.8. Despite the current underestimation for the behavior of sugars and asparagine, the proposed model gives a reasonable estimation for the formation of acrylamide in an aqueous model system. The kinetic model supports the observations (11) that for the formation of acrylamide from asparagine a carbonyl source is needed, and our results suggest an important role for fructose, especially at the higher temperatures. Fructose is invariably formed from glucose

isomerization. Furthermore, the experimental observations and the kinetic modeling suggested that acrylamide is not an end product in the Maillard reaction. The behavior of acrylamide suggests that it is an intermediate. The multiresponse model derived in this study is a first step into the realization of a tool that can be used to predict how acrylamide reduction in foods containing asparagine and reducing sugars can be accomplished. Further research is on the way to determine intermediate reaction products in order to extend the kinetic model. In addition, the formation of compounds via the Strecker degradation route and the degradation of sugars into carbohydrate fragments will be investigated to improve the current kinetic model in estimating the behavior of sugars and asparagine.

3.5 ACKNOWLEDGMENTS

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Chapter 4

Unraveling the kinetics of the formation of acrylamide in the Maillard reaction by multiresponse modeling

A kinetic model for the formation of acrylamide in a fructose-asparagine reaction system at pH 5.5 is proposed based on an approach called multiresponse kinetic modeling. The formation of acetic acid and formic acid from the degradation of fructose and its isomer glucose was included in the proposed kinetic model. The kinetic model suggests that the effect of temperature on acrylamide formation with fructose is more due to the preceding steps. The higher yield of acrylamide with fructose as reducing sugar at pH 5.5 as opposed to the yield of acrylamide with glucose at pH 6.8 suggests that both pH and type of sugar can play a role in lowering acrylamide formation. Furthermore, these models have shown that at high temperatures (120–200˚C) the Maillard reaction rapidly goes into the advanced stages, forming high amounts of organic acids and high molecular weight melanoidins. Overall, these mechanistic models provided more insight in the formation of acrylamide in a quantitative way.

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4.1 INTRODUCTION

The Maillard reaction or non-enzymatic browning, reported almost a century ago by Louise Camille Maillard (1), has still not been unraveled or understood completely. The comprehensive reaction scheme presented by John E. Hodge (2) in 1953 was a major achievement in showing the complexity of the underlying chemistry of the Maillard reaction and scientists have developed and elaborated this original scheme, advancing steadily the knowledge about the Maillard reaction. Traditionally, the main focus has been on components affecting color, flavor and taste development as these play an important role in quality of foods. However, another aspect of the Maillard reaction has gained more attention recently: the formation of mutagens and carcinogens in the Maillard reaction (3). The discovery of acrylamide in foods has greatly contributed to this renewed interest in the Maillard reaction (4). Acrylamide, probably carcinogenic to humans, was found to be present in heat-treated foods, such as French fries, potato crisps, coffee and bread (4, 5). The Maillard reaction has been found to be the main source for the formation of acrylamide in foods with asparagine and reducing sugars as the main precursors (6-8).

To control the formation of acrylamide in the Maillard reaction, the steps of interest need to be studied in a quantitative way. Kinetic knowledge about individual reaction steps would make it possible to describe and predict the formation of acrylamide at any time-temperature combination. The kinetics of acrylamide formation has been studied earlier, by fitting simple kinetic models (first- and second-order reactions) to only the formation and degradation reaction of acrylamide while neglecting preceding reaction steps (9, 10). Although these studies give insight in how time, temperature and pH can influence the formation of acrylamide, these models do not give insight in the actual reaction mechanism. Applying multiresponse modeling techniques to estimate kinetic parameters helps in building mechanistic models (11, 12). The basic idea of multiresponse modeling is to take into account all measured concentration changes (responses) of reactants, intermediates and end products simultaneously as opposed to only one response (*e.g.* acrylamide) in simple kinetics. The main advantage of such an approach is that models can be tested more rigorously and, once the goodness of fit is deemed acceptable, estimation of parameters can be done much more precisely, by applying appropriate statistical techniques to optimally use the information that is contained in all available data (13). This multiresponse modeling approach has been successfully used for modeling the Maillard reaction in different reaction systems (13-17).

The aim of the present study was to develop a comprehensive kinetic model for the formation of acrylamide in the Maillard reaction between fructose and asparagine at pH 5.5 using the multiresponse modeling approach. In an earlier study (**Chapter 3**) we have shown by multiresponse modeling that fructose had a higher reactivity with asparagine in the formation of acrylamide; therefore, we have focused further on the behavior of this reducing sugar. Furthermore, the degradation of glucose and asparagine was underestimated by this earlier proposed model (**Chapter 3**), as the possible degradation pathways of these components were not incorporated in the model. The formation of acetic acid and formic acid, a major pathway (18) for the degradation of sugars, was therefore included in the present study. The pH of the

system was adjusted to pH 5.5, which is more in line with the pH that is found in potatoes (19). For the development of the multiresponse models, firstly, the main reaction products were identified and quantified in heated fructose-asparagine reaction systems. Secondly, the main reaction pathways were established. Finally, the proposed kinetic models were put through the iterative process of kinetic modeling:

- Propose a model
- Confront the model with experimental data
- Adjust the model until a satisfactory model has been obtained

The parameters (rate constants and activation energies) of the kinetic model were estimated by simultaneously fitting the proposed reaction network to the experimental data obtained at five temperatures (120, 140, 160, 180 and 200°C). The information obtained from these models can then be used to address the problem of acrylamide formation in more realistic and complex systems, namely real foods.

4.2 MATERIAL AND METHODS

4.2.1 Chemicals

The following compounds were obtained from commercial sources: Acetonitril, acetic acid, D-glucose, D-fructose, formic acid, KH_2PO_4, Na_2HPO_4, sulfuric acid (Merck, Darmstadt, Germany); acrylamide, L-asparagine (Fluka, Buchs, Switzerland). All chemicals used in this study were of analytical grade.

4.2.2 Preparation of reaction mixtures

Equimolar solutions of fructose and asparagine (0.1 M) were prepared in phosphate buffer (0.1 M, pH 5.5). Samples (10 mL) were heated in hermetically closed screw-capped stainless steel tubes (Workshop, Wageningen University, The Netherlands) at 120, 140, 160, 180 and 200°C in a heating block (Liebisch, Bielefeld, Germany). At predetermined heating times (0, 1, 2, 4, 8, 16, 32 and 64 min), samples were taken and immediately cooled on ice and stored at –20°C prior to analysis. Experiments were carried out in duplicate (0, 16, 32 and 64 min) or triplicate (1, 2, 4, 8 min).

4.2.3 Analysis of acrylamide

The analysis of acrylamide was based on the HPLC-UV method used in **Chapter 3**, which was adapted from Barber *et al.* (20). Prior to analysis, samples were diluted in Millipore water (1:5 or 1:10) and filtered on a 0.2 μm filter (Grace, Deerfield, IL, USA). The HPLC system used

for the analysis consisted of a P2000 pump, an AS3000 autosampler, and a UV2000 detector (Thermo Scientific, Waltham, MA, USA). A Synergi 4μ Hydro Reversed Phase C18 column (80 Å, 250 × 2.00 mm) with AJ0-4286 guard column (Phenomenex, Torrance, CA, USA) was used for the analysis. Samples (20 μL) were eluted isocratically at 20 °C with a solution containing 6% acetonitril and 94% Millipore water at a flow rate of 0.2 mL/min. Acrylamide (t_r = 4.8 min) was detected by its absorbance at 210 nm with a UV-detector, and quantified by the external standard procedure using a calibration curve.

4.2.4 Analysis of asparagine

The diluted samples (1:10 or 1:20) were analyzed for asparagine by HPLC-ELS. The HPLC system used for the analysis consisted of a P2000 pump, an AS3000 autosampler (Thermo Scientific, Waltham, MA, USA), and an Alltech 3300 ELSD (Grace, Deerfield, IL, USA). A Prevail C18 5μ column (250 × 4.6 mm) with guard column (Grace, Deerfield, IL, USA) was used for analysis. 5mM heptafluorobutyric acid in Millipore with 0.7% trifluoroacetic acid (A) and acetonitril (B) were used as mobile phase with the gradient: 0% B until 6 min, from 6 to 8 min from 0% B to 15% B and from 8 to 25 min from 15% B to 35% B. The flow rate of the mobile phase was 1 mL/min; column temperature was ambient (20˚C). Asparagine was detected with the following settings of the ELS detector: temperature drift tube 60˚C, gas flow 2.0 L/min, gain 1. Quantification was done by external standard procedure using a calibration curve.

4.2.5 Analysis of sugar

The diluted samples (1:10) were analyzed for sugars by HPLC-RI using the method of Martins *et al.* (21). The HPLC system used for the analysis consisted of a P2000 pump, an AS3000 autosampler, and a RI-71 detector (Thermo Scientific, Waltham, MA, USA). An IOA-1000 Organic Acids column (300 × 7.8 mm) with guard column (Grace, Deerfield, IL, USA) was used for the analysis at 85˚C. Sulphuric acid (2.5 mM) in Millipore water was used as eluent with a flow rate of 0.4 mL/min. Sugars were detected by monitoring the refractive index and quantified by the external standard procedure using a calibration curve. The formation of sugar isomers in the Maillard reaction has been confirmed by HPLC and electrochemical detection previously (22, 23).

4.2.6 Analysis of organic acids

The diluted samples (1:5 or 1:10) were analyzed for organic acids by HPLC-DAD. The HPLC system used for the analysis consisted of a P2000 pump, an AS3000 autosampler, and a UV6000 diode array detector (Thermo Scientific, Waltham, MA, USA). A Prevail Organic Acids 5μ column (250 × 4.6 mm) with guard column (Grace, Deerfield, IL, USA) was used for the analysis at 20˚C. 25 mM KH_2PO_4 acidulated to pH 2.5 with phosphoric acid was used a mobile phase with a flow rate of 1.0 mL/min. Organic acids were detected by monitoring

the UV spectrum between 180–380 nm for the UV-absorbance profile and at 210 nm for quantification by external standard procedure using a calibration curve.

4.2.7 Analysis of melanoidins

Melanoidins were quantified by measuring their absorbance at 470 nm using a Cary 50-Bio spectrophotometer (Varian, Palo Alto, CA, USA). When necessary, the samples were diluted with Millipore water. The concentration of melanoidins was calculated using the Lambert-Beer equation with an extinction coefficient of 282 L/mol·cm (24); a value derived for melanoidins formed from glucose and asparagine. The concentration of melanoidins is thus expressed as moles of sugar incorporated in the brown polymers. Although melanoidins are, of course, complex mixtures of various molecules, several studies have shown that they can be quantified in this way (13, 16, 25, 26).

4.2.8 Kinetic modeling

Kinetic models were derived from the proposed reaction networks. For each reaction step, a differential equation was set up, and these were translated into a mathematical model. The differential equations were solved by numerical integration. For both numerical integration and parameter estimation, the software package Athena Visual Studio v. 11.0 was used (27). The parameters of the model, *i.e.*, the rate constants and activation energies were estimated by non-linear regression using the determinant criterion (13). Although the experimental conditions were non-isothermal in the first minutes of the experiments (**Figure 4.1**), the system was considered isothermal for modeling purposes, which has been done in previous studies (13, 16). For modeling, individually measured concentrations were used. For the visualization of the experimental results (**Figures 4.2 – 4.9**), the average values with their standard deviation (represented by the error bars) were used.

4.3 RESULTS AND DISCUSSION

4.3.1 Identification and quantification of reactants and main products

During heating of the fructose-asparagine reaction mixtures, the concentrations of the two reactants decreased over time. The rate of degradation increased with temperature, as was expected. Almost a complete loss of fructose was observed at 180 and 200°C after 32 and 16 min, respectively (**Figure 4.2**). The loss of fructose was comparable at 120 and 140°C, although it should be noted that the results for the first minutes at 120 and 140°C showed large variability. The loss of asparagine was comparable to the loss of fructose at 120°C and

200˚C (**Figure 4.3**). At 140, 160 and 180˚C the loss of asparagine was, however, somewhat quicker than the loss of fructose. Almost a complete loss of asparagine was observed at 180 and 200˚C after 32 and 8 min, respectively. In our earlier study with a glucose-asparagine reaction system we did not found a faster decrease of asparagine compared to the sugar compound (**Chapter 3**). A possible explanation is a lower regeneration of asparagine from the initial condensation products or an increased degradation of asparagine at pH 5.5. Heating of asparagine without the presence of sugar at 200˚C for 8 min resulted in a variety of unidentified fragments, which were noticed on the HPLC chromatogram obtained with the method described for the analyses of organic acids. In a study by Talley *et al.* (28, 29) fumaramic and aspartic acid were identified as major breakdown products of asparagine. However, we were not able to identify and quantify these breakdown products of asparagine.

Isomerization of reducing sugars in aqueous model systems has been reported earlier (16, 21), and with fructose the formation of glucose and mannose was expected. Analysis showed no formation of mannose, but glucose was indeed formed (**Figure 4.4**). This corresponds with the earlier studies where only fructose was found as isomerization product of glucose (13, 16, 30). A clear maximum in glucose formation was found at 160 and 200˚C at 32 and 4 min, respectively. At 180˚C the maximum formation of glucose was between 8 and 16 min. For the reaction mixtures heated at 180 and 200˚C, these maximum values were followed by almost a complete loss after 32 and 16 min, respectively. The decrease in glucose concentration can be explained by reversible isomerization, the participation of glucose in the formation of acrylamide and by degradation to sugar fragments and organic acids (**Chapter 3**). The isomerization of fructose to glucose was three times lower compared to the isomerization of glucose to fructose at pH 6.8 (**Chapter 3**). A possible explanation for the lower isomerization of fructose to glucose can be the higher reactivity of fructose with asparagine in the formation of the Schiff base, as we found previously in a glucose-asparagine reaction model at pH 6.8 (**Chapter 3**) or an effect of the lower pH. Martins *et al.* have shown that the isomerization of fructose to glucose decreased with decreasing pH (13). Incidentally, it has been shown by Brands and van Boekel (14) that the reaction between fructose and casein to form advanced Maillard products is faster than with glucose-casein, which is in line with the present finding.

The initial rate of acrylamide formation increased with temperature (**Figure 4.5**). At 160, 180 and 200˚C the increase of acrylamide was followed by a slow decrease or steady state in which the formation of acrylamide and degradation of acrylamide were probably in balance. The maximal acrylamide formation at 160, 180 and 200˚C corresponded with the time at which the asparagine concentration and to a certain degree also the fructose concentration were almost reduced to zero (**Figures 4.2, 4.3**). The yield of acrylamide was higher compared to the yield in a glucose-asparagine reaction system, reaching almost 3% of the initial asparagine concentration (**Chapter 3**). The higher yield of acrylamide could be the result of the higher reactivity of fructose or the result of a lower pH. However, in another study lowering the pH from 8 to 6 resulted in a lower acrylamide formation in a phosphate buffered glucose-asparagine reaction system (10), which suggests that fructose is more reactive than glucose.

Regarding the formation of organic acids in the fructose-asparagine reaction system, acetic acid was always formed in higher concentrations than formic acid, independent of the temperature (**Figures 4.6, 4.7**), which is in line with the observations of Martins and van Boekel (13). The rate of formation of formic acid increased with temperature (**Figure 4.6**). At 180 and 200˚C the formation of formic acid reached a steady state, where the formation and degradation of formic acid could be in balance or a precursor for the formation of formic acid is not present anymore. The formation of formic acid never exceeded more than 15% of the degraded D-fructose. This is three times as much as has been reported for a glucose-glycine system heated for 4 h at 100˚C (13), which is probably due to the higher reaction temperatures (>120˚C). The formation of acetic acid also increased with increasing temperatures (**Figure 4.7**). At 160, 180 and 200˚C there was a maximum in the formation of acetic acid. At 160˚C this was followed by a steady state between 32 and 64 min and at 180 and 200˚C this was followed by a decrease after 16 and 8 min, respectively. The formation of acetic acid was considerable, reaching a maximum yield of 120% (% mmol/mmol fructose). Davídek *et al.* have shown that there are several pathways for the formation of acetic acid from the degradation of glucose (18). D-fructose can be degraded at high temperatures into acetic acid and formic acid and other carbohydrate fragments, which can also be further degraded into small organic acids (13, 18, 31). Furthermore, acetic acid can be formed via the pathway of 1-deoxyosone and formic acid via 3-deoxyosone (13). The considerable formation of acetic acid and other organic acids that can be formed in the Maillard reaction and degradation of asparagine (29) had an influence on the pH of the reaction system.

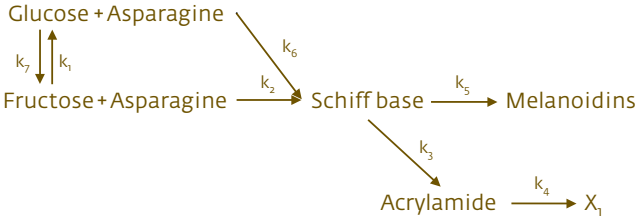
The pH decreased with increasing temperature and the maximum in the decrease was also attained after shorter heating times (**Figure 4.8**). In the reaction systems heated at 180 and 200˚C the pH showed a maximum decrease after 32 and 16 min, respectively. In the case of 200˚C, the final pH even exceeded the initial pH. The maximum decreases in pH corresponded with the acetic acid reaching its maximum formation. The increase of pH at 180 and 200˚C also corresponded with the decrease of acetic acid. It should be noted that the buffering capacity of the 0.1M phosphate buffer was not strong enough, dissolving the reactants lowered the initial pH of the buffer already from 5.5 to 5.3. However, increasing the phosphate buffer concentration would enhance the acrylamide formation more (10). Another aspect that should be taken into consideration is the fact that the pH of the system was measured after cooling down the samples to room temperature, therefore, the exact pH at the actual temperatures is unknown. The effect of the changes in pH on the course of the reactions in the fructose-asparagine reaction systems is unclear. In principle a reduction of pH slows down the Maillard reaction. However, it was also found, under different conditions, that if the pH decrease during an experiment stays within one pH unit, the effects are not considerable (13). Due to technical restrictions it was impossible to perform the experiments at constant pH as the reaction systems are heated at high temperatures (>120˚C) in hermetically closed reaction tubes. For modeling purposes the change in pH has not been taken into account, which implies that possible pH effects, if any, are hidden in the parameters obtained from the models.

The structure of melanoidins (low and high molecular weight, brown, nitrogenous chromophores), also known as Maillard reaction end products responsible for color formation, is largely unknown, which makes quantification difficult. Previous studies have shown that it is possible to relate the absorbance caused by the melanoidins to the number of sugar molecules incorporated in the melanoidins in aqueous model systems, though this was not studied at the high temperatures used in this study (13, 24, 25). The effect of temperature on melanoidin formation is generally in line with the literature (**Figure 4.9**). As reported earlier, at high temperatures insoluble high molecular weight melanoidins can be formed, which hinder absorbance measurements by the scattering effect and can cause an underestimation of the melanoidins concentration (**Chapter 3**). This was the case for the reaction mixtures heated at 180 and 200˚C for more than 16 and 8 min, respectively. In foods color formation and acrylamide formation can be correlated (32, 33). In our experiments, the formation of acrylamide could not be associated with the formation of melanoidins. In aqueous model systems reactants can meet each other readily. However, in foods other factors such as concentration gradients, diffusion, evaporation and the presence of other amino acids and sugars can have an effect on relation between the formation of melanoidins and acrylamide.

Figures 4.10 – 4.14 show the results of the mass balance calculations for the five different temperatures. There are some shortcomings in the mass balance calculations; the mass balance is calculated as the percentage of the initial fructose concentration, products can also be formed from the breakdown of asparagine concentration, which could lead to higher recoveries. Another aspect is the melanoidin concentration, which is expressed as moles of sugar incorporated and therefore is not a really accurate quantification. Unfortunately, there are no methods for the precise quantification of the mol concentration of melanoidins. However, these mass balance calculations do provide some insight into the recovery of reactants and main products in the reaction system, which are useful for the process of kinetic modeling. Too high recoveries will undoubtedly lead to problems. At 120˚C, the sum of all the identified and quantified products reached about 80% of the fructose initial concentration (**Figure 4.10**). The difference is likely the result of not having identified and quantified the numerous intermediates that can be formed in the Maillard reaction. At 140˚C, the same result can be seen in the first minutes of the experiments, later on the recovery comes to almost 100% (**Figure 4.11**). At 64 min, however, the calculated mass balance reaches almost 120%, which can be the result of the shortcomings of the way the mass balance is calculated. At 160, 180 and 200˚C, the recovery is almost a 100% in the first 4-8 minutes (**Figures 4.12 – 4.14**). At the longer reaction times, the recoveries also exceed 100%, with the exception at 64 min at 180 and from 16 min to 64 min at 200˚C where the recovery decreases to about 85% and 65%, respectively. These results indicate that at the higher temperatures, the Maillard reaction rapidly proceeds to the formation of stable end products such as melanoidins and organic acids.

4.3.2 Proposal of a kinetic model based on a reaction network

By analyzing the reactants' degradation and the formation of the main products in the Maillard reaction between fructose and asparagine, a kinetic model could be proposed based on earlier reported reaction networks for the Maillard reaction and the formation of acrylamide in particular. For the formation of acrylamide in the Maillard reaction several reaction networks have been proposed (6, 7, 34-37) of which the one proposed by Stadler *et al.* (34) is the most comprehensive and supports the work of Zyzak *et al.* (36) and Yaylayan *et al.* (35) and emphasizes the formation of *N*-glycosides in the early Maillard reaction as the key intermediate. The hypothesis proposed by Mottram and co-workers (6, 37) emphasizes the pathway of the Strecker degradation. **Figure 4.15** gives an overview of the pathways that could lead to the formation of acrylamide in the Maillard reaction. This reaction network was simplified and building on the work of Brands *et al.* (14, 15) resulted in a first kinetic model (**Scheme 4.1**), which is also based on our earlier proposed model (**Chapter 3**).



Scheme 4.1 Simplified kinetic model proposed for the formation of acrylamide from asparagine through early Maillard reaction.

The formation pathway leading to acrylamide has been simplified because the intermediates such as *N*-glycosyl conjugate, Schiff base and imines were not quantified and therefore the number of parameters describing the fate of these compounds would be too high compared to the number of responses. The kinetic model, next to the formation of acrylamide, takes into consideration the degradation of acrylamide (X_1), the responses for asparagine, fructose, glucose, and melanoidins. The formation of glucose was suggested by including a reversible isomerization reaction between fructose and glucose and the role of glucose in the formation of acrylamide was also included. Furthermore, the formation of melanoidins was suggested to result from further reaction of the Schiff base. Perhaps, it should be noted that the Amadori product is not modeled here. In the reaction of ketoses, such as fructose, one would expect the Heyns product, but Brands and van Boekel (14) were not able to show a Heyns product in the reaction of ketoses with casein. We therefore bypass the formation of the Heyns product altogether.

4.3.3 Test of the hypothesized mechanism

The proposed reaction model presented in **Scheme 4.1** was used as the starting point of the iterative process of kinetic modeling. The reaction network was translated into a mathematical model and for each reaction step, a differential equation was set up by use of the law of mass action, and the obtained coupled differential equations were solved by numerical integration. To test the temperature dependence, which plays a major role in the Maillard reaction, an Arrhenius relationship was also taken into account for the rate constants (k) of the different reactions. The Arrhenius equation is:

$$k = k_o \cdot \exp\left(\frac{-E_a}{R \cdot T}\right) \tag{4.1}$$

k_o is the so-called frequency factor, R is the gas constant [8.314 J/(mol·K)], E_a is the activation energy (J/mol), and T is the absolute temperature (K). The Arrhenius equation was reparametrized to avoid statistical problems in estimation (11) as follows:

$$k = X \cdot \exp\left(-Y \cdot E_a\right) \tag{4.2}$$

where

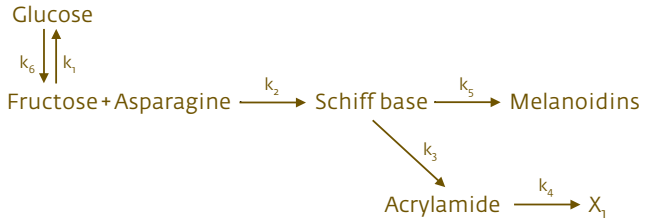
$$X = k_o \cdot \exp\left(\frac{-E_a}{R \cdot T_{av}}\right) \tag{4.3}$$

$$Y = \frac{1}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{av}}\right) \tag{4.4}$$

$$T_{av} = \frac{\Sigma T}{n} \tag{4.5}$$

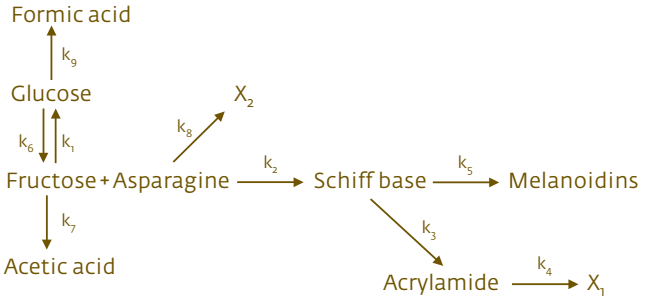
The model was fitted to the data obtained at 120, 140, 160, 180 and 200°C at pH 5.5 simultaneously by substituting **Eq. 4.2** for the rate constants.

The model of reaction network presented in **Scheme 4.1** resulted in negative values (results not shown) for the formation of the Schiff base from glucose and asparagine (k_6) and for the isomerization reaction of glucose to fructose (k_7). In our previous study we have shown that fructose has a higher reactivity in the reaction with asparagine to form the Schiff base (**Chapter 3**). The results suggest that the formation of the Schiff base at pH 5.5 from glucose and asparagine is not significant in a fructose-asparagine reaction system. The results forced us to reconsider the kinetic model. **Scheme 4.1** was adjusted to **Scheme 4.2** by the removal of the reaction pathway from glucose and asparagine leading to the Schiff base.



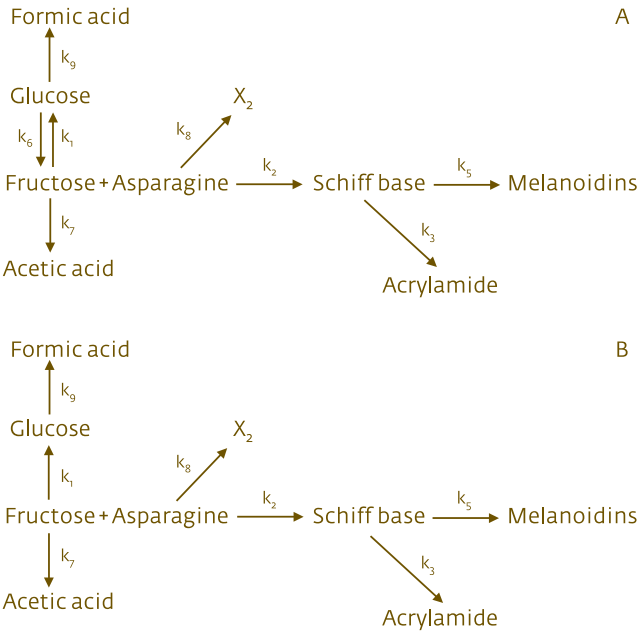
Scheme 4.2 Modified kinetic model proposed for the formation of acrylamide from asparagine through early Maillard reaction.

The model of the reaction network of **Scheme 4.2** showed better results. Although the result for E_{a4} was negative and the ±95% highest posterior density could not be determined for k_3, E_{a3} and E_{a5}, the model fitted the data at 120, 140 and 160˚C reasonably well for the sugars, asparagine, melanoidins and acrylamide (results not shown). At 180 and 200˚C the model underestimated the degradation of fructose and asparagine, which was expected, as degradation routes for fructose and asparagine were not included in the model. The fit of the formation and subsequent decrease of glucose was also problematic at 180 and 200˚C. The kinetic model was therefore again changed, resulting in **Scheme 4.3** in which the degradation of fructose and glucose was included by the pathways leading to acetic acid and formic acid, respectively.



Scheme 4.3 Modified kinetic model proposed for the formation of acrylamide from asparagine through early Maillard reaction.

For both acetic acid and formic acid we applied the rule that 1 mole of fructose or glucose can, theoretically, be broken down to 3 moles acetic or formic acid. The degradation of asparagine was incorporated by including the putative formation of unknown reaction products (X_2). As discussed above, heat-induced degradation of asparagine does occur, though we were not able to identify the reaction products. The fate of reactants and end products was described well by the model based on **Scheme 4.3** (results not shown). However, the activation energy for the breakdown of acrylamide was negative, which suggests that with increasing temperature the degradation of acrylamide would decrease and this is of course not true. Therefore, the degradation route of acrylamide was omitted in **Scheme 4.4**.



Scheme 4.4 Modified kinetic model proposed for the formation of acrylamide from asparagine through early Maillard reaction. **A**) Model with reversible isomerization of glucose to fructose (k_6); **B**) Model without reversible isomerization of glucose to fructose.

The degradation of acrylamide in the fructose-asparagine reaction system (shown in **Figure 4.5**) was not as significant as reported earlier in a glucose-asparagine system with pH 6.8 (**Chapter 3**) and apparently the model has difficulty in estimating the breakdown process. Therefore, this step was omitted in the next model. We also applied model discrimination to see if the model could be further optimized by removing the reversible isomerization reaction of glucose to fructose (**Scheme 4.4B**). Model discrimination can be used to provide information about the most plausible model (13). In this case we used the Akaike criterion (the model with the lowest value is preferred) and the posterior probability (the model with the highest posterior probability is preferred) to assess the most plausible model (38, 39). The results from our test, shown in **Table 4.1**, support the model of **Scheme 4.4B** without the reversible sugar isomerization reaction.

The multiresponse model based on **Scheme 4.4B** described the fate of reactants and end products reasonably well (**Figures 4.16 – 4.22**). The formation of and subsequent decrease of formic acid and melanoidins was fitted well by the model for all temperatures (**Figures 4.20, 4.22**). The formation of acrylamide was fitted well at 120 and 140˚C, at 160, 180 and 200˚C from 4 tot 32 min the formation was underestimated. For the reactants, including breakdown pathways did improve the predictions of the model. However, the decrease of fructose and asparagine was still underestimated at 180 and 200˚C. For acetic acid and glucose, the

Table 4.1

Model discrimination results for the proposed model based on **Scheme 4** with (**A**) or without (**B**) the reversible isomerization reaction from glucose to fructose (k_6).

Model	p	SS	n	AIC_c	ΔAIC_c	PPB	PPS
A	18	$1.14 \cdot 10^5$	700	18182.7	48.2	-74.5	0.05
B	16	$1.13 \cdot 10^5$	700	18134.6	0.0	-73.2	0.95

p (number of parameters); SS (Residual Sum of Squares); n (number of data points including the replicates); AIC_c (corrected Akaike Criterion); ΔAIC_c (AIC_c difference taking the smallest value as reference); PPB (Log10 of Posterior Probability); PPS (Normalized Posterior Probability Share).

Table 4.2

Estimates of rate constants (k) at 120, 140, 160, 180 and 200 °C and activation energies (E_a) ± 95% Highest Posterior Density (HPD) interval as found by kinetic modeling for the proposed kinetic model presented in **Scheme 4B**.

k	120°C	140°C	160°C	180°C	200°C	E_a (kJ/mol)
k_1*	0.55 ± 0.1	1.4 ± 0.2	3.1 ± 0.4	6.5 ± 1	13 ± 3	61 ± 8
k_2**	0.020 ± 0.01	0.078 ± 0.03	0.27 ± 0.06	0.86 ± 0.2	2.4 ± 0.6	93 ± 12
k_3*	1.9 ± 2	3.1 ± 3	5.0 ± 3	7.8 ± 3	12 ± 4	35 ± 27
k_5*	30 ± 37	55 ± 48	95 ± 52	157 ± 51	246 ± 82	40 ± 26
k_7*	1.7 ± 0.7	5.2 ± 1	14 ± 3	36 ± 7	84 ± 21	75 ± 10
k_8*	3.9 ± 2	8.3 ± 3	16 ± 5	30 ± 10	53 ± 23	50 ± 16
k_9*	2.0 ± 1	7.1 ± 2	22 ± 5	62 ± 13	159 ± 48	84 ± 14

* ($\cdot 10^{-3}$ min^{-1}); ** ($\cdot 10^{-3}$ $l \cdot mmol^{-1} \cdot min^{-1}$)

model was able to predict the trends in the formation, but also at the higher temperatures (160–200°C) the formation of these compounds was underestimated. The over- and underestimations of the model are the results of not including into the kinetic model all possible degradation and formation routes. Identifying and quantifying more reaction products in the reaction between fructose and asparagine could improve the model. Nevertheless the results from the present model show some remarkable phenomena that are worth discussing.

The estimated parameters, that is, rate constants and activation energies, are shown in **Table 4.2.** The rate constants for the different reaction temperatures have been derived from the estimated values found for X. The estimated values for X are the same as the derived values for the rate constants at 160°C, as T_{av} in this case is 160°C. The temperature dependence of the present model is based on an Arrhenius relationship, which implies that the present reaction network applies for the temperature range of 120–200°C and the temperature induced change in rate constants fit this Arrhenius relationship. The precision of some of the parameters is low, which indicates that the number of data points should increase to improve precision. Nevertheless, the model seems to perform well overall and several remarks can be made from the results.

The rate constant for the formation of the Schiff base from fructose and asparagine (k_2) at pH 5.5 is lower than at pH 6.8 (**Chapter 3**). This can be the effect of protonation of asparagine amino groups, which blocks the nucleophilic addition of the carbonyl compound, preventing the formation of the Schiff base (40-42). The rate constant for the formation of acrylamide from the Schiff base (k_3) at pH 5.5 was at 120˚C about a factor 2 higher compared to pH 6.8 (**Chapter 3**). The estimated activation energies were in the same range as earlier reported values for the reaction at pH 6.8, only the activation energy for the formation of acrylamide from the Schiff base was almost a factor 3 lower (**Chapter 3**). This suggests that the effect of temperature on acrylamide formation with fructose is more due to preceding steps. Indeed, the E_a for the Schiff base formation is the highest in this network. However, it should be noted that the precision of the estimated values for the activation energies was rather low, so we cannot draw definitive conclusions yet.

4.4 CONCLUDING REMARKS

The aim of this study was to work towards a more comprehensive kinetic model for the formation of acrylamide in the Maillard reaction of fructose and asparagine at an initial pH of 5.5. The proposed kinetic model was able to describe well the fate of reactants and main products. The model, therefore, does give more insight into the reaction network for the formation of acrylamide in a quantitative way, though more data are needed to improve the precision of some parameters. This would be the next iteration step in the modeling process: now that an adequate model is obtained, attention should be given to obtain better estimates. The higher yield (3% of the initial asparagine concentration) of acrylamide at pH 5.5 with fructose compared to pH 6.8 with glucose with a yield of 1.5% suggests that in food systems the type of reducing sugar and pH can play a role in lowering acrylamide formation. The high yield of acetic acid suggests that the Maillard reaction at high temperatures can rapidly go into the advanced stages with the formation of organic acids and high molecular weight melanoidins. Further elucidation of this advanced stage of the Maillard reaction by identifying and quantifying these advanced degradation products could contribute to further knowledge about the Maillard reaction at higher processing temperatures.

4.5 ACKNOWLEDGMENTS

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Chapter 5

A study on the use of empirical models to predict the formation of acrylamide in potato crisps

The formation of acrylamide in potato crisps was fitted by empirical mathematical models. Potato slices were fried under the same experimental conditions for different times. Besides the content of precursors in the raw potato slices, acrylamide and water content in the potato crisps were quantified after predetermined times (2–6 min). The temperature developments in the surrounding oil and outer cell layer of the potato slices were monitored, giving more insight in the frying process and making future comparisons between studies possible. The pattern found for the formation of acrylamide, which was similar to earlier studies, was fitted to three empirical models. Statistical methods were used to compare the performance of the models, with the 'Logistic-Exponential' and 'Empirical' model performing equally well. The obtained model parameters were in the range of earlier reported studies, although this comparison is not unequivocal as the experimental conditions differed between studies. The precision of parameter estimates was problematic; this should be improved by better experimental design. Nevertheless, the approach of this study will make it possible to truly compare acrylamide formation patterns and model parameters in the future, with the ability to develop a tool to predict acrylamide formation in potato crisps.

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5.1 INTRODUCTION

During the last decades, consumers and the food industry have been confronted with the discovery of several food-borne toxicants, *i.e.*, potential carcinogens that are not naturally present in foods, but may have developed during preservation or cooking. Well-known examples of these food-borne toxicants are nitrosamines, heterocyclic amines and polycyclic aromatic hydrocarbons (1–3). The latest addition to this list of food-borne toxicants is acrylamide. Acrylamide, classified by the International Agency for Research on Cancer (IARC) as 'probably carcinogenic to humans', was found to be present in heat-treated carbohydrate-rich foods (4, 5). This discovery led to worldwide concern due to the presence of acrylamide in products with a high level of consumption such as French fries, potato crisps, coffee, and bread. Although the amounts of acrylamide in some of these products are low, they can contribute significantly to the overall intake of acrylamide (6). The intake of acrylamide depends consequently on the consumption pattern and the acrylamide levels in foods.

Acrylamide levels in food and the reaction pathways leading to its formation have been widely investigated. Most investigations point towards the Maillard reaction as the source of acrylamide in heated food (7–9). Some studies suggest that there are different pathways next to the Maillard reaction (10–12), but these seem to be of less importance in the overall formation of acrylamide.

The kinetics and mechanisms of acrylamide formation are mostly studied in aqueous or dry model systems. The advantage of this approach is that relevant information is obtained about the actual mechanism of the reaction, especially when the technique of multiresponse modeling is applied to the various Maillard reaction pathways that lead, among others, to the formation of acrylamide (**Chapter 3** and **4**). These fundamental mechanistic studies cannot, however, be applied easily to real food systems. To predict the formation of acrylamide in real foods with these mechanistic models, it needs to be taken into account that reactants cannot meet each other readily and that there maybe temperature and concentration gradients. Hence, mass and heat transfer models need to be incorporated as well. On the other hand, studies (14–16) on the influence of different processing conditions on the formation of acrylamide in food do not often include the kinetics of acrylamide formation. Recently, an empirical modeling approach was suggested that would get round these difficulties (17). That is to say, the confounding effects of heat and mass transfer effects are taken up in empirical constants. It would also allow incorporating isothermal and non-isothermal kinetics in a relatively easy way. The objectives of this study were therefore to investigate the kinetics of acrylamide formation in real foods, namely fried potato crisps, under well-controlled conditions and to apply the empirical mathematical approach suggested by Corradini and Peleg (17) to study the kinetics of acrylamide formation in potato crisps. Furthermore, we have evaluated these models statistically and compared them as far as possible with the results from other studies. Although these empirical models will not give much insight in the mechanism behind the formation of acrylamide, as opposed to our mechanistic models (**Chapter 3** and **4**), empirical models can be quite useful in developing tools to control or reduce acrylamide in potato crisps in the short term.

5.2 MATERIAL AND METHODS

5.2.1 Preparation of crisps and frying conditions

Potatoes (*Solanum tuberosum* L.) of the variety Bintje grown in the south of Sweden were harvested in September 2005, and stored at 6°C from December 2005 until April 2006. Crisps were prepared as described by Viklund *et al.* (18). Briefly, potatoes (6–10 cm of diameter and weighing around 100–250 g) were washed and divided into halves lengthwise. One half of each potato was used for analysis of acrylamide precursors, and the other half was cut into 1.5 mm slices that were deep fried in rapeseed oil in three net cages placed on top of each other. Thermocouples (type K, 0.1 mm) were connected to a computer and used to monitor the temperature of the oil and potato crisps. One thermocouple was placed in each net cage to measure the oil temperature within the cage, one thermocouple was placed at the bottom of the oil bath, one as close as possible to the surface of a potato slice and one thermocouple was fixed to a potato slice with the tip of the thermocouple placed in the outer cell layer. The oil was preheated to 180°C. The potato slices were fried for 2, 3, 4, 5 and 6 min. When the potato slices were submerged in the oil bath, the oil temperature decreased rapidly. When the oil reached 160°C after about 1.5–2.0 min, the thermostat setting was changed and the temperature was kept constant at 160°C for the rest of the experiment. After frying, the crisps were cooled to room temperature on paper, and stored in plastic bags at –18°C until analysis.

5.2.2 Chemical analyses

The dry matter contents of the tubers and crisps were determined as described by Viklund *et al.* (18). The tubers were analyzed with regard to asparagine using HPLC and a fluorescence detector, and for glucose, fructose and sucrose using GC and a flame ionization detector (19). The acrylamide content in the crisps was analyzed using LC-MS/MS (18).

5.2.3 Empirical mathematical models

It is recognized that acrylamide is not only formed but also degraded during heat treatments above 160°C (13, 20–22). Therefore, models are needed that can handle this phenomenon. Corradini and Peleg (17) proposed three empirical models to describe the formation and degradation of acrylamide. These three models can be divided in two semi-empirical models 'Logistic-Fermi' and 'Logistic-Exponential', and one total empirical model 'Empirical'. The semi-empirical models have characteristic time parameters for the inflection points in the formation or degradation of acrylamide and steepness parameters around these inflection points. The total empirical model has abstract parameters and is simpler in appearance. The simplicity of this model, as opposed to the semi-empirical models, could lead to less computational problems in the estimation of parameters (17).

The Logistic-Fermi model (**Eq. 5.1**) describes the formation of acrylamide by a logistic function and the degradation by a Fermi-type function:

$$C(t)=\left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}}-\frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]}\right]\cdot\frac{1}{1+\exp\{k_2(T)[t-t_{c2}(T)]\}} \quad (5.1)$$

where $C(t)$ is the concentration of acrylamide, $t_{c1}(T)$ and $t_{c2}(T)$ are temperature-dependent time characteristics for the inflection points in the formation (t_{c1}) and degradation (t_{c2}) of acrylamide, $k_1(T)$ and $k_2(T)$ are temperature-dependent steepness parameters around the inflection points for the formation (k_1) and degradation (k_2) of acrylamide and $a(T)$ serves as a temperature-dependent 'scale factor' for the acrylamide concentration.

The Logistic-Exponential model (**Eq. 5.2**) differs from the Logistic-Fermi model in the part that describes the degradation of acrylamide. The Logistic-Fermi function predicts that at prolonged heating times the acrylamide concentration will become zero. However, the Logistic-Exponential function with its exponential term for the degradation predicts a residual acrylamide concentration at prolonged heating times:

$$C(t)=\left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}}-\frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]}\right]\cdot\exp\left(-\frac{t}{\tau(T)}\right) \quad (5.2)$$

where $\tau(T)$ is a temperature-dependent characteristic time. The Empirical model is expressed as:

$$C(t)=\frac{a(T)\cdot t^{n(T)}}{b(T)+t^{m(T)}} \quad (5.3)$$

where $a(T)$, $b(T)$, $m(T)$ and $n(T)$ are temperature-dependent constants.

5.2.4 Parameter estimation and statistical methods

The semi-empirical models 'Logistic-Fermi' and 'Logistic-Exponential' and the total empirical model 'Empirical', proposed by Corradini and Peleg (17), were fitted to the obtained experimental data for the acrylamide formation using the software program Mathcad® version 13.1 (PTC, Needham, MA, USA).

For the estimation of the model parameters, the command 'Minerr' was used to minimize the sum of squares. This command makes use of nonlinear regression and to determine the search direction in the iterative process the software used Quasi-Newton methods. The SD of the model parameters were estimated by linear approximation using the variance-covariance

matrix and mean squares. The Akaike information criterion (AIC) was used in the model discrimination to investigate statistical differences between models. The AIC gives a penalty for models that have more parameters (23). This is useful because a model with more parameters fits better in general but leads to more imprecision in parameter estimates. The general equation for the AIC is:

$$AIC = n \cdot \ln\left(\frac{SS}{n}\right)^2 + 2(p+1) \tag{5.4}$$

where n is the number of data points, p the number of estimated parameters and SS the residual sum of squares. The number of data points (n) was relatively small compared to the number of estimated parameters (p) (n/p<40) for all cases, therefore the corrected AIC, AIC_c, was used:

$$AIC_c = AIC + 2(p+1)\left(\frac{p+2}{n-p}\right) \tag{5.5}$$

The model with the lowest Δ_{AIC}, the difference between the AIC values with the lowest value as reference, performs the best.

5.3 RESULTS AND DISCUSSION

5.3.1 Temperature development in the potato crisps

The set-up used in these experiments has been described comprehensively by Viklund *et al.* (18). The laboratory frying conditions mimicked industrial processing and gave reproducible results regarding color, water content and acrylamide levels with no significant differences between duplicate batches (18). The temperature profile during frying plays an important role in the production of potato crisps. **Figure 5.1** shows the temperature profile of the oil at the bottom of the fryer and the temperature of the oil within the three net cages. The profile is in line with the temperature profile commonly found in industry where potato slices are immersed in oil having a starting temperature of around 180–190˚C and an end temperature of around 150–175˚C (24). The temperature within the cages was lower than in the rest of the oil bath. Within the first seconds, the temperature fluctuated between 150 and 170˚C and after this period stabilized around 160˚C. The temperature in the three cages was similar. **Figure 5.2** shows the temperature profile obtained by the thermocouple that was fixed to a potato slice with the tip placed in the outer cell layer. The results of this temperature measurement differed between experiments. It was reported earlier by Yildiz *et al.* (25) that obtaining a reliable temperature at the surface of potato slices is difficult and could lead to potential errors. They proposed another way of determining the surface temperature

by establishing the heat transfer coefficient based on measurements of time-dependent temperature and moisture content of potato slices. However, this approach could also lead to potential errors in the estimation, as the thermo-physical properties of the potato slices were assumed constant throughout the potato (25). In our approach, we tried to fix the tip of thermocouple into the outer cell layer to gain insight in the actual temperatures in this region. The observed differences in the measurements could be the result of the depth of the tip within the outer cell layer, but the temperature profiles could also be influenced by the changes that occur at the outer cell layer. Costa *et al*. (26) have illustrated the structural changes and shrinkage of potato slices during frying, which could lead to changes in the placement of the tip. The tip could be exposed to the oil instead of measuring the temperature within the potato cell layer. Another factor that could influence the temperature measurements in the cell layer is the release of water vapor from deeper parts of the potato slice. Because of the difficulties to obtain accurate temperature measurements in the outer cell layer, we have averaged the temperature profile to estimate the temperature in the part of the potato slice where the crust is formed, see **Figure 5.3**. Within the first seconds, the temperature increased to 120˚C, and from 10 to 35 s, the temperature increased to 140˚C. This rapid temperature increase was followed by a slight decrease to 135˚C, which could be caused by release of water vapor. After 45 s, the temperature steadily increased to 160˚C at about 210 s; from this moment on the temperature remained stable at 160˚C. Within the first 2 min of frying, the temperatures of the oil and the outer cell layer differed substantially, after 2 min, the temperature of the slices and the oil was almost constant at 160˚C. Although the temperature measurements are perhaps not as reliable as one would like, these measurements do give insight in the temperature development during the frying process of potato crisps. With this knowledge, one could truly compare the formation patterns of acrylamide with the results from studies where the temperature profile during frying of potato crisps has also been studied. Currently, these details are seldom mentioned or even studied for potato crisps (14–16, 27) making comparison of published results difficult and could lead to wrong conclusions. Gökmen *et al*. (28), however, have reported these details for the frying of French fries, creating the opportunity to compare the results of acrylamide formation in French fries, as this study does for the formation of acrylamide in potato crisps.

5.3.2 Chemical analyses

As explained in the introduction, the aim of this study was to investigate the use of empirical models; therefore, the results from the chemical analysis are described briefly to provide information about the precursor and water content, so that the results from modeling can be put into context with other studies. The fresh potatoes were analyzed for their sugar and asparagine content (n = 2). The fructose, glucose and sucrose concentration were 10.4 ± 0.11 mg/g dry matter (dm), 15.4 ± 0.26 mg/g dm and 5.9 ± 0.17 mg/g dm, respectively. The asparagine concentration was 11.7 ± 0.47 mg/g dm. It has to be mentioned that the variety Bintje is not a potato normally used for the production of potato crisps due to its high sugar content. The sugar content also increases during storage and declines in April-May (19).

Figure 5.4 shows the acrylamide and water content for potato slices fried for 2, 3, 4, 5 and 6 min. Due to the high concentration of precursors, the acrylamide concentration was high compared to acrylamide levels reported in literature (5, 10). Between 2 and 4 min, the concentration of acrylamide increased rapidly from 2.3 mg/kg dm to 18.6 mg/kg dm and stabilized between 4 and 6 min. At 6 min, the concentration of acrylamide was slightly lower than that at 4 and 5 min, probably reflecting the earlier mentioned breakdown of acrylamide (13, 21, 22). The initial water content of the raw potato slices was around 75% and decreased with frying time from 7% at 2 min to 1% at 4 min and remained around 1% from 4 to 6 min. During the decrease of water content from 7% to 2%, the formation of acrylamide rapidly increased. A similar pattern was found in an earlier study on acrylamide formation in crisps when slices of Saturna potatoes were deep fried for 2–4.5 min at 160°C (18).

5.3.3 Mathematical modeling

The precursors for acrylamide formation are not homogeneously distributed within potatoes and vary between potatoes (29). Subsequently, these concentrations change during processing due to different reactions and physical processes that occur, such as degradation reactions, water evaporation, starch gelatinization and competing Maillard reactions. Therefore, the use of mechanistic models derived from aqueous model systems to describe the formation of acrylamide in real food systems is not possible without combining these models with heat and mass transfer models to account for these changes. Heat and mass transfer models have been developed for potato products (25), but most of these models need to be adapted or modified to account for the physical changes that occur during frying (26), and the inhomogeneous distribution and diffusion of precursors within potatoes. Using models that only give a mathematical description of the formation or degradation of acrylamide bypasses the problem of having to consider all the mechanisms that occur during processing of foods. The semi-empirical and the total empirical models proposed by Corradini and Peleg (17) describe the formation and degradation of acrylamide by mathematical functions rather than by underlying mechanisms. The concentration versus time relationship of acrylamide in the potato crisps was 'force-fitted' with these models, meaning that the empirical parameters are chosen in such a way that the residual sum of squares is minimized without any mechanistic restraints, such as knowledge of the underlying reaction networks. **Figure 5.5** shows the fit of these three models with the data from the potato crisp models. The estimated parameters found for the three models are shown in **Table 5.1**. The SD, obtained by linear approximation in the nonlinear regression routine, of some parameters was found to be quite high. This is the case for k_2 of the Logistic-Fermi model, τ of the Logistic-Exponential and a and b of the Empirical model. These high SD could be due to the limited timescale. Especially the parameter estimates for the degradation part in the Logistic-Fermi and Logistic-Exponential model strongly depend on the availability of data points during the degradation of acrylamide. Monte Carlo simulations revealed that the parameter estimates were approximately normally distributed and correlation plots of the simulated parameter estimates showed no strong correlation between model parameters (results not shown). Therefore, the low precision of the parameter estimates is mainly due to the fact that the

Table 5.1

Estimates of parameters with their approximate SD (by linear approximation) for the Logistic-Fermi, Logistic-Exponential and Empirical models.

		Logistic-Fermi					Logistic-Exponential				Empirical			
Source	Initial temp.	a (μg/kg)	k_1 (min^{-1})	t_{c1} (min)	k_2 (min^{-1})	t_{c2} (min)	a (μg/kg)	k_1 (min^{-1})	t_{c1} (min)	τ (min)	a	n	b	m
Knol *et al.* (this study) 2-6 min, 1.5 mm slices	180 °C	$1.9 \cdot 10^4 \pm 8 \cdot 10^2$	3.0 ± 0.5	2.7 ± 0.1	3.5 ± 8	6.6 ± 1	$2.6 \cdot 10^4 \pm 6 \cdot 10^3$	2.8 ± 0.5	2.8 ± 0.1	15 ± 10	$4.1 \cdot 10^4 \pm 2 \cdot 10^4$	6.2 ± 1	$1.1 \cdot 10^3 \pm 1 \cdot 10^3$	6.7 ± 1
Elmore *et al.* (30) 10-60 min, 3 mm cakes	180 °C	$6.6 \cdot 10^3 \pm 5 \cdot 10^2$	0.38 ± 0.09	16 ± 0.6	0.093 ± 0.05	68 ± 5	$9.1 \cdot 10^3 \pm 1 \cdot 10^3$	0.31 ± 0.07	17 ± 0.9	94 ± 31	$1.1 \cdot 10^5 \pm 7 \cdot 10^4$	3.2 ± 0.5	$2.0 \cdot 10^5 \pm 2 \cdot 10^5$	4.0 ± 0.4
Gökmen *et al.* (27) 0-60 min, 2 mm slices	150 °C	$5.2 \cdot 10^3 \pm 9 \cdot 10^2$	0.33 ± 0.05	9.3 ± 0.5	0.050 ± 0.01	39 ± 8	$7.2 \cdot 10^3 \pm 6 \cdot 10^2$	0.29 ± 0.02	11 ± 0.5	37 ± 3	$4.3 \cdot 10^5 \pm 3 \cdot 10^5$	1.8 ± 0.2	$1.1 \cdot 10^4 \pm 6 \cdot 10^3$	3.2 ± 0.2
Gökmen *et al.* (27) 0-60 min, 2 mm slices	170 °C	$1.6 \cdot 10^4 \pm 2 \cdot 10^4$	1.8 ± 2	3.3 ± 0.7	0.033 ± 0.02	25 ± 82	$1.2 \cdot 10^4 \pm 9 \cdot 10^2$	1.5 ± 0.4	3.5 ± 0.3	50 ± 8	$3.2 \cdot 10^4 \pm 4 \cdot 10^3$	4.0 ± 0.5	$6.0 \cdot 10^2 \pm 3 \cdot 10^2$	4.5 ± 0.4
Kim *et al.* (14) 0.5-10 min, 1 mm slices	180 °C	$8.7 \cdot 10^4 \pm 7 \cdot 10^4$	2.3 ± 1	-0.025 ± 0.7	0.080 ± 0.2	77 ± 150	$1.2 \cdot 10^6 \pm 2 \cdot 10^6$	1.5 ± 0.1	-2.2 ± 1	$5.5 \cdot 10^3 \pm 2 \cdot 10^4$	$4.6 \cdot 10^4 \pm 2 \cdot 10^3$	1.4 ± 0.1	0.37 ± 0.07	1.5 ± 0.1
Kita *et al.* (31) 3-7 min, 1.5 mm slices	160 °C	$2.1 \cdot 10^3 \pm 1 \cdot 10^3$	1.0 ± 0.1	4.8 ± 0.4	0.26 ± 0.4	12 ± 7	$2.0 \cdot 10^3 \pm 7 \cdot 10^2$	1.1 ± 0.09	4.7 ± 0.3	30 ± 43	$2.4 \cdot 10^4 \pm 6 \cdot 10^4$	3.3 ± 0.5	$3.7 \cdot 10^3 \pm 7 \cdot 10^3$	4.5 ± 0.7
Kita *et al.* (31) 2-5 min, 1.5 mm slices	185 °C	$5.4 \cdot 10^3 \pm 7 \cdot 10^3$	1.3 ± 1	2.2 ± 0.3	0.013 ± 0.1	13 ± 96	$2.8 \cdot 10^3 \pm 6 \cdot 10^2$	1.3 ± 0.9	2.2 ± 0.2	$4.3 \cdot 10^3 \pm 1 \cdot 10^5$	$9.4 \cdot 10^2 \pm 3 \cdot 10^1$	24 ± 5	$5.7 \cdot 10^6 \pm 2 \cdot 10^7$	23 ± 5

data do not contain enough information to extract precise model parameters. Of course, this is not strange because these datasets were not collected with the tested models in mind. However, if these models are going to be used for prediction, future experiments should be directed to an optimal experimental design with regard to precision of parameter estimates. The fit of the three models, however, is practically indistinguishable. The Logistic-Fermi model behaves differently in that it shows a strong decrease after 6 minutes. Unfortunately, there are no data points to validate this. The three models seem to be indistinguishable up until 6 min. We applied model discrimination, using the AIC, to investigate whether a statistical analysis would show differences. The model with the lowest Δ_{AIC}, the difference between the AIC values with the lowest value as reference, performs the best from a statistical point of view (23). The results are shown in **Table 5.2**. The model with the highest number of parameters (Logistic-Fermi) performs less, though not substantial, but based on this we prefer the Logistic-Exponential and Empirical model. These two perform equally well. Based on the simplicity and the lowest score on model discrimination parameters, one could say that the Empirical model is favored.

Comparison with other studies, where the precise circumstances of the experiment are not well known, should be done cautiously. Nevertheless, we have fitted these three models to data from other studies (14, 27, 30, 31) to compare the results for the parameters and to perform model discrimination. **Figures 5.6–5.9** show the fit of these three models with the data taken from literature. The estimated parameters found for the models are also shown in **Table 5.1** next to our own results. The three models fitted the data of Kim *et al.* (14) visibly the same (**Figure 5.6**), but the two Logistic models have very large deviations in their parameter estimation, which is probably caused by the absence of the degradation of acrylamide and the relatively low number of data points. The parameter estimation also resulted in negative time characteristics for the inflection point in the formation of acrylamide; this is probably caused by the relatively rapid formation of acrylamide. The fit of the three models to the data of Kita *et al.* (31) showed similar results (**Figure 5.7**), both Logistic models showed large deviations in the parameters estimation because there was no degradation of acrylamide. The Empirical model for the data of 185°C has a deviant appearance, which is the results of the total empirical character of the model and the few number of data points. The larger timescale of the experiments by Elmore *et al.* (30) and Gökmen *et al.* (27) and the noticeable degradation of acrylamide (**Figures 5.8** and **5.9**) resulted in a better parameter estimation for these studies. The Logistic-Fermi model, however, did show some irregularities with fitting the degradation of acrylamide.

Overall, one could say that our results are in the same order of magnitude with the other studies, but there are differences and these are probably due to the different experimental conditions, such as different temperature profiles, crisps thickness and timescale. Nevertheless, the models perform in a similar way. As with the results of our experiment, the approximate SD in some cases are high. The short timescale of some experiments and the low number of data points could be the reason for this high SD. The results for model discrimination are shown in **Table 5.2**. The Empirical and Logistic-Exponential model performed in all cases the best and of these two the Empirical one in all but one case, which

Table 5.2

Model discrimination results for the Logistic-Fermi, Logistic-Exponential and Empirical models.

		Logistic-Fermi						Logistic-Exponential						Empirical					
Source	Initial temp.	*p*	*n*	MS[a]	SS[b]	AIC_c[c]	Δ_{AIC}	*p*	*n*	MS	SS	AIC_c	Δ_{AIC}	*p*	*n*	MS	SS	AIC_c	Δ_{AIC}
Knol *et al.* (this study) 2-6 min, 1.5 mm slices	180 °C	5	20	$3.3 \cdot 10^6$	$5.0 \cdot 10^7$	311.3	3.0	4	20	$3.3 \cdot 10^6$	$5.3 \cdot 10^7$	308.5	0.2	4	20	$3.3 \cdot 10^6$	$5.2 \cdot 10^7$	308.3	0.0
Elmore *et al.* (30) 10-60 min, 3 mm cakes	180 °C	5	12	$1.6 \cdot 10^5$	$1.1 \cdot 10^6$	160.0	2.7	4	12	$1.9 \cdot 10^5$	$1.5 \cdot 10^6$	157.7	0.4	4	12	$1.9 \cdot 10^5$	$1.5 \cdot 10^6$	157.3	0.0
Gökmen *et al.* (27) 0-60 min, 2 mm slices	150 °C	5	10	$2.2 \cdot 10^4$	$1.1 \cdot 10^5$	120.6	16.8	4	10	$8.0 \cdot 10^3$	$4.8 \cdot 10^4$	103.8	0.0	4	10	$1.9 \cdot 10^4$	$1.1 \cdot 10^5$	112.3	8.5
Gökmen *et al.* (27) 0-60 min, 2 mm slices	170 °C	5	10	$1.3 \cdot 10^6$	$6.5 \cdot 10^6$	161.7	27.7	4	10	$6.5 \cdot 10^5$	$3.9 \cdot 10^6$	147.7	13.6	4	10	$1.7 \cdot 10^5$	$9.9 \cdot 10^5$	134.1	0.0
Kim *et al.* (14) 0.5-10 min, 1 mm slices	180 °C	5	6	$7.0 \cdot 10^6$	$7.0 \cdot 10^6$	178.8	80.3	4	6	$7.1 \cdot 10^5$	$1.4 \cdot 10^6$	113.3	14.8	4	6	$6.1 \cdot 10^4$	$1.2 \cdot 10^5$	98.5	0.0
Kita *et al.* (31) 3-7 min, 1.5 mm slices	160 °C	5	6	$1.8 \cdot 10^3$	$1.8 \cdot 10^3$	129.2	58.1	4	6	$9.2 \cdot 10^2$	$1.8 \cdot 10^3$	73.3	2.2	4	6	$6.3 \cdot 10^2$	$1.3 \cdot 10^3$	71.1	0.0
Kita *et al.* (31) 2-5 min, 1.5 mm slices	185 °C	5	6	$9.5 \cdot 10^4$	$9.5 \cdot 10^4$	153.0	84.4	4	6	$4.7 \cdot 10^4$	$9.3 \cdot 10^4$	96.9	28.3	4	6	$4.2 \cdot 10^2$	$8.3 \cdot 10^2$	68.6	0.0

a) Residual mean squares; b) residual sum of squares; c) corrected Akaike information criterion.

is in line with our results and support our conclusions that the Logistic-Exponential and Empirical model are preferred for modeling the formation and degradation of acrylamide in potato crisps.

As discussed earlier, the parameters only apply for the specific experimental conditions; time-temperature profile of frying, potato variety, slice thickness and initial concentration of precursors. These factors are the key parameters affecting acrylamide formation from a viewpoint of heat and mass transfer facts. By keeping the experimental conditions the same, one could establish relationships between the mathematical parameters and the physical or chemical parameters that can influence acrylamide formation. Secondly, the same experimental conditions make it possible to really compare the results from modeling. This could perhaps even give a mechanistic meaning to the parameters in the Logistic models. Although this still has to be validated, one could suspect that parameter '*a*' or the 'scale factor' could be related to the initial sugar content. Earlier studies showed the correlation between sugar content and acrylamide formation (32). Furthermore, by establishing the temperature dependence of the mathematical parameters, one could predict the influence of different temperature profiles during frying. Serpen and Gökmen (33) have suggested the use of artificial neural networks to accomplish the same objective, namely the relationship between initial precursor content, time, temperature and acrylamide. Their study is based on the assumption of an isothermal process at the initial temperature, the actual temperature profile during frying is however based on the oil/potato ratio, the type of equipment and therefore in almost any case a non-isothermal process, with big differences in the actual temperature of the product and the oil surrounding it. The heating rate could also play a significant role in the formation of acrylamide and therefore should be included in the development of predictive models (17).

5.4 CONCLUDING REMARKS

The present study shows that the formation of acrylamide in potato crisps can be modeled by applying Logistic-Fermi, Logistic-Exponential or Empirical models in accordance with an earlier study (17). The Empirical model is preferred as it performed the best in the statistical evaluation. However, it also appeared that the precision of the estimates obtained is quite bad, and this puts a serious constraint on the use of these models if they are going to be used for prediction: the precision of predictions made with such imprecise parameters will go quickly out of hand. While we found two models to perform reasonably well in fitting, the next step should be to put much more emphasis on the precision of parameters. This calls for much more attention for experimental design to obtain precise estimates. Further research is on the way to establish the relationship between the model parameters and precursors content and to determine the influence of the temperature dependence on the model parameters. Once these relationships are established and with more precision in parameter estimation, the development of simple to use mathematical models to predict the formation of acrylamide in potato crisps could be realized. These tools could be useful for industry and manufactures to develop new mitigation strategies.

5.5 ACKNOWLEDGMENT

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Chapter 6

Kinetic modeling: a tool to predict the formation of acrylamide in potato crisps

Three empirical models were used to fit the formation of acrylamide in crisps of three different cold-sweetened potato genotypes, fried under the same experimental conditions. Statistical methods were used to compare the performance of the models, with the 'Logistic-Exponential' model performing the best. The obtained model parameters for the formation of acrylamide showed improvement in precision compared to an earlier study, the precision of the parameter estimates for the degradation of acrylamide was still problematic. Nevertheless, the predictive capacity of the 'Logistic-Exponential' model was tested, as this model showed a strong correlation between parameter '*a*' and the reducing sugar content of the raw potato. The predictions from this model for the formation of acrylamide in potato crisps were close to earlier reported experimental values. Therefore, the use of the 'Logistic-Exponential' model as a tool to predict acrylamide in potato crisps seems promising and should be developed further.

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6.1 INTRODUCTION

The discovery of acrylamide, classified by the International Agency for Research on Cancer as 'probably carcinogenic to humans', in heat-treated carbohydrate-rich foods led to worldwide concern (1, 2). Products with a high level of consumption, such as French fries, potato crisps, coffee and bread, contribute significantly to the overall intake of acrylamide (3). Next to the consumption pattern, the acrylamide levels in these foods play an important role in the intake of acrylamide (3). Although there is enough evidence about the neurotoxic, genotoxic and carcinogenic effects of acrylamide, epidemiological studies have opposed outcomes about the risks associated with the current intake levels of dietary acrylamide (4-9). These epidemiological studies, however, are based on food frequency questionnaires and where not designed to measure actual acrylamide exposure. In a recent study a positive association was found between the risk for breast cancer and acrylamide-hemoglobin adducts in red blood cells, a biomarker for acrylamide exposure (10). It should however be noted that there are also limitations to use of biomarkers as these do not distinguish the source of acrylamide intake. There is, consequently, need for further research to clearly establish the impact of the presence of acrylamide in foods on the health of consumers. However, from the principle of precaution, the Joint FAO/WHO Expert Committee on Food Additives advises to continue appropriate efforts to reduce acrylamide concentrations in food in the meantime (11).

The main mechanism of acrylamide formation in heated foods is the Maillard reaction between sugars and amino acids and several pathways have been proposed (12-17). Most studies to unravel the reaction networks of the formation of acrylamide used aqueous or dry model systems. The advantage of this approach is that relevant information is obtained about the actual mechanisms of the reaction, especially with the technique of multiresponse modeling (**Chapter 3** and **4**). However, these mechanistic studies in model systems are not easily applied to real food systems, as most foods are complex mixtures of a number of compounds. Because of the complex food matrices, reactants cannot meet each other readily and their activity will be different from model experiments; furthermore, generally there are temperature and concentration gradients within the food during heating. Hence, mechanistic models have to be combined with mass and heat transfer models, and adapted or modified to account for the physical changes in the food product, such as cell shrinkage and damage, cell separation and starch gelatinization that occur in potato products during processing (19). Using models that only give a mathematical description of the formation or degradation of acrylamide in food bypasses the problem of considering all the mechanisms that occur during processing of foods. Corradini and Peleg (20) demonstrated that the formation and degradation of acrylamide could be successfully modeled by mathematical functions, where confounding effects of heat and mass transfer effects are taken up in empirical constants. We already successfully demonstrated the use of these empirical models for modeling the formation of acrylamide in potato crisps (**Chapter 5**). The precision of the obtained parameters was however problematic, which puts a serious constraint on the use of these models for prediction.

The main objectives of the present study were to obtain more precise estimates of the model parameters and to apply and evaluate three empirical models. Using well-controlled frying conditions it was possible to study the relationships between model parameters and precursor content, which may be used for the development of a simple tool to predict the formation of acrylamide in potato crisps and which be applied in mitigation strategies.

6.2 MATERIAL AND METHODS

6.2.1 Preparation of samples

The potato genotypes (*Solanum tuberosum* L.) Hulda, Lady Rosetta and SW 91 102 grown in the south of Sweden were harvested in September 2006 (22). After wound healing at 15˚C for two weeks and a slow reduction of storage temperature, the tubers were stored at 4˚C at a relative humidity of 90–95% for 4 weeks from the beginning of November 2006 until the end of November 2006. Crisps were prepared as described by Viklund *et al*. (23). Briefly, potatoes (6-10 cm of diameter and weighing around 100-250 g) were washed and cut into 1.5 mm slices and divided randomly into three batches per genotype. One batch was used for the frying experiments. The raw potato slices were deep-fried in rapeseed oil in three net cages placed on top of each other. The temperature of the oil and potato crisps was measured by thermo-couples as described in **Chapter 5**. The oil was preheated to 180˚C. The potato slices were fried for different times (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8 and 12 min). Duplicate experiments were carried out at 2, 2.5, 3 and 4 min of frying. After submerging the potato slices in the oil bath, the oil temperature decreased rapidly. After about 1.5–2.0 min, the oil reached a temperature of 160˚C, and the thermostat setting was then changed to keep the temperature constant at 160˚C for the rest of the frying session. After frying, the crisps were cooled to room temperature, and stored in closed plastic bags at -18˚C until analysis. The other two batches were used for the analysis of acrylamide precursors (fructose, glucose, sucrose, and asparagine) in the raw potato slices. The frying experiments were carried out in one day; to check for changes in the precursors content during the day, one batch was taken for analysis at the start (morning) and one batch at the end of the experiments (evening).

6.2.2 Analyses

The water content of the potato tubers and potato crisps was determined gravimetrically by the AOAC-984.25 method as described earlier by Viklund *et al*. (23). The homogenized raw potato samples were analyzed with regard to glucose, fructose and sucrose using GC-FID, and for asparagine using HPLC and fluorescence detection (24). Duplicate analyses were made. The acrylamide content in the crisps was analyzed using LC-MS/MS (23). One extraction was made on each sample and the extractions were analyzed in duplicate. The color of the potato crisps was measured using a nondestructive computer vision system (23).

6.2.3 Empirical models

Three empirical models as proposed by Corradini and Peleg (20) were used to describe the formation and degradation of acrylamide. These models can be divided in two semi-empirical models 'Logistic-Fermi' and 'Logistic-Exponential', and one total empirical model 'Empirical'. The Logistic-Fermi model (**Eq. 6.1**) describes the formation of acrylamide by a logistic function and the degradation by a Fermi-type function:

$$C(t)=\left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}}-\frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]}\right]\cdot\frac{1}{1+\exp\{k_2(T)[t-t_{c2}(T)]\}} \quad (6.1)$$

where $C(t)$ is the concentration of acrylamide, $t_{c1}(T)$ and $t_{c2}(T)$ are temperature-dependent time characteristics for the inflection points in the formation (t_{c1}) and degradation (t_{c2}) of acrylamide, $k_1(T)$ and $k_2(T)$ are temperature-dependent steepness parameters around the inflection points for the formation (k_1) and degradation (k_2) of acrylamide and $a(T)$ serves as a temperature-dependent 'scale factor' for the acrylamide concentration.

The Logistic-Exponential model (**Eq. 6.2**) differs from the Logistic-Fermi model in the part that describes the degradation of acrylamide. The Logistic-Fermi function predicts that at prolonged heating times the acrylamide concentration will become zero. However, the Logistic-Exponential function with its exponential term for the degradation predicts a residual acrylamide concentration at prolonged heating times:

$$C(t)=\left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}}-\frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]}\right]\cdot\exp\left(-\frac{t}{\tau(T)}\right) \quad (6.2)$$

where $\tau(T)$ is a temperature-dependent characteristic time.

The Empirical model is expressed as:

$$C(t)=\frac{a(T)\cdot t^{n(T)}}{b(T)+t^{m(T)}} \quad (6.3)$$

where $a(T)$, $b(T)$, $m(T)$ and $n(T)$ are temperature-dependent constants.

6.2.4 Parameter estimation and statistical methods

The semi-empirical models 'Logistic-Fermi' and 'Logistic-Exponential' and the total empirical model 'Empirical', proposed by Corradini and Peleg (20), were fitted to the obtained experimental data for the acrylamide formation using the software program Mathcad® version 13.1 (PTC, Needham, MA, USA).

For the estimation of the model parameters, the command 'Minerr' was used to minimize the sum of squares. This command makes use of nonlinear regression and to determine the search direction in the iterative process the software uses Quasi-Newton methods. The SD of the model parameters were estimated by linear approximation using the variance-covariance matrix and mean squares. The Akaike information criterion (AIC) was used in the model discrimination to investigate statistical differences between models. The AIC gives a 'penalty' for models that have more parameters (25). This is useful because a model with more parameters fits better in general but leads to more imprecision in parameter estimates. The general equation for the AIC is:

$$AIC = n \cdot \ln\left(\frac{SS}{n}\right)^2 + 2(p+1) \tag{6.4}$$

where n is the number of data points, p the number of estimated parameters and SS the residual sum of squares. In the present study, the number of data points (n) was relatively small compared to the number of estimated parameters (p) ($n/p<40$) for all cases, therefore the corrected AIC, AIC_c, was used:

$$AIC_c = AIC + 2(p+1)\left(\frac{p+2}{n-p}\right) \tag{6.5}$$

The model with the lowest Δ_{AIC}, the difference between the AIC values with the lowest value as reference, performs the best. For the statistical analyses of the differences in precursor content, Minitab Statistical Software Version 13 (Minitab Inc., State College, PA, USA) was used. Differences were evaluated with the general linear model followed by Tukey's multiple comparison test. A value of $p \leq 0.05$ was considered significant.

6.3 RESULTS AND DISCUSSION

6.3.1 Temperature development in the potato crisps

The laboratory frying conditions of the experiments have been set-up to mimic industrial processing and gave reproducible results regarding color, water content and acrylamide levels also at longer frying times (23). **Figure 6.1** shows the average temperature profiles of the oil, the outer cell layer of the potato slices and the differences between those temperatures in the present study and in an earlier study (21). The temperature profile of the frying oil was the same for both studies and in line with the temperature profile commonly found in industry where potato slices are immersed in oil having a starting temperature of around 180-190°C and an end temperature of around 150-175°C (26). Also the temperature profile for the outer

cell layer of the potato slices in general followed the same trend. The small differences in the temperature profiles could be the results of the placement of the tip of the thermocouples or could be influenced by the structural changes of the potato slices during frying, which was also noticeable in individual temperature measurements of potato slices (19, 21). Viklund *et al*. used the same experimental frying conditions, which makes it possible to compare the acrylamide formation patterns from this study and the results reported in literature (19, 21-23). Furthermore, the consistent conditions of our experiments make it possible to test the predictive capabilities of the empirical models from the present study on the results obtained by Viklund *et al*. (22).

6.3.2 Chemical analyses

Table 6.1 shows the contents of fructose, glucose, sucrose and asparagine for the three different potato genotypes at the start of the experiments (morning) and at the end of the experiments (evening). Changes in the concentration of precursors might occur as the potatoes were sliced and cells were damaged, which could lead to physiological and chemical reactions in the potato tissue that could affect the precursor content. Generally, the differences in the concentrations of acrylamide precursors between morning and evening samples were not significant (p >0.05) except for the sucrose concentration of Hulda slices (p =0.0072). Because of the similarities, the average concentrations of all the measurements were used for the empirical modeling.

Figure 6.2 shows the acrylamide concentration for the potato slices fried for 1 to 12 min. The highest concentration of acrylamide was measured in the Lady Rosetta potato crisps, followed by the Hulda and sw 91 102 crisps, respectively. In crisps fried for 1 min, the acrylamide concentrations were below the detection limit of 50 µg/kg dry matter (dm). Frying between 1.5 and 4 min made the acrylamide concentrations increase rapidly for all three genotypes. After 4 min, the acrylamide concentration decreased slowly, with the largest decrease found in the Lady Rosetta crisps. The decreased acrylamide concentrations are probably a result of breakdown reactions that have previously been reported (18, 27, 28). The acrylamide formation in the crisps was not linearly related to the concentration of the sugars in the raw potatoes: the fructose and glucose concentrations of sw 91 102 genotype were higher than those of Hulda. One would, therefore, expect a higher acrylamide formation in sw 91 102 crisps than in Hulda crisps as studies have shown a strong correlation between sugar concentration and acrylamide formation in potato products (23, 29, 30). However, in a previous study, low concentrations of asparagine gave crisps with the lowest concentration of acrylamide (22). Apparently, there are other factors affecting the acrylamide formation such as genotype specific factors like asparagine concentration and chemical composition, although in the present study the asparagine concentration did not vary to a large extent. This reinforces our earlier statement that in the attempt to model the formation of acrylamide one should realize that the parameters are only applicable for specific experimental conditions such as time-temperature profile of frying, potato genotype, slice thickness and initial concentration of precursors (21).

Table 6.1

Acrylamide precursor concentrations [mg/g dm] with their variation ($n = 2$) of the raw potato slices from the three different genotypes used at the beginning of the experiments (morning) and at the end of all experiments (evening).

Compound	Genotype	Morning	Evening
Fructose	Hulda	3.33 ± 0.06	3.97 ± 0.13
	Lady Rosetta	7.28 ± 0.08	7.78 ± 0.36
	SW 91 102	4.88 ± 0.15	4.72 ± 0.05
Glucose	Hulda	5.94 ± 0.11	6.21 ± 0.23
	Lady Rosetta	8.09 ± 0.03	8.03 ± 0.06
	SW 91 102	7.34 ± 0.30	7.00 ± 0.04
Sucrose	Hulda	6.89 ± 0.16	5.26 ± 0.12[a]
	Lady Rosetta	7.39 ± 0.32	7.34 ± 0.15
	SW 91 102	10.45 ± 0.36	11.51 ± 0.39
Reducing sugars	Hulda	9.27 ± 0.05	10.17 ± 0.10
	Lady Rosetta	15.37 ± 0.11	15.80 ± 0.42
	SW 91 102	12.21 ± 0.45	11.71 ± 0.01
Asparagine	Hulda	10.53 ± 0.42	10.47 ± 0.25
	Lady Rosetta	11.60 ± 0.04	11.42 ± 0.04
	SW 91 102	9.93 ± 0.07	10.05 ± 0.01

[a] = significant difference between morning and evening samples, $p \leq 0.05$

The decrease in water content followed the same trend for all three genotypes (**Figure 6.3**). The initial water contents were 78% for Lady Rosetta and sw 91 102 and 80% for Hulda. After 1 min of frying, the potato crisps from Lady Rosetta had the highest water content, 41%, followed by sw 91 102, 34% and Hulda 30%. Despite the small differences at 1 min, the rapid decrease of water between 1 and 3 min of frying resulted in almost the same water content of the crisps after 3 min frying. At longer frying times, crisps from sw 91 102 had a slightly higher water content, 5%. Industrially manufactured crisps normally have a water content of around 2% (26). The rapid increase of acrylamide in the crisps took place after the rapid decrease of the water content and when the temperature of the outer cell layer of the potato crisps had reached 160°C. Similar patterns were found in earlier studies on the formation of acrylamide in crisps when slices of Saturna and Bintje potatoes were deep-fried at 160°C (21, 23).

Table 6.2

Estimates of parameters with their approximate SD (obtained by linear approximation) for the Logistic-Fermi, Logistic-Exponential and Empirical models.

	Logistic-Fermi					Logistic-Exponential				Empirical			
Genotype	a (µg/kg)	k_1 (min^{-1})	t_{c1} (min)	k_2 (min^{-1})	t_{c2} (min)	a (µg/kg)	k_1 (min^{-1})	t_{c1} (min)	τ (min)	a	n	b	m
Bintje*	$1.9{\cdot}10^4 \pm 8{\cdot}10^2$	3.0 ± 0.5	2.7 ± 0.1	3.5 ± 8	6.6 ± 1	$2.6{\cdot}10^4 \pm 6{\cdot}10^3$	2.8 ± 0.5	2.8 ± 0.1	15 ± 10	$4.1{\cdot}10^4 \pm 2{\cdot}10^4$	6.2 ± 1	$1.1{\cdot}10^3 \pm 1{\cdot}10^3$	6.7 ± 1
Lady Rosetta	$2.7{\cdot}10^4 \pm 4{\cdot}10^4$	3.0 ± 0.4	2.8 ± 0.1	0.053 ± 0.06	0.60 ± 52	$1.4{\cdot}10^4 \pm 9{\cdot}10^2$	3.0 ± 0.4	2.8 ± 0.1	31 ± 9	$2.5{\cdot}10^4 \pm 2{\cdot}10^4$	4.9 ± 2	$3.5{\cdot}10^2 \pm 4{\cdot}10^2$	5.3 ± 2
Hulda	$8.5{\cdot}10^3 \pm 2{\cdot}10^2$	2.8 ± 0.3	2.2 ± 0.04	1.7 ± 4	13 ± 2	$9.6{\cdot}10^3 \pm 5{\cdot}10^2$	2.5 ± 0.3	2.3 ± 0.1	42 ± 14	$1.3{\cdot}10^4 \pm 7{\cdot}10^3$	4.9 ± 3	$9.2{\cdot}10^1 \pm 2{\cdot}10^2$	5.1 ± 2
sw 91 102	$7.1{\cdot}10^3 \pm 9{\cdot}10^2$	1.6 ± 0.4	3.0 ± 0.2	0.63 ± 1	14 ± 3	$9.3{\cdot}10^3 \pm 2{\cdot}10^3$	1.5 ± 0.4	3.1 ± 0.3	22 ± 15	$2.6{\cdot}10^4 \pm 3{\cdot}10^5$	3.2 ± 13	$1.8{\cdot}10^2 \pm 2{\cdot}10^3$	3.8 ± 9

* Data from **Chapter 5**.

Table 6.3

Model discrimination results for the Logistic-Fermi, Logistic-Exponential and Empirical models.

	Logistic-Fermi						Logistic-Exponential						Empirical					
Genotype	p	n	MS[a)]	SS[b)]	AIC_c[c)]	Δ_{AIC}	p	n	MS	SS	AIC_c	Δ_{AIC}	p	n	MS	SS	AIC_c	Δ_{AIC}
Bintje*	5	20	$3.3{\cdot}10^6$	$5.0{\cdot}10^7$	311.3	3.0	4	20	$3.3{\cdot}10^6$	$5.3{\cdot}10^7$	308.5	0.2	4	20	$3.3{\cdot}10^6$	$5.2{\cdot}10^7$	308.3	0.0
Lady Rosetta	5	32	$9.0{\cdot}10^5$	$2.4{\cdot}10^7$	447.4	3.0	4	32	$8.7{\cdot}10^5$	$2.4{\cdot}10^7$	444.4	0.0	4	32	$1.5{\cdot}10^7$	$2.9{\cdot}10^7$	450.6	6.2
Hulda	5	32	$2.8{\cdot}10^5$	$7.7{\cdot}10^6$	410.5	0.0	4	32	$3.5{\cdot}10^5$	$9.9{\cdot}10^6$	415.6	5.1	4	32	$4.7{\cdot}10^6$	$8.9{\cdot}10^6$	412.4	1.9
sw 91 102	5	32	$1.6{\cdot}10^6$	$4.3{\cdot}10^7$	465.9	2.7	4	32	$1.6{\cdot}10^6$	$4.4{\cdot}10^7$	463.1	0.0	4	32	$2.2{\cdot}10^8$	$4.4{\cdot}10^7$	463.5	0.4

* Data from **Chapter 5**; a) Residual mean squares; b) residual sum of squares; c) corrected Akaike information criterion.

6.3.3 Mathematical modeling

The formation and degradation of acrylamide was fitted with three empirical models, the semi-empirical models 'Logistic-Fermi' and 'Logistic-Exponential' and the total empirical model 'Empirical'. Corradini and Peleg demonstrated that these models can be useful in quantitative characterization of acrylamide formation patterns in foods and might be used to simulate and predict non-isothermal generation of acrylamide (20). We successfully demonstrated the application of these models in modeling the formation of acrylamide in potato crisps in **Chapter 5**.

The parameters for the models were estimated by the software in such a way that the residual sum of squares was minimized without any mechanistic restraints, such as knowledge of the underlying reaction networks. **Figures 6.4 – 6.6** show the fit of these three models with the data from the three potato crisps experiments. The estimated parameters found for the three models are shown in **Table 6.2**. The standard deviation, obtained by linear approximation in the nonlinear regression routine, of some parameters was found to be quite high. This is the case for k_2 and t_{c2} of the Logistic-Fermi model, τ of the Logistic-Exponential model and for all the parameters of the Empirical model. The high standard deviations of the parameter estimates for the degradation part in both Logistic models was not expected as the setup of the new experiments included a longer timescale to include more data points for the part where acrylamide degradation in the crisps takes places as compared to our first study (**Chapter 5**). Monte Carlo simulations revealed that the parameter estimates were approximately normally distributed and correlation plots of the simulated parameter estimates showed no strong correlation between model parameters (data not shown). Therefore, especially for the estimation of the Empirical model parameters, the low precision of the parameter estimates must be mainly due to the fact that the data do not contain enough information to extract precise model parameters for the degradation. The Logistic-Exponential model, except for the characteristic time parameter τ, was overall the model with the best precision in the estimation of the model parameters for the three crisps and therefore the most suitable to be used for prediction of acrylamide formation.

The fit of the three models, however, is practically indistinguishable until 12 min. The Logistic-Fermi model behaves differently in that it shows a strong decrease after 12 min for the SW 91 102 and Hulda potato crisps. Unfortunately, there are no data points to validate this. We applied model discrimination, using the AIC, to investigate whether a statistical analysis would show differences. The model with the lowest Δ_{AIC}, the difference between the AIC values with the lowest value as reference, performs the best from a statistical point of view (25). The results are shown in **Table 6.3**. The model with the highest number of parameters (Logistic-Fermi) performs less, though not substantially, but based on this we prefer the Logistic-Exponential and Empirical model. These perform equally well. These results are in line with our earlier study (21). If we take the results from the discrimination test and the precision of the parameter estimation the Logistic-Exponential model is favored.

Data from earlier studies on Bintje potatoes are also included in **Tables 6.2** and **6.3** for comparison as the experimental set-up was the same as in the present study (21). The characteristic time parameter t_{c1} of the Logistic models was almost the same for all four potato genotypes. This temperature-dependent parameter t_{c1} stands for the inflection point in the formation of acrylamide and is dependent of the experimental conditions such as the temperature profile, oil-crisp ratio and thickness of the slices. This can also be visually concluded from **Figures 6.4 – 6.6** where the acrylamide formation for the three genotypes follows the same trend. The other parameters were in the same order of magnitude and no clear relationship could be found between the model parameters and the precursor concentration of the fresh potato (data not shown). However, there was one exception, parameter '*a*' of the Logistic-Exponential model showed a strong relationship with the initial concentration of fructose (R^2=0.91), glucose (R^2=0.97) and reducing sugars (R^2=0.98) in the raw potato slices (**Figure 6.7**), which was expected as earlier studies have shown a correlation between sugars and acrylamide formation (23, 29, 30). These relationships give a more mechanistic meaning to the empirical model, and make it possible to predict the formation of acrylamide based on the initial sugar concentrations. It should be noted that the relationship was established with potatoes containing high sugar concentrations; the predictability of the empirical model could therefore be only valid for potatoes with high initial sugar concentrations. As the concentration of reducing sugars is the sum of the fructose and glucose concentrations and showed the highest correlation with parameter '*a*', the reducing sugar concentration is used to predict parameter '*a*'. This relationship can be described by

$$a = 1.06 \cdot C_{red.sugars} - 2.04 \qquad (6.6)$$

where $C_{red.sugars}$ is the total concentration (mg/g dm) of the reducing sugars in the raw potato slices. Data from a study on crisps from Viklund *et al*. (22) were used to test the predictive capacity of the Logistic-Exponential model based on the parameters k_1, t_{c1} and τ obtained in the present study for Hulda combined with parameter '*a*' as a function of the initial concentration of reducing sugars. **Figure 6.8** shows the predictions made by the Logistic-Exponential model plotted against the experimental data of the study of Viklund *et al*. (22). The estimates of the model are close to the experimental values with some exceptions where the difference between the values was more than 15%. The model could be used to make predictions in this case, as the experimental conditions were exactly the same, such as the temperature profile during frying, the oil-crisps ratio and the thickness of the crisps. It should, however, also be mentioned that the relation between parameter '*a*' and the reducing sugar concentration is based on four different genotypes. Ideally, this relationship should be established by using only one genotype with a large range of different sugar concentrations. Nevertheless, as the frying conditions are mostly kept constant in the industry, these models may easily be established for the specific processes and could serve as a simple tool to make predictions about the formation of acrylamide. Once the parameters are obtained, the formation of acrylamide could be reduced by selecting potatoes below a maximum amount of reducing sugars or by altering this concentration during the processes before frying.

Figure 6.9 shows the L^* a^* b^* values obtained for the potato crisps made from Hulda plotted against the acrylamide concentration. The results are in agreement with the results from Viklund *et al*. (23) and to some extent in agreement with results from Pedreschi *et al*. (30). The correlations (R^2) obtained by linear regression were 0.28, 0.82 and 0.85 for L^*, a^* and b^*, respectively. If this relationship between color development and acrylamide formation is determined more precisely, one could integrate this in the 'acrylamide' prediction tool to establish a trade-off between color formation and acrylamide formation and even apply this model in quality assurance protocols for potato crisps.

6.4 CONCLUDING REMARKS

The present study shows that the formation of acrylamide in potato crisps can be modeled by applying empirical models with the Logistic-Exponential model as the best performing model. The precision of the parameters was greatly improved as opposed to our previous study (**Chapter 5**) and it was possible to use the Logistic-Exponential model to make predictions for the acrylamide formation based on the strong relationship between the initial reducing sugar concentration and parameter '*a*'. The predictive capacity of this model has not been tested exhaustively and should be extended to other processing conditions. The results of this study clearly show that the use of empirical models such as the Logistic-Exponential model are promising tools for the crisps industry to mitigate acrylamide formation in their products and even become part of quality assurance protocols.

6.5 ACKNOWLEDGMENTS

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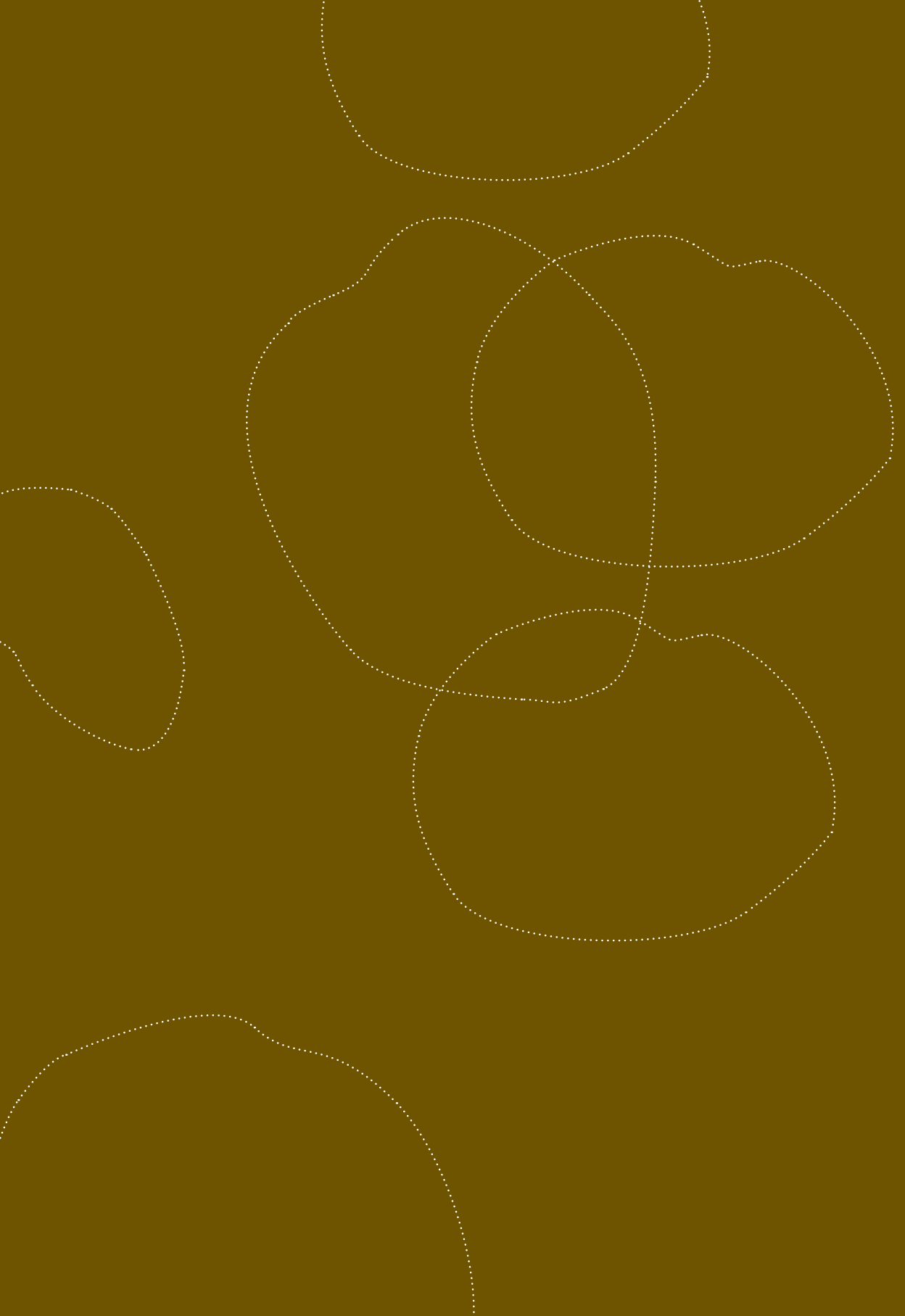
Chapter 7
General discussion



7.1 INTRODUCTION

Mitigating the content of acrylamide in foods, which is formed during heating of foods, is of importance as acrylamide is known to be neurotoxic, genotoxic and carcinogenic (1, 2). Although there is a 500-fold margin between the estimated average intake of dietary acrylamide and the NOAEL-level (no observable adverse effect level) for acrylamide of 0.5 mg/kg bodyweight per day, the health effects associated with especially the long-term intake of dietary acrylamide are still unclear (3). Epidemiological studies about the health risks associated with dietary acrylamide have shown contradictory outcomes, although recent studies by Hogervorst *et al*. have given more indications about a positive association between high acrylamide intake and various types of cancer (4-9). The European project HEATOX was set-up to assess the health risks associated with acrylamide and other hazardous components in heat-treated food and to find methods of minimizing the formation of acrylamide. This PhD study, part of the HEATOX project, focused on the reaction mechanisms and kinetics of formation of acrylamide. In this chapter the main achievements in this PhD study are discussed. An overview is given on how kinetic modeling has become a powerful tool to get insight in the formation of acrylamide in the Maillard reaction and how it can be used in mitigation strategies for acrylamide in foods. The achievements of the whole HEATOX project are summarized to place this study in a wider perspective. Finally, a future perspective is given about the role kinetic modeling could play in food science.

7.2 MAIN ACHIEVEMENTS OF THIS STUDY

The aim of the present thesis was the development of mathematical models that describe quantitatively the formation of acrylamide as a function of several parameters. The aim resulted in five objectives (**Chapter 1**), which can be divided into two parts: the development of mechanistic multiresponse models (OBJECTIVES 1-3) and the development of empirical models (OBJECTIVES 4-5). The development of mechanistic models gave more insight in the reaction networks for the formation of acrylamide and described the fate of reactants and main products (**Chapter 3** and **4**). Furthermore, this work showed that empirical models can be used to describe the formation of acrylamide in real food and can be used as a predictive tool for the mitigation of acrylamide (**Chapter 5** and **6**). In the following sections the main achievements will be further discussed.

7.2.1 Mechanistic mathematical models

For the development of mechanistic mathematical models we have applied the approach of multiresponse modeling (10). The first three steps that should be taken into account with multiresponse modeling are also the first three objectives of this study and will be discussed in this section. The final step in multiresponse modeling is to test the hypothesized mechanism, which will be discussed finally.

Objective 1 *- Identify and quantify the main intermediates and end products in the Maillard reaction of reducing sugars and asparagine*

The formation of acrylamide in the Maillard reaction between reducing sugars and asparagine encompasses not one reaction pathway, but a whole network of various reactions in which beside acrylamide other components are formed. Next to reactants and acrylamide, the following components were identified and quantified: the isomerization product of the reducing sugar, formic acid, acetic acid and melanoidins. The experimental observations and kinetic modeling suggested that acrylamide is not an end product in the Maillard reaction. The behavior of acrylamide suggested that it is an intermediate. In general, the formation of acetic acid indicates a major degradation pathway of sugars and is thought to be an end product that contributes much to the mass balance in the Maillard reaction (11). Results of earlier work have shown that at around 100˚C at pH 6.8 considerable amounts of acetic acid are formed after prolonged reaction times (12), however, the experiments in the present study were conducted at higher reaction temperatures (120-200˚C). In **Chapter 4** we have shown that acetic acid contributed much to the mass balance, but also that acetic acid at high temperatures (>180˚C) will react further in the reaction system, suggesting that at high temperatures acetic acid is also an intermediate in the Maillard reaction. Identification of the reaction products of the degradation of acrylamide and acetic acid appeared however not possible with the methods that we had available. Although we were able to observe possible reaction products by HPLC, we were not able to fractionate enough purified material for identification with NMR or MS. To our knowledge, nobody has yet identified the reaction products of the degradation of acrylamide. Polymerization and Michael type addition reactions with amino acids have been suggested for the degradation of acrylamide, but these theories have not been confirmed yet. Heating of asparagine without the presence of sugar at 180˚C resulted in a variety of unidentified fragments, which were observed by HPLC during the analysis for organic acids (**Chapter 4**). Also in this case, fractionation of the material didn't result in enough material necessary for identification. One possibility could be the formation of fumaramic acid, which was identified by Talley *et al.* as one of the major breakdown products of asparagine (13, 14). Unfortunately this component is not commercially available, which hindered us from identifying this component by chromatography. The identification of these unknown reaction products would contribute to the improvement of the reaction network for the formation of acrylamide in the Maillard reaction between reducing sugars and asparagine. In general the identification and quantification will also contribute significantly to the understanding of the Maillard reaction at higher reaction temperatures (120-200˚C).

Objective 2 *- Establish the reaction pathways and propose a mechanistic model from a quantitative point of view*

A major achievement of this thesis is the establishment of simplified kinetic models for the reaction of glucose/fructose and asparagine. Based on the reaction pathways that have been proposed in literature for the formation of acrylamide (15-18), kinetic models were suggested

based on the multiresponse models that have been proposed earlier for the reactions between proteins and sugar and glycine and glucose in the Maillard reaction (19, 20). The kinetic models were simplified as much as possible to reduce the number of parameters, but were still in line with the chemistry behind it. The number of parameters should not be too high compared to the number of responses as this increases model bias. Despite the current underestimation for the behavior of sugars and asparagine, which is due to the unknown degradation reactions, the proposed models gave reasonable estimations for the fate of reactants and reaction products in aqueous model systems. The multiresponse models resulted in relevant kinetic parameters (rate constants and activation energies) for all the reaction steps in the proposed reaction networks. Because the kinetic parameters are derived by monitoring the concentrations of reactants and reaction products, they are based on additional information, which cannot be derived from the fate of acrylamide concentration alone. These single response models have been proposed for the formation of acrylamide both in aqueous and dry reaction systems (21-24). Comparison of models should, however, be done with caution as the conditions of the systems (*e.g.* buffer type, concentration of reactants, temperature profile) and the number of parameters may differ. **Table 7.1** shows the results of the study of Claeys *et al*. (21) on the one hand and the kinetic parameters established in **Chapter 3** by multiresponse modeling on the other hand. For the comparison, we have also applied the technique of single response modeling for the formation and degradation of acrylamide as has been reported in **Chapter 3**. The model that was used for the single response modeling was:

$$\frac{dC_{Acrylamide}}{dt} = k_f \cdot C_{Glucose} \cdot C_{Asparagine} - k_e \cdot C_{Acrylamide} \qquad (7.1)$$

In which k_f and k_e are the rate constants for the formation and elimination of acrylamide, respectively. At time t=0, the concentration of glucose ($C_{Glucose}$) and asparagine ($C_{Asparagine}$) were considered to be equal to the initial concentration (0.2 M) and the concentration of acrylamide ($C_{Acrylamide}$) was considered to be zero.

The fit of the models to the experimental data is shown in **Figures 7.1 – 7.3**. In spite of the fewer possible limitations (other responses and reaction pathways), the single response model fitted our data less well as opposed to the multiresponse model. The single response model of Claeys *et al*. (21) was able to fit the data well. The differences between the fit of these models can be the result of that we observed more degradation of acrylamide than Claeys *et al*. (21), which makes it more difficult for the software program to fit both formation and degradation of acrylamide. The results of Claeys *et al*. (21) contain too little information about the degradation and therefore the program can fit the results better. As they only reported the uncertainty of k_{ref}, the rate constant at reference temperature 160°C, the goodness of fit of these models cannot be compared in detail. Although in our study the formation of acrylamide consists of several steps as opposed to Claeys *et al*. (21) with only one step, the rate constants for the formation of acrylamide at 180 and 200°C found by Claeys *et al*. (21) correspond nicely with the rate constants found for the formation of acrylamide from the Schiff base. At the lower temperatures the rate constants that we found with multiresponse

Table 7.1

Kinetic parameters of acrylamide formation and degradation in aqueous reaction systems of asparagine-glucose.

	Claeys *et al*. (21)* Single response		This study (Chapter 3)** Single response		This study (Chapter 3)** Multiresponse			
Temp.	k_f [a]	k_e [a]	k_f [b]	k_e [a]	k_1 [b]	k_3 [b]	k_4 [a]	k_6 [a]
140°C	0.047	12	0.0014 ± 0.0005	35 ± 19	0.31 ± 0.05	0.37 ± 0.1	0.71 ± 0.2	28 ± 5
160°C	0.45 ± 0.02	111 ± 9	0.0055 ± 0.001	111 ± 36	0.67 ± 3	1.5 ± 0.4	2.5 ± 0.5	88 ± 25
180°C	3.5	862	0.020 ± 0.004	313 ± 61	1.4 ± 0.4	5.0 ± 2	8.1 ± 1	250 ± 45
200°C	23	5629	0.063 ± 0.01	812 ± 177	2.6 ± 0.9	16 ± 6	23 ± 4	650 ± 136
E_a (kJ/mol)	168 ± 4	167 ± 4	104 ± 13	85 ± 17	58 ± 8	102 ± 14	94 ± 11	85 ± 14

* 0.01 M L-asparagine and 0.01 M D-glucose dissolved in 0.05 M citrate buffer with pH 6.0; k_f is the rate constant for the formation of acrylamide and k_e is the rate constant for the degradation or elimination of acrylamide; ** 0.2 M L-asparagine and 0.2 M D-glucose dissolved in 0.1 M phosphate buffer with pH 6.8; k_1 and k_3 are the rate constants for the formation of the Schiff base from asparagine and glucose or fructose, respectively; k_4 is the rate constant for the formation of acrylamide from the Schiff base and k_6 is the rate constant for the degradation of acrylamide; [a] 10^{-3} min^{-1}; [b] 10^{-3} $l \cdot mmol \cdot min^{-1}$

modeling were slightly higher. The rate constants found for the formation of acrylamide in our reaction systems by single response modeling differed strongly from the results of Claeys *et al*. (21) and our multiresponse models. The rate constants found for the degradation of acrylamide in our reaction systems by single response and multiresponse modeling did not differ much. At 140 and 160°C all the rate constants were in the same range. At 180 and 200°C, the rate constants found in our study were lower than the ones found by Claeys *et al*. (21). The reported activation energies differed a factor 2 between our study and the study of Claeys *et al*. (21), the single response modeling of our acrylamide data resulted in activation energies that were comparable to the results as found by multiresponse modeling. The different results can have different causes. The differences between single and multiresponse models are based on the fact that multiresponse models use more information that has been obtained by monitoring multiple responses; the loss of reactants and the formation of other products are taken into account by estimating the rate constants and activation energies. The differences between single response models can be caused by other factors. The different buffer types that were used also have their effect on the reaction; the phosphate buffer can have a stimulating effect on the Maillard reaction (22). The pH difference of the systems was 0.8; this also can have its influence on the formation of acrylamide (**Chapter 4**). Finally, the temperature profiles can also differ between the experimental set-ups of both studies. Overall, it can be concluded that it is difficult to compare modeling results especially if different systems are used in combination with different modeling techniques. This means that the results for kinetic parameters that are published are not just valid for any situation, but only for specific conditions under which the experiments were performed. The multiresponse models do, however, provide more insight in the individual steps in the reaction network for

acrylamide formation in the Maillard reaction, and therefore provide more insight into the reaction mechanisms of acrylamide formation. However, the translation of the results from these models to general conditions could also be problematic and needs to be studied further.

Objective 3 *- Establish how processing conditions interfere with the reaction routes and influence the final composition of reaction mixture*

The experiments with the model reaction systems were conducted at different temperatures, times and pH. In this way it was established how temperature, time and pH influence the reaction routes and the fate of reactants and main products. In **Chapter 3** and **4** detailed explanations are given about these differences. The main conclusions that can be made from these observations are:

- The loss of reactants increased with increasing temperature.
- At higher reaction temperatures (>180˚C) the rate of degradation of acrylamide surpasses the rate of its formation in a glucose-asparagine system at pH 6.8.
- At pH 5.5 the degradation of acrylamide was, however, almost negligible, also at the higher temperatures.
- Glucose is able to isomerize to fructose and vice versa.
- Fructose has a higher reactivity with asparagine in the formation of the Schiff base.
- At pH 5.5 with fructose the activation energy for the formation of acrylamide from the Schiff base was a factor 3 lower compared to pH 6.8 with glucose in the aqueous reaction systems, which suggests that the temperature dependence of acrylamide formation with fructose as reducing sugar is much stronger for the precursor formation of acrylamide than acrylamide formation itself, whereas with glucose as reducing sugar acrylamide formation is much stronger temperature dependant.
- In the temperature range of 120–200˚C the Maillard reaction goes rapidly into the advanced stages with the formation of organic acids and high molecular weight melanoidins as main end products.

Test of the hypothesized mechanisms

An important step in modeling is to criticize a model to see how robust it is. If a model is only valid for the conditions on which it was built, its use is limited. If the model also holds for other conditions, it becomes much more useful. The kinetic models were tested at five different temperature and the estimated parameters showed Arrhenius-like temperature dependence. The kinetic models that were proposed are, therefore, at least for the systems we have studied, valid for all the temperatures within the range of 120-200˚C.

7.2.2 Empirical mathematical models

The main advantage of multiresponse kinetic models is that they give relevant information about the actual mechanisms of the reaction as in this case with the formation of acrylamide. However, these mechanistic models are not easily applied to real food systems as has been discussed in **Chapters 5** and **6**. Therefore we have applied the use of empirical models to elucidate the kinetics of acrylamide formation in a real food system.

Objective 4 *- Elucidate the kinetics of acrylamide formation in a real food system by mathematical models*

Three different empirical models were used to describe the kinetics of acrylamide formation in potato crisps. The Logistic-Fermi, Logistic-Exponential and Empirical models were able to fit the formation and degradation of acrylamide in potato crisps. The Logistic-Exponential model was favored after statistical evaluation and comparison of the precision of parameters. The precision of the parameters for the models in **Chapter 5** was, however, quite bad, which put a constraint on the use of these models for the prediction of acrylamide formation. By changing the experimental design, the precision of the parameters improved in the study described in **Chapter 6**.

Objective 5 *- Develop predictive models for the formation of acrylamide in real food systems*

The improved precision of parameters in **Chapter 6** and the strong correlation that was found between the 'scale factor' or parameter '*a*' of the Logistic-Exponential model and the initial reducing sugar concentration in the potatoes, made it possible to use this model for the prediction of acrylamide formation. Although the predictive capacity of the model has not been tested exhaustively, and should also be extended to other processing conditions, the use of this model seems promising to be used in tools by the crisp industry to mitigate acrylamide formation in their products. The use of empirical models to describe the formation of acrylamide in model reaction systems has been tested by Corradini and Peleg (25). They have modeled our results presented in **Chapter 3** for the formation and degradation of acrylamide with the empirical model 'Logistic-Fermi'. The conclusion from this paper is that empirical models can be used to describe the formation of acrylamide in model reaction systems, but that mechanistic models are stronger and more enlightening (25). The precision of the parameters of the empirical models was not taken into consideration in this study (25). We have applied the Logistic-Exponential model, which performed the best in **Chapter 5** and **6**, to the results for acrylamide formation (**Chapter 3**). **Table 7.2** shows the precision of the obtained parameters.

Table 7.2.

Estimates of parameters with their approximate SD (by linear approximation) for the Logistic-Exponential model

Temp (°C)	a (mmol·l^{-1})	k_1 (min^{-1})	t_{c1} (min)	τ (min)
120	0.36 ± 0.05	0.18 ± 0.07	30 ± 2	931 ± 1007
140	1.2 ± 0.03	0.32 ± 0.04	13 ± 0.5	2995 ± 1824
160	1.7 ± 0.07	0.63 ± 0.1	6.2 ± 0.5	22800 ± 96950
180	3.1 ± 0.2	1.9 ± 0.6	2.6 ± 0.3	53 ± 9
200	4.6 ± 0.3	4.0 ± 2	1.9 ± 0.07	18 ± 2

The precision of the parameters for the 'formation part' of the acrylamide concentration (k_1 and t_{c1}) and the 'scale factor' parameter 'a' are good, however the precision of parameter (τ) for the 'degradation part' is quite bad, especially at 120–160°C. Therefore the Logistic-Exponential model is less preferable for lower reaction temperatures where the degradation of acrylamide is not significant. In **Chapter 5** and **6** it was also shown that the precision of the parameters for the degradation was quite bad, which was the result of too little information about the degradation in the data. Although the precision of the 'formation parameters' of the Logistic-Exponential model showed a good precision, the parameters do not give any insight in the mechanism of formation: the parameters are only fitting parameters for the curve of acrylamide formation.

Model reaction systems are, however, designed to investigate reaction mechanisms; multiresponse modeling is for this the best technique as its extracts the most information from the measured responses and results in estimations for rate constants and activation energies for the different steps in the reaction. Empirical models, however, are very useful to model for instance the formation of acrylamide in foods, as they ignore all the chemical and physical mechanisms that occur during processing of foods. Foods are complex mixtures of a number of compounds and because of the complex food matrices reactants cannot meet each other readily and their activity will be different from model experiments. Furthermore, there are temperature and concentration gradients within the food during heating, which require the use of mass and heat transfer models that are also adapted or modified to account for physical changes in the food product.

7.3 MAIN ACHIEVEMENTS OF HEATOX

This PhD study was part of the HEATOX project, and only focused on one small part of it: the chemical reaction mechanism and kinetics of the formation of acrylamide. The HEATOX project encompassed more, namely the identification, characterization and risk minimization of heat-generated food toxicants. The three-year project HEATOX included

24 partners from 14 countries, mostly universities and research institutes, but also authorities and a European consumer organization. The main achievements are listed below (26):

Identified risk

- The HEATOX risk characterization concluded that the evidence of acrylamide posing a cancer risk for humans has been strengthened.
- Experiments have improved the scientific basis for estimating the health risk of low acrylamide doses for humans from high dose animal tests.
- Acrylamide levels in bread and potatoes have been reduced in laboratory experiments. Thus, human exposure can potentially be decreased.
- Food frequency questionnaires, as used in epidemiological studies, often give an imprecise measure of actual acrylamide exposure. The best way to estimate dietary exposure is the analysis of biomarkers in blood or urine.
- Acrylamide is not the only genotoxic compound formed when heating food. Furan, HMF and other compounds have been investigated. A database of more than 800 heat-induced compounds, of which around 50 have been highlighted as potential carcinogens based on their chemical structure, has been compiled to aid future research.

Managing the risk

Food industry

A large proportion of acrylamide intake comes from industrially prepared food. Mitigation methods can be efficient, since raw materials and production processes are well controlled. HEATOX has been a contributor to the European food industry's approach to minimize acrylamide formation – called the CIAA Toolbox.

- Formation factors in potatoes have been elucidated, illustrating the importance of raw material selection, additives, and processing, *e.g.* blanching and vacuum frying.
- The importance of heat transfer and oil/potato ratio in semi-industrial fryers has been investigated.
- Acrylamide formation in bread can be minimized by long yeast fermentation. New baking technologies have been evaluated.
- The influence of raw material and baking conditions on acrylamide levels in bread has been shown.

Research on mitigation methods need to be continued and the applicability in real food production need to be tested by industries. HEATOX scientists have calculated that successful application of all presently known methods would reduce the acrylamide intake by 40 % at the very most.

Home cooking

HEATOX has estimated that the acrylamide contribution from home-cooked food is in general relatively small, when compared with industrially or restaurant-prepared foods. However, high intake risk groups might exist. Furthermore, minimizing acrylamide formation in home cooking is a national challenge, since cooking and eating habits vary considerably between countries. General advice, resulting from this project, is to avoid overcooking when baking, frying or toasting carbohydrate rich foods.

Consumption

By following the general dietary recommendations (*i.e.* a balanced diet without excessive fat or calorie intake) a further reduction of the acrylamide intake can be achieved. Consumers should also avoid eating overcooked baked/fried food.

This PhD study has contributed to the gained knowledge about the chemical mechanisms and kinetics of acrylamide formation and has resulted in models that can be used in the mitigation of the content of acrylamide.

7.4 FUTURE PERSPECTIVES

As mentioned in the previous sections, the kinetic models that were derived gave more insight into the formation of acrylamide and can contribute to strategies to reduce acrylamide in foods. However, the multiresponse models for acrylamide formation should be further improved by elucidating more reaction routes by identifying and quantifying more products in the Maillard reaction and by introducing competing amino acids into the reaction system; working towards a more realistic model system. In general, this technique could also be used to gain more insight into the formation of other hazardous compounds, such as the list found by the HEATOX project. The use of empirical models seems promising by the simplicity of these models to mathematically describe a phenomenon such as acrylamide formation. These models can therefore be used for more processes in the food industry to describe the fate of certain quality parameters. If one is able to link the parameters to certain initial conditions, one could develop predictive models that can be used in quality assurance protocols.

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SUMMARY

The presence of acrylamide, classified as probably carcinogenic to humans in foods, has led to worldwide concern. Products such as French fries, crisps, coffee and bread contribute significantly to the overall intake of acrylamide. Although the health risks associated with dietary acrylamide are still not clearly established, the evidence of dietary acrylamide posing a cancer risk for humans has been strengthened. Mitigation of the content of acrylamide in foods is therefore of importance. This study was part of the HEATOX project, a European Union-funded project on the identification, characterization and risk minimization of heat-generated food toxicants. The present thesis was designed to increase the understanding of the reaction networks for the formation of acrylamide and to describe and predict the formation of acrylamide in a real food system, contributing to the efforts that are being made to mitigate acrylamide content in foods. The aim of this thesis was to derive mathematical models that describe quantitatively the formation of acrylamide as a function of several parameters. In three parts, the necessary steps are taken to achieve the aim and objectives, which are described in **Chapter 1**.

Part I (**Chapter 2**) provides the reader background information about acrylamide and its associated health risk, the Maillard reaction as main mechanism for the formation of acrylamide in food, the production process of potato crisps and strategies to reduce acrylamide formation and finally information about kinetic modeling.

Part II (**Chapter 3-4)** deals with the development of mechanistic models. The main mechanism of acrylamide formation is the Maillard reaction. The technique of multi-response modeling was used to unravel the reaction networks of the formation of acrylamide in the Maillard reaction using aqueous reaction systems. In **Chapter 3** a first kinetic model for the formation of acrylamide in a glucose-asparagine reaction system at pH 6.8 is proposed. The model resulted in a reasonable estimation for the formation of acrylamide in an aqueous model system, although the behavior of sugars and asparagine was slightly underestimated. Our kinetic model supported the observations that for the formation of acrylamide from asparagine a carbonyl source is needed, and the results also suggested an important role for fructose, especially at the higher temperatures. Furthermore, the experimental observations and the kinetic modeling suggested that acrylamide is an intermediate in the Maillard reaction rather than an end product, which implies that it is also subject to a degradation reaction. In **Chapter 4** the model from Chapter 3 was used as a starting point for the development of a more comprehensive model for the formation of acrylamide in a fructose-asparagine aqueous reaction system at pH 5.5. After the iterative process of developing a kinetic model, a final model was proposed that described the fate of reactants and main products. The formation of organic acids from sugar degradation was also included in the new kinetic model. The kinetic model suggests that the effect of temperature on acrylamide formation with fructose is more due to the preceding steps. The higher yield of acrylamide with fructose as reducing sugar at pH 5.5 as opposed to the yield of acrylamide with glucose at pH 6.8 suggests that both pH and type of sugar can play a role in lowering acrylamide formation. Furthermore, these models have shown that at high temperatures (120 –200˚C) the Maillard reaction rapidly goes into the advanced stages, forming high

amounts of organic acids and high molecular weight melanoidins. Overall, these mechanistic models provided more insight in the formation of acrylamide in a quantitative way.

Part III (**Chapter 5-6**) is devoted to the use of empirical models to describe and predict the formation of acrylamide in potato crisps. In **Chapter 5** it was shown that the formation of acrylamide in potato crisps could be modeled by applying Logistic-Fermi, Logistic-Exponential or Empirical models. Statistical methods were used to compare the performance of the models, with the 'Logistic-Exponential' and 'Empirical' model performing equally well. The precision of parameter estimates was problematic, which put a constraint on the use of these models to be used for prediction. Special attention was given in this study to the temperature developments in the surrounding oil and outer cell layer of the potato slices, providing more insight in the frying process and to make future comparisons between studies possible. In **Chapter 6** improvements were made to the precision of the parameters of the empirical models. The relationships between the parameters of the best performing model and the precursors content were studied. Parameter '*a*' of the Logistic-Exponential model showed a strong correlation with the reducing sugar concentration of the raw potatoes. Based on this relationship, the predictive capacity of the Logistic-Exponential model was tested. The predictions of the model for the formation of acrylamide in potato crisps were close to earlier reported experimental values. Therefore, the use of the 'Logistic-Exponential' model as a tool to predict acrylamide in potato crisps seems promising and needs further development.

In **Chapter 7** the main achievements of this PhD study are discussed. An overview is given of how multiresponse modeling has become a powerful tool to get insight in the formation of acrylamide in the Maillard reaction. Furthermore, the use of empirical models to describe and predict the formation of acrylamide and their potential use in mitigation strategies is discussed. Finally, the main achievements of the HEATOX project are summarized to place this study in a wider perspective and a future perspective is given about modeling.

SAMENVATTING

Aangezien acrylamide als potentieel carcinogeen voor mensen is geclassificeerd, heeft het voorkomen van acrylamide in voedingsmiddelen wereldwijd tot grote ongerustheid geleid. Vooral producten zoals frites, chips, koffie en brood dragen bij tot de totale inname van acrylamide. Hoewel de gezondheidsrisico's van de inname van acrylamide via voedsel nog niet duidelijk zijn vastgesteld, komen er steeds meer aanwijzingen dat acrylamide in voedingsmiddelen het risico op verschillende typen kanker zou kunnen verhogen. Daarom is het terugdringen van de vorming van acrylamide in voedingsmiddelen van belang. Deze promotiestudie maakte deel uit van het HEATOX project, een door de Europese Unie gesponsord project gericht op de identificatie, karakterisering en het verlagen van risico's van toxische componenten die tijdens de verhitting van levensmiddelen worden gevormd. Het doel van dit promotieonderzoek was om meer inzicht te verkrijgen in de reactienetwerken voor de vorming van acrylamide in modelsystemen en om de vorming van acrylamide in een levensmiddelsysteem te beschrijven en te voorspellen om daarmee een bijdrage te leveren aan de inspanningen die in gang zijn gezet om acrylamide vorming in levensmiddelen te reduceren. Er werd naar gestreefd om te komen tot de ontwikkeling van mathematische modellen die kwantitatief de vorming van acrylamide beschrijven als functie van een aantal parameters. In drie delen worden de noodzakelijke stappen genomen om dit doel en de bijbehorende doelstellingen, welke in **Hoofdstuk 1** worden besproken, te bereiken.

Deel I (Hoofdstuk 2) verstrekt de lezer achtergrond informatie met betrekking tot acrylamide en de daarbij behorende gezondheidrisico's, de Maillard reactie als belangrijkste mechanisme voor de vorming van acrylamide in levensmiddelen, het productieproces van aardappelchips en strategieën voor de verlaging van acrylamidevorming en tenslotte informatie over kinetisch modelleren. Het dilemma met de Maillard reactie is dat deze niet alleen leidt tot vorming van het *ongewenste* acrylamide maar ook tot de vorming van allerlei *gewenste* smaak- en geurstoffen en een vaak gewenste bruine kleur. Er moet dus een compromis gevonden worden tussen zo weinig mogelijk acrylamide en zoveel mogelijk gewenste stoffen. Het idee is dat een dergelijk compromis bestudeerd kan worden door gebruik te maken van computermodellen.

Deel II (Hoofdstukken 3 en **4)** beschrijft de ontwikkeling van mechanistische modellen. De Maillard reactie is het belangrijkste mechanisme voor de vorming van acrylamide. De techniek van 'multiresponse' modelleren is toegepast om de reactie netwerken te ontrafelen voor de vorming van acrylamide in de Maillard reactie gebruikmakend van waterige modelsystemen. In **Hoofdstuk 3** wordt een eerste model gepresenteerd voor de kinetica van de vorming van acrylamide in een glucose-asparagine reactie systeem met pH 6.8. Het model resulteerde in een redelijke schatting voor de vorming van acrylamide in het waterige modelsysteem, hoewel het verloop van suikers en asparagine lichtelijk werd onderschat door het model. Ons kinetisch model onderschrijft de observaties dat voor de vorming van acrylamide naast asparagine ook een carbonyl bron nodig is. Isomerizatie van glucose leidde tot de vorming van fructose en de resultaten suggereren dat fructose, voornamelijk bij de hogere temperaturen, een belangrijkere rol speelt dan glucose in de vorming van acrylamide.

Daarnaast doen de experimentele waarnemingen vermoeden dat acrylamide geen eindproduct is in de Maillard reactie, maar een intermediair, waardoor acrylamide onderhevig is aan afbraakreacties. In **Hoofdstuk 4** is het model van Hoofdstuk 3 gebruikt als startpunt voor de ontwikkeling van een uitgebreider model voor de vorming van acrylamide in een fructose-asparagine waterig modelsysteem met pH 5.5. Na het iteratieve proces van het ontwikkelen van een kinetisch model is een uiteindelijk model voorgesteld dat het lot van reactanten en eindproducten beschrijft. De vorming van organische zuren door suikerdegradatie is ook verwerkt in het nieuwe kinetische model. Het kinetisch model suggereert dat het effect van temperatuur op de vorming van acrylamide uit fructose en asparagine meer effect heeft op stappen voorafgaand aan de eigenlijke acrylamidevorming dan op de vormingsreactie zelf. Dit is in tegenstelling tot de vorming van acrylamide uit glucose en asparagine waar de temperatuur vooral effect heeft op de eigenlijke vormingsreactie zelf. De hogere opbrengst van acrylamide met fructose als reducerende suiker bij pH 5.5 in tegenstelling tot de opbrengst van acrylamide met glucose bij pH 6.8 suggereert dat zowel pH als het type suiker een rol kunnen spelen bij het verlagen van acrylamide vorming. Daarnaast tonen deze modellen aan dat bij hoge temperaturen (120-200˚C) de Maillard reactie zeer spoedig overgaat in de vergevorderde stadia, waarbij organische zuren en melanoidinen (een verzamelnaam voor de bruine kleurstoffen) in grote mate worden gevormd. Over het algemeen kan worden gezegd dat deze mechanistische modellen op een kwantitatieve manier meer inzicht hebben gegeven in de vorming van acrylamide.

Deel III (Hoofdstukken 5 en **6)** is gewijd aan het gebruik van empirische modellen om vorming van acrylamide in aardappelchips te beschrijven en te voorspellen. De reden om over te stappen op empirische modellen is dat met levensmiddelen de samenstelling en structuur te complex is om zonder meer mechanistische modellen te kunnen gebruiken. Bovendien komen er vraagstukken t.a.v. stof- en warmtetransport om de hoek kijken die niet eenvoudig op te lossen zijn. Met empirische modellen worden al deze complicaties min of meer verborgen in de gebruikte parameters. In **Hoofdstuk 5** is aangetoond dat de vorming van acrylamide in aardappelchips kan worden gemodelleerd door het toepassen van Logistische-Fermi, Logistische-Exponentiële en Empirische modellen. De prestaties van deze modellen zijn vergeleken met behulp van statistische methoden, waarbij het Logistische-Exponentiële en het Empirische model even goed presteerden. De precisie van de schattingen voor de parameters was enigszins teleurstellend, hetgeen een restrictie oplegde voor het gebruik van deze modellen voor het voorspellen van acrylamide vorming. Speciale aandacht is besteed aan de temperatuurprofielen in de omringende olie en de buitenste laag cellen van de aardappel chips. Hierdoor werd meer inzicht verkregen in het frituur proces en is het mogelijk om in de toekomst verschillende studies beter met elkaar te vergelijken. In **Hoofdstuk 6** is beschreven hoe een betere precisie van de parameters van de empirische modellen is bereikt. De relaties tussen de parameters van het best presterende model en de beginconcentraties van suikers en asparagine in de rauwe aardappelchips is onderzocht. Parameter '*a*' van het Logistische-Exponentiële model toonde een sterke correlatie met de concentratie reducerende suikers in de rauwe aardappel. Op grond van deze relatie is het voorspellende karakter van het Logistische-Exponentiële model getest. De voorspellingen van het model voor de vorming van acrylamide in aardappelchips waren dicht in de buurt van eerder gerapporteerde

experimentele waarden. Het gebruik van het Logistische-Exponentiële model als instrument om acrylamide vorming te voorspellen in aardappelchips ziet er daarom veelbelovend uit en verdient verdere ontwikkeling.

In **Hoofdstuk 7** worden de belangrijkste resultaten en conclusies van deze promotiestudie besproken. Er wordt een overzicht gegeven over hoe 'multiresponse' modelleren een krachtig instrument is geworden om inzicht te verkrijgen in de vorming van acrylamide in de Maillard reactie. Daarnaast wordt het gebruik van empirische modellen om acrylamide vorming te beschrijven en te voorspellen besproken en hoe deze mogelijk kunnen worden toegepast in strategieën om acrylamide vorming te reduceren. Een kritische vergelijking wordt gemaakt met de literatuur en geconcludeerd wordt dat het niet zomaar mogelijk is om resultaten te vergelijken. Dit heeft praktische consequenties voor het toepassen van modelleerwerk. Tot slot worden de belangrijkste uitkomsten van het HEATOX project samengevat om deze studie in een breder perspectief te plaatsen en wordt een toekomstperspectief gegeven over de rol van modelleren in de levensmiddelentechnologie.

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Jeroen Jaap Knol was born in Breda, the Netherlands, on the 5th of March 1975. He received his diploma at Municipal Grammar School Hilversum in 1994. In the same year he started at the Wageningen Agricultural University with the study Bioprocess Technology. In 1995 he switched from Bioprocess Technology to Food Technology and received his propaedeutic in Food Technology in 1998. After a temporary leave of 2 years, in which he worked at the department Operations Electronic Banking of ABN AMRO BANK N.V., Amsterdam, he returned to Wageningen University and continued his BSc in Food Technology in 1999. In 2002 he received his BSc degree in Food Technology, followed by his MSc degree in Food Technology in 2004. He carried out his internship at the strategic marketing company Innovaction B.V., Leiden. He completed two majors during his MSc. The first MSc thesis was done at the Product Design & Quality Management Group, Wageningen University. The second MSc thesis was done part-time at Innovaction B.V., Leiden for the Marketing and Market Research Group, Wageningen University. Besides his second MSc thesis, he worked part-time at Innovaction B.V. in different projects in the field of market and trend analysis for the food industry. In 2004 he started his PhD at the Product Design & Quality Management Group, Wageningen University. Within this period he carried out two research periods at the Department of Food Technology, Engineering and Nutrition, Lund University, Sweden. During his PhD he was also active as chair of the VLAG PhD Council and member of the Wageningen PhD Council. Since 2006 he is also active for the Dutch Society for Nutrition and Food Technology (NVVL). Since April 2008 he works as product innovator / nutritionist at AGF Innovations B.V.

PUBLICATIONS

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Jeroen J. Knol, Wil A. M. van Loon, Jozef P. H. Linssen, Anne-Laure Ruck, Martinus A. J. S. van Boekel, and Alphons G. J. Voragen. Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system. *Journal of Agricultural and Food Chemistry* **2005**, 53, 6133-6139

OVERVIEW OF COMPLETED PHD TRAINING ACTIVITIES

International courses and trainings

- Short Term Scientific Mission at Lund University, COST action 927, Lund, Sweden, 2006
- International Advanced Course 'Food & Health', Socrates Intensive Program, Paris, France, 2005
- Reaction Kinetics in Food Science, VLAG, Wageningen, the Netherlands, 2004

International symposia, workshops and meetings

- Workshop COST action 927 - Thermally Processed Foods: Possible Health Implications, Ebersberg, Germany, 2007
- 9th International Symposium on the Maillard Reaction, Munich, Germany, 2007
- Workshop COST action 927 - Thermally Processed Foods: Possible Health Implications, Sofia, Bulgaria, 2007
- PhD study tour, University of Massachusetts (Amherst, MA); Cornell University (Ithaca, NY); Penn State University (State College, PA); Heinz R&D (Warrendale, PA); Ohio State University (Columbus, OH); University of Wisconsin-Madison (Madison, WI), Kraft Foods Global Research (Glenview, IL), USA, 2007
- Symposium 'Multidisciplinary Approaches to Reducing the Levels of Acrylamide in Food', Hertfordshire, United Kingdom, 2006
- Meetings for the EU project 'HEATOX' (Heat-generated Food Toxicants - Identification, Characterization and Risk Minimization), Copenhagen, Denmark; Uppsala, Sweden; Bologna, Italy; Wageningen, the Netherlands; Istanbul, Turkey; Graz, Austria; Prague, Czech Republic, 2004-2007

National courses and trainings

- Career Perspectives, WGS, Wageningen, 2007
- Media Training, WGS, Wageningen, 2006
- Supervising of Undergraduate Students, OWU, Wageningen, 2006
- Philosophy and Ethics of Food Science, VLAG, Wageningen, 2005
- Scientific Writing, CENTA, Wageningen, 2005
- Predictive Modeling, Wageningen University, Wageningen, 2004
- PhD Introduction Course, VLAG, Bilthoven, 2004

National symposia, workshops and meetings

- Symposium 'Salt Reduction', NVVL, Wageningen, 2008
- Symposium 'Passion or Passive', Wageningen University, Wageningen, 2007
- Symposium 'Food for Innovation', Wageningen University, Wageningen, 2005
- Symposium 'Science is Cooking', Wageningen University, Wageningen, 2005

ACKNOWLEDGEMENT

Presumably the most read piece in most dissertations is the 'acknowledgment'. Most readers won't be interested to read about mechanistic multiresponse models for the formation of acrylamide in aqueous reaction systems or about the kinetics of acrylamide formation in potato crisps. They would, rather, feast upon a big bag of those potato crisps if they forgot to read Chapter 2 and 7 (there is still time to do so!). Anyhow, the 'acknowledgment' is also an important part of the thesis, acknowledging all the persons that have had their hand in the process that finally led to this tangible proof of my PhD study.

Where does one start? If it weren't for Dirk Balfoort for instance, I would not have studied Food Technology in the first place and without the support of my parents I would not have finished my Food Technology study, which can be seen as rather important requisites for doing a PhD study in Food Technology. Furthermore, a lot of people have contributed in the past years, during my studies and work, to my personal and scientific development, each of course in its own way and all of inestimable value. Mentioning everybody will make this acknowledgment rather lengthy, so I will limit myself.

Dear Tiny, when you heard that I was considering a PhD study, you approached me with this wonderful project about modeling acrylamide. After our first conversation about the project, you and Jozef gave me the confidence that I have relied on during these years. Your enthusiasm and profound knowledge of kinetic modeling have contributed greatly to this work. Despite your role as Scientific Director of VLAG and all your other obligations, you had always the time to assist me. Comments on articles were returned within the shortest possible time. A PhD student could not wish for more. Beside your 'scientific role', I enjoyed your company on the 'business trips' to the HEATOX meetings and especially enjoyed our trip to Sofia together with Marieke and your wife Corrie. My apologies to you, Corrie, for all the times that I confiscated your valuable time with Tiny.

Dear Jozef, as I mentioned already, your confidence contributed to the completion of this PhD study. During these four years I had some experimental drawbacks: the crash of the hard disk of the HPLC computer, erasing the work of weeks; the fruitless attempts of purifying and identifying the breakdown products of asparagine and other unknown components; the uncooperativeness of the GC-MS. Your commonsense has helped to overcome these obstacles and work effectively with the results that were already accomplished. Thanks for all your valuable input during these years!

During my stay at Lund University, I had the pleasure of working with Gunilla, Ingegerd and Kerstin. Our meetings about the frying of potato crisps and the possibilities of modeling acrylamide formation have been very fruitful. But foremost I remember your warm hospitality, the nice dinners and the inspirational atmosphere at your department. Thank you! I would also like to thank all the other students for a wonderful time with the innerbandy games, the lunches and the wonderful cocktail party. Thank you all! I would also like to thank Susanna and Joakim for lending me their very nice apartment and of course Henrik and Cecillia for your hospitality.

As my study was part of a large European project, I had the pleasure of working with a great number of fine scientists. I enjoyed our meetings and cooperation, especially with all the other PhD students and young scientist. Thank you all for the great times! I am also grateful to Helga and Hanne Mari for your valuable lessons on communication. Finally I would like to thank Kerstin and Anki for their important role in coordinating the HEATOX project.

All these years of research would not have been possible without the financial support from the European Union, the same goes for my scientific mission to Lund University which was funded by COST action 927, for which I am very grateful.

During these years I had also the pleasure of working with lots of others. Wil, thank you for your cooperation, which resulted in Chapter 3. Prof. Peleg and Maria, Chapter 5 and 6 are based on your work with empirical models, thank you for your mathematical assistance. The very short visit to your department in Amherst has been very fruitful. I am looking forward to our future collaboration. At the department of Organic Chemistry, I have had the pleasure of working with Prof. Südholter, Ton, Teris, Frank and Elbert. Although our collaboration did not produce the results that we were looking for, I have appreciated our discussions and gained a lot from the analyses done at your department. Pieter thank you for the NMR analyses. I am also very grateful for all the work that has been done by the Technical Workshop. Your work is indispensable!

I would also like to thank all the people from the VLAG PhD Council, Wageningen PhD Council and the VLAG office with whom I have worked.

I have done my work at the department of Product Design & Quality Management, where I enjoyed working all those years. All the activities, especially the drinks with our colleagues from Food Physics and the PhD trip to the USA, have made the process of doing a PhD study more comfortable. Thank you all for the fun times during the parties and drinks! And to all my roommates of 211B, I will not miss the crowdedness, but will miss our discussions about the 'more important' things in live! Jeroen, it hasn't happened for some years now, but this season Feyenoord will become champion! Mirko, next year we should drink a nice bottle of 'Kasteel' beer on your PhD! Gao, all the best, hopefully we can stay in contact. A special thanks goes to Frans, Charlotte and Henk for all their support during these years. I would also like to thank the few students that have worked for me, Anneke, Els and Yvette, thank you for your contribution.

The strange thing of doing a PhD study is that four years of good work is being rewarded with discharge, in that way the word 'promotion' does seem to be out of place. So, during the final months I had to think about the direction of my career. The nice discussions during all the job interviews and with Mr. van Lede have helped me choosing the right direction. Thank you all for your valuable advises.

Furthermore, I would like thank the Executive Board of Proost en Brandt for their generous contribution by supplying the paper for this dissertation. Gertie, I appreciate all your work on

designing the complete lay-out of this thesis, without you it wouldn't look this good! Finally, I will address myself to my close friends and family. During the past years my work sometimes impacted on my social life, especially the last months. Hopefully, I can make up for the lost time. Jasper, I hope we can find the time to make up for the important times I couldn't be there for you. Joris, I will miss our coffee breaks on the Dreien, gossiping about Wageningen UR. Quirine and Wouter, thank you for your patience, as you are reading this, you should have your photo book of your wedding, if I can combine it with taking care of ~~Emma~~ Annemijn. Jan, Eleonore, the rest of the family (in law) and friends thank you for all your interest in my research!

Jochem, it means a great deal to me that you are there again for me on an important day in my life. Your friendship is invaluable! I do hope that we can find more time to come to Maastricht enjoying the company of you, Anja, Jelle and Sanne. Willem, as our big brother, you have paved the way for Maurits and me in multiple ways. You always looked after us, for which I am very grateful. Thank you for being my paranimf. I look forward to spend more time with you, Sytske, Olivier and Thijmen; moving away from The Hague has affected our relation. Maurits, my little brother, it was great to see how you found your vocation. Hopefully, Vienna will not be an obstacle to see you and Dieuwertje regularly.

Dear parents, your patience has finally paid off. Without your support and your faith in me I could not have come this far. After all those years I am finally ready with studying. You can focus completely on your new role as grandparents! I appreciate your good care for me all these years.

Dear Kika, I have met you just in the right time. Your support helped me finishing my MSc. Although my MSc study took a bit longer then I promised you in the beginning, I have made it up with my PhD. You supported me in the decision to do this PhD study, giving up your job and moving back with me to Wageningen. I have admiration for how you did the PABO next to your job at the ABN AMRO. During these years you supported me when I had setbacks in my research and kept me on target. The last period, during your pregnancy, you were still there for me to give me neck massages and doing the domestic chores, so I could continue my work. You are the most cheerful person that I know; being with you makes live more wonderful. I look forward to spent more time with you; the last few months my study has taken too much time away from us. Thank you for all your good care and for your love! Annemijn, you're just in time to be included in this book, you are the best thing that has ever happened to your mother and me. Your presence in your mothers belly was a great stimulant to finish everything before your birth. Thank you for being so wonderful!

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FIGURES

KINETIC MODELING OF ACRYLAMIDE FORMATION IN AQUEOUS REACTION SYSTEMS AND POTATO CRISPS

Chapter 2

Acrylamide, the Maillard reaction, potato crisps and modeling

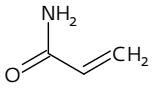


Figure 2.1 Molecular structure of acrylamide

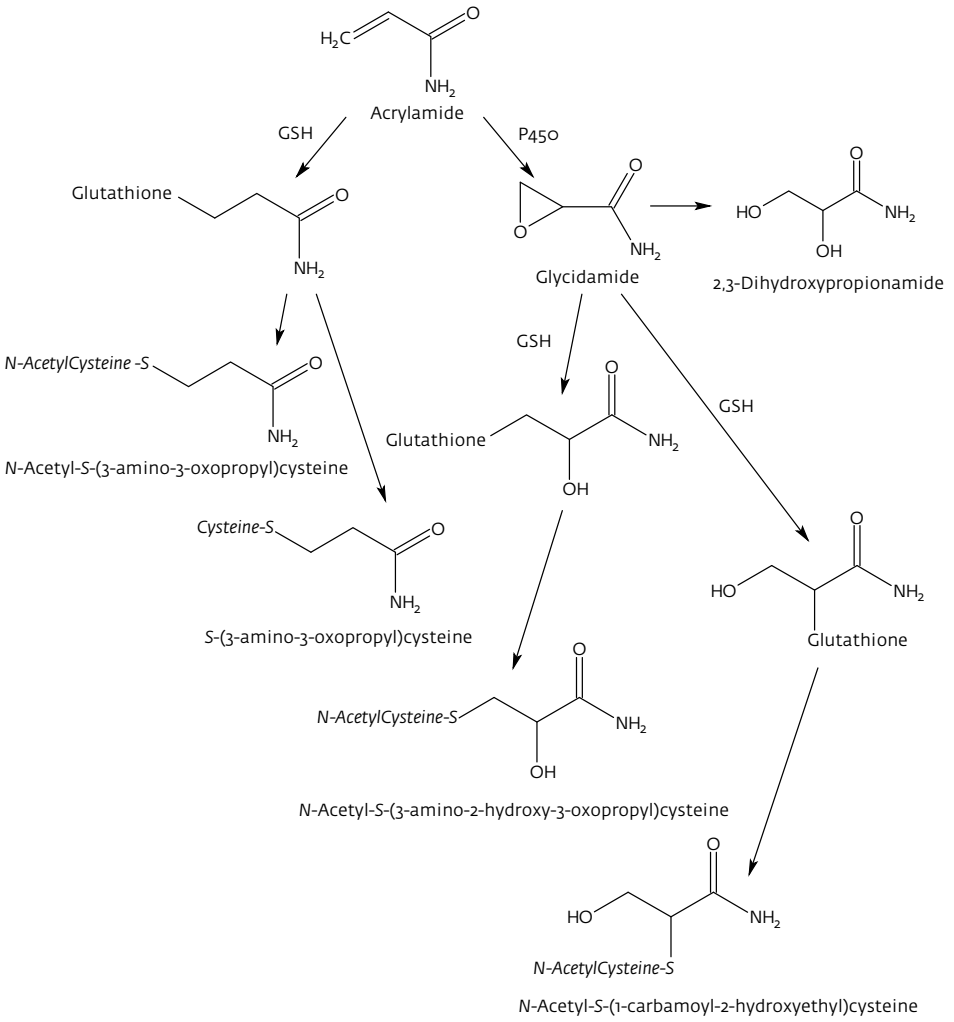


Figure 2.2 Proposed metabolism of acrylamide. Adapted from Sumner *et al*. (10, 11).

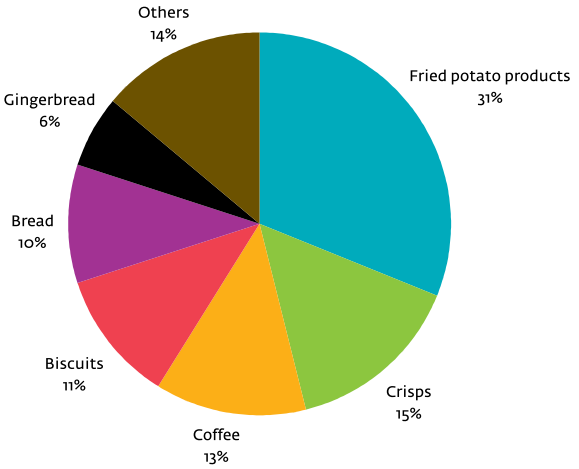


Figure 2.3 Contribution (%) of the most important food groups to the dietary exposure to acrylamide in the total Dutch population (1 – 97 years) taken from Boon *et al*. (29).

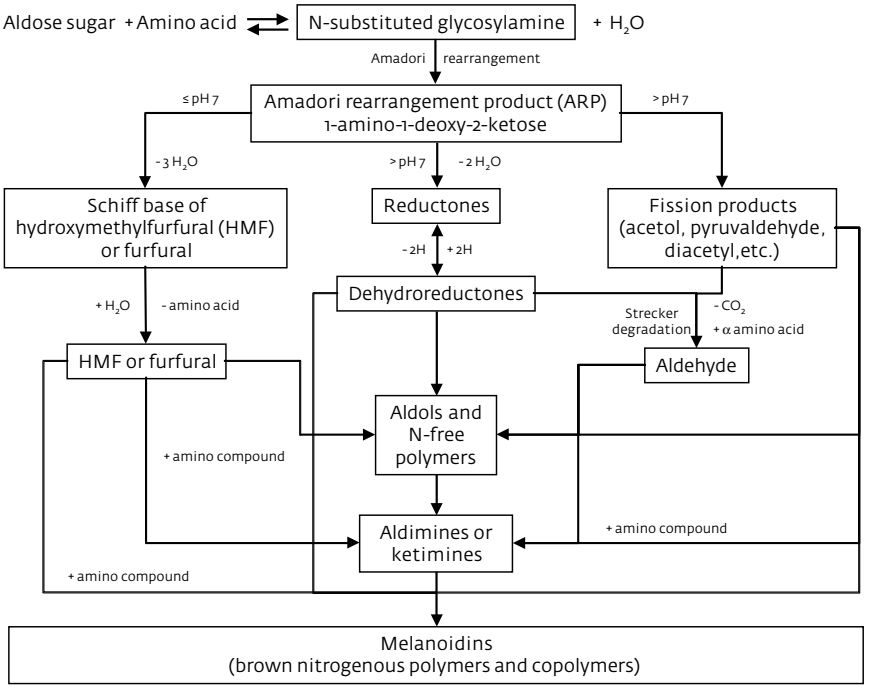


Figure 2.4 Maillard reaction scheme adapted from Hodge (43).

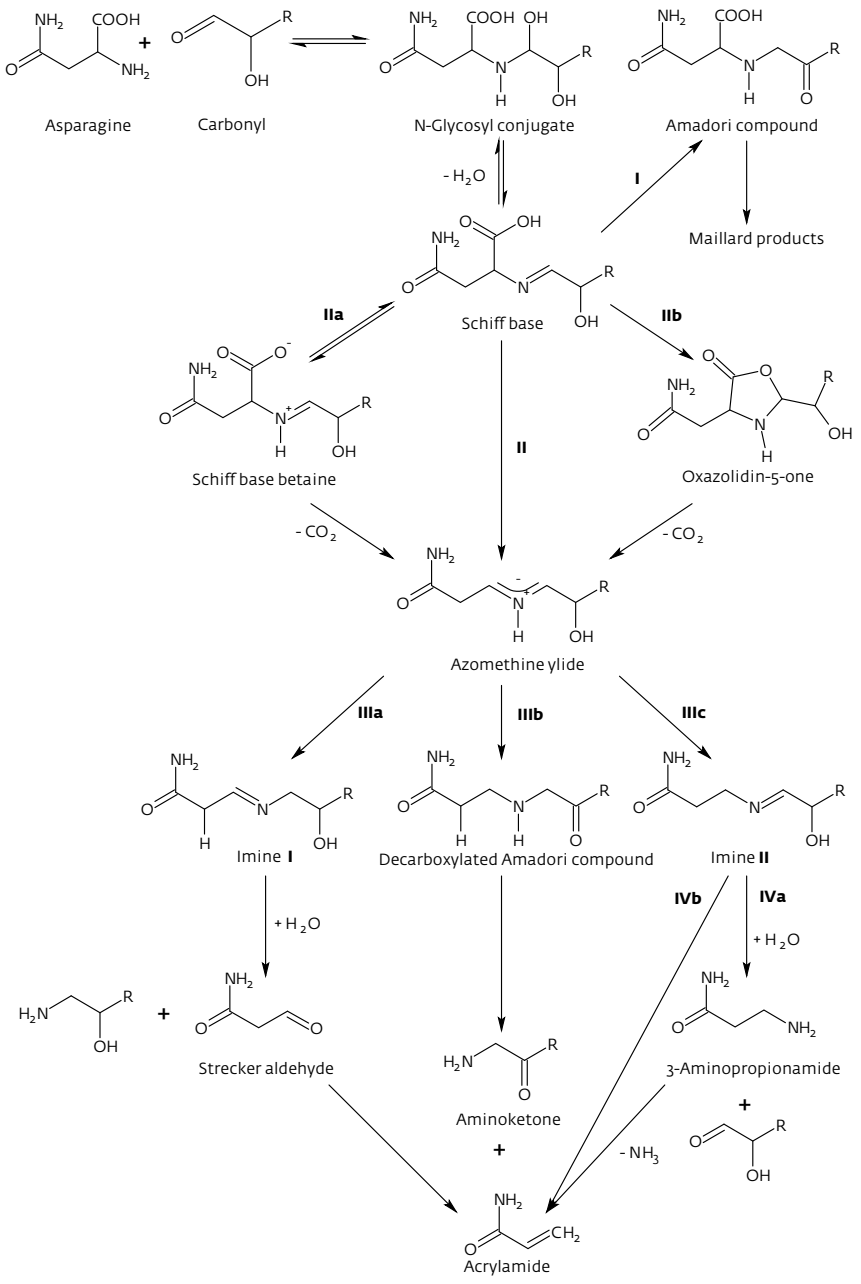


Figure 2.5 Proposed reaction scheme for the formation of acrylamide in the Maillard reaction adapted from Stadler *et al*. (58).

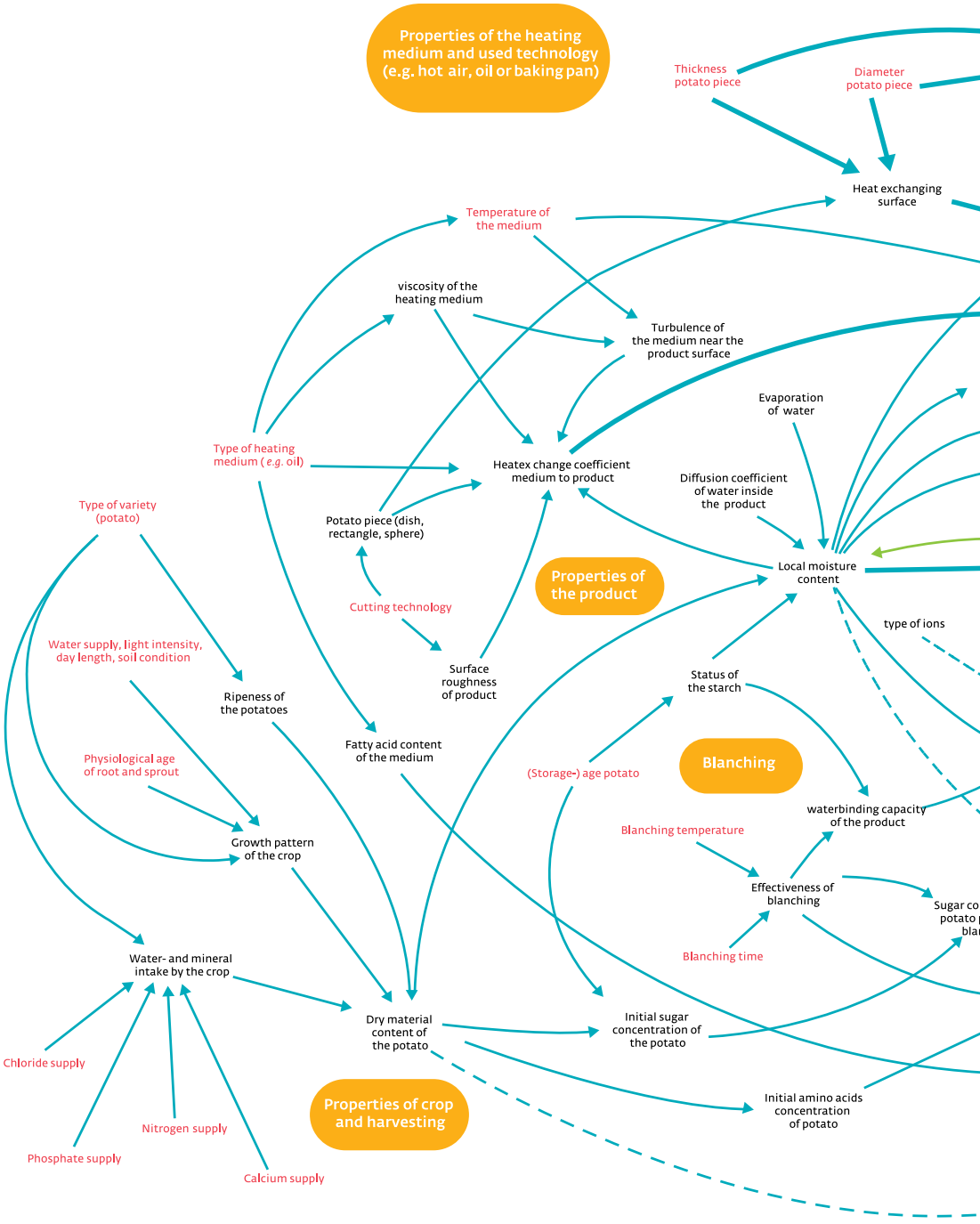
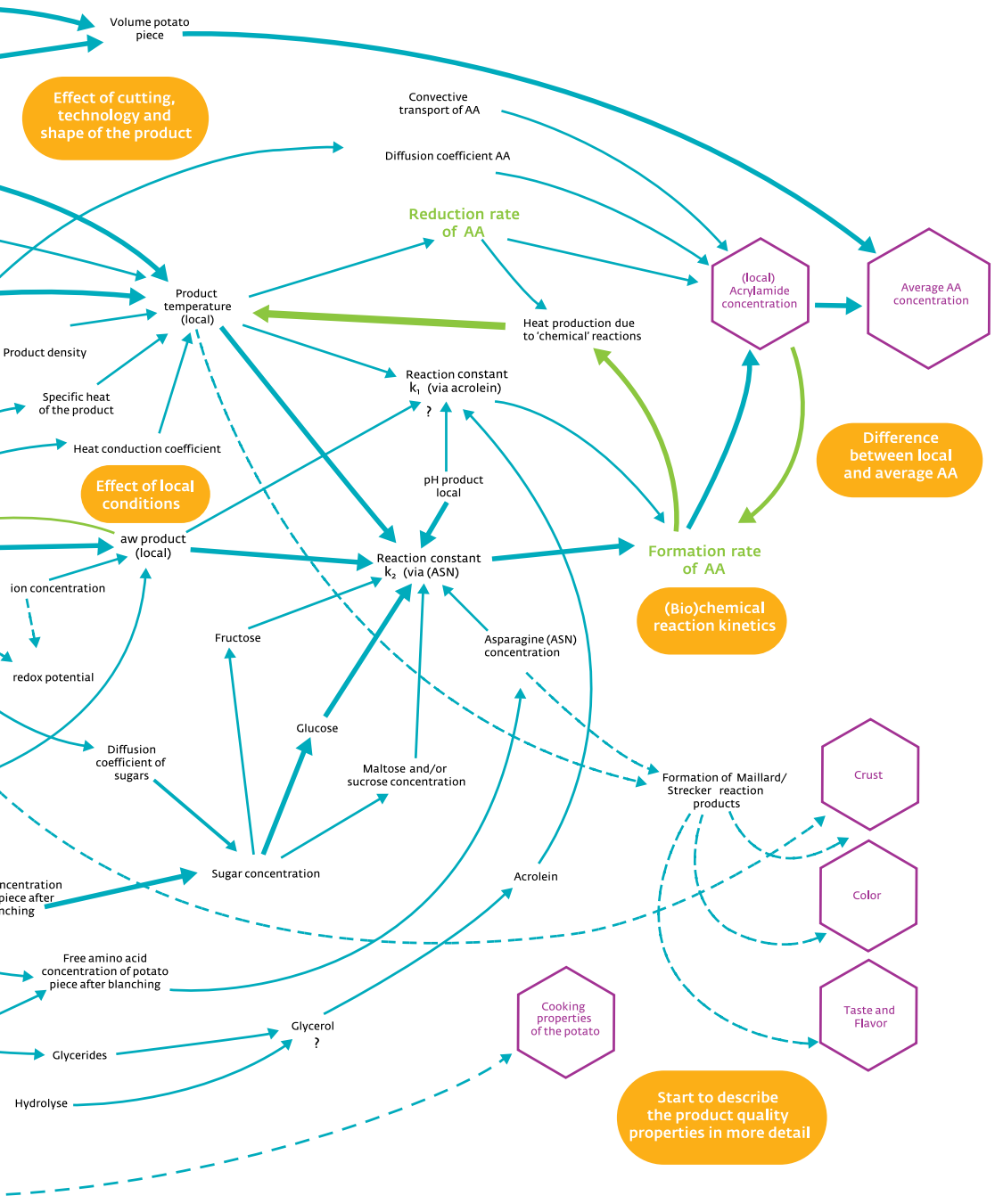


Figure 2.6 Mind map of the factors describing the formation of acrylamide in relation to the production of fried potato products (75).


Volume potato piece
Effect of cutting, technology and shape of the product
Convective transport of AA
Diffusion coefficient AA
Reduction rate of AA
(local) Acrylamide concentration
Average AA concentration
Product temperature (local)
Heat production due to 'chemical' reactions
Product density
Specific heat of the product
Reaction constant k_1 (via acrolein)
?
Heat conduction coefficient
Difference between local and average AA
Effect of local conditions
pH product local
aw product (local)
Reaction constant k_2 (via ASN)
Formation rate of AA
(Bio)chemical reaction kinetics
ion concentration
Fructose
Asparagine (ASN) concentration
redox potential
Glucose
Diffusion coefficient of sugars
Maltose and/or sucrose concentration
Formation of Maillard/ Strecker reaction products
Crust
Color
Sugar concentration
Acrolein
Free amino acid concentration of potato piece after blanching
Cooking properties of the potato
Taste and Flavor
Glycerol
?
Glycerides
Hydrolyse
Start to describe the product quality properties in more detail

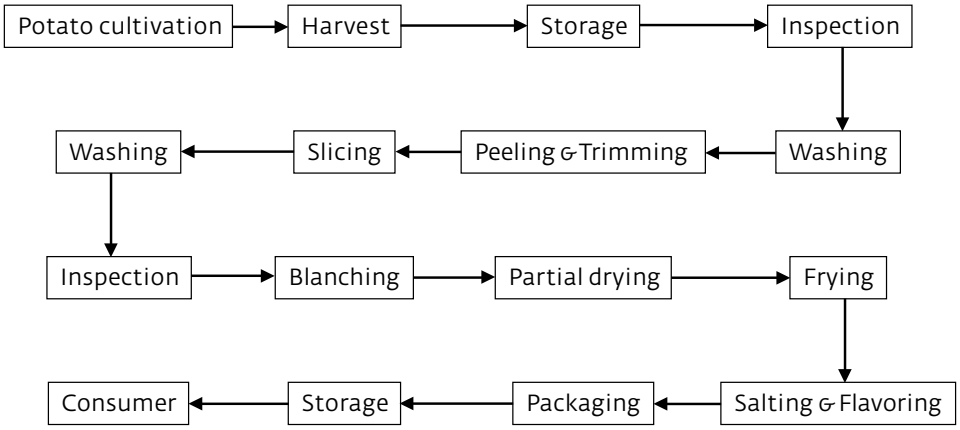
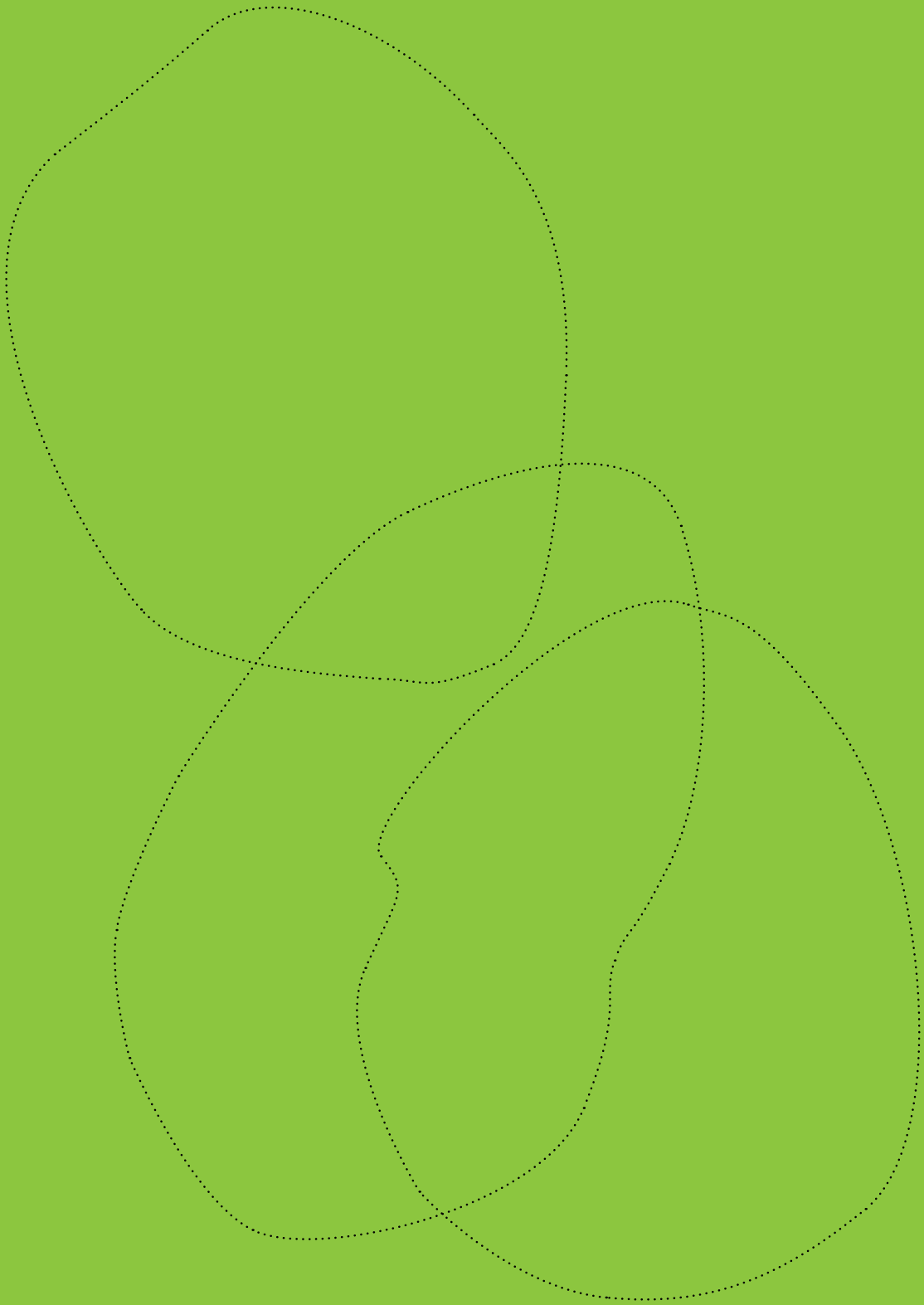


Figure 2.7 Overview of the production process of potato crisps.

Chapter 3

Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system



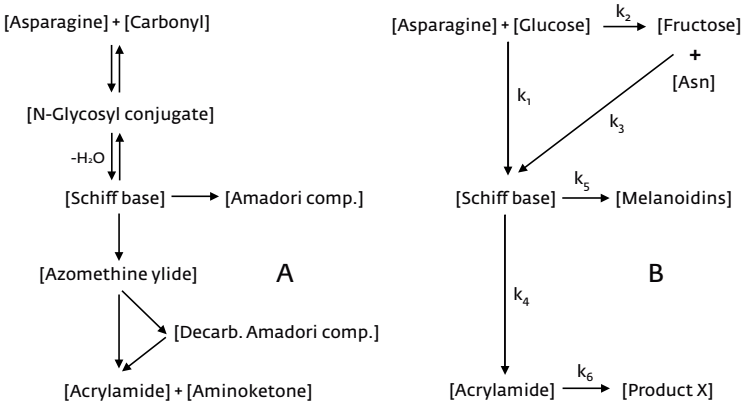


Figure 3.1 (**A**) Reaction network for the formation of acrylamide from asparagine through early Maillard reaction adapted from Stadler *et al*. (33). (**B**) Simplified reaction network for the formation of acrylamide from asparagine and glucose/fructose through early Maillard reaction. Asn, asparagine; Decarb. Amadori comp., decarboxylated Amadori compound; Product X, assumed product(s) formed from acrylamide.

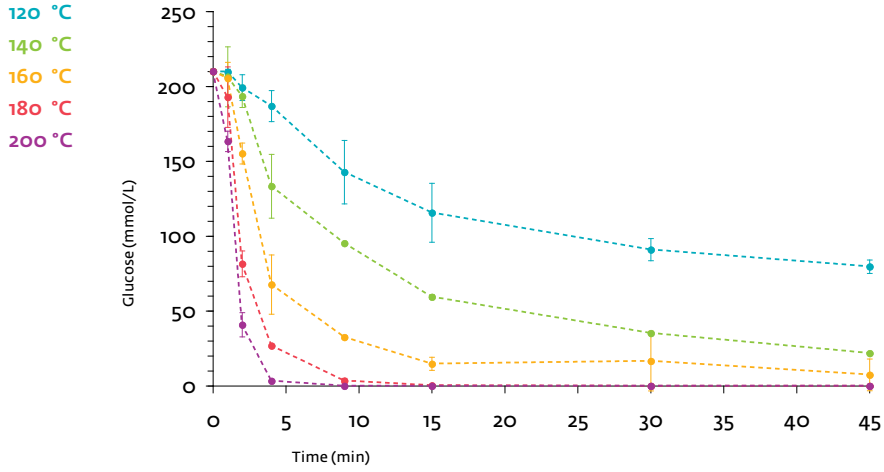


Figure 3.2 Experimental data of the loss of glucose in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

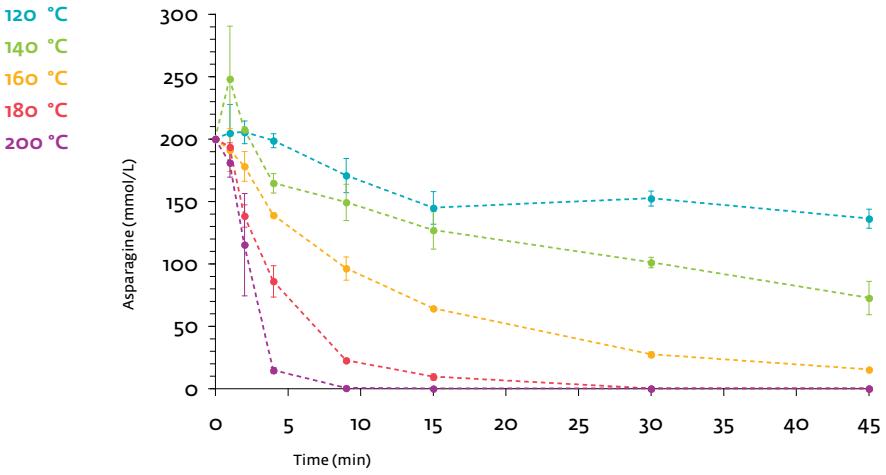


Figure 3.3 Experimental data of the loss of asparagine in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

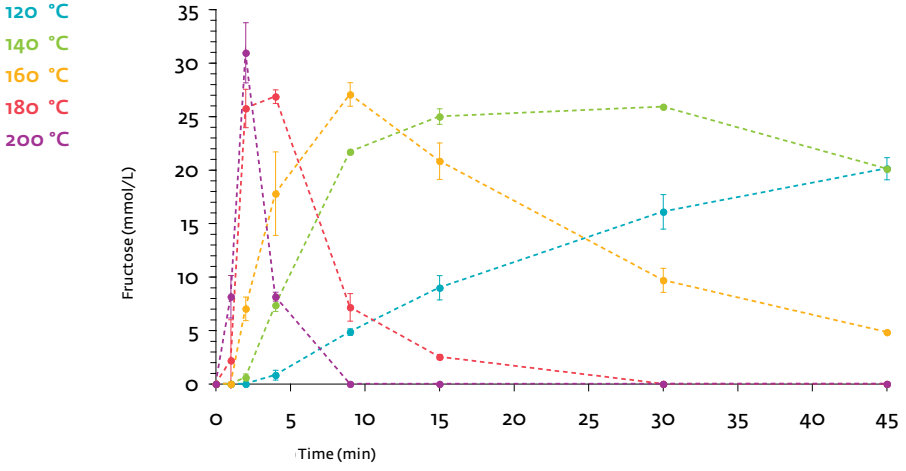


Figure 3.4 Experimental data of the formation of fructose in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

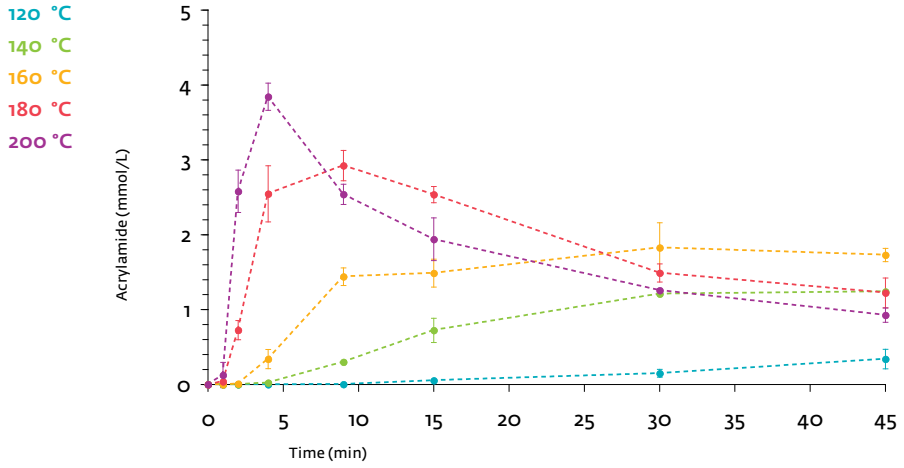


Figure 3.5 Experimental data of the formation of acrylamide in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

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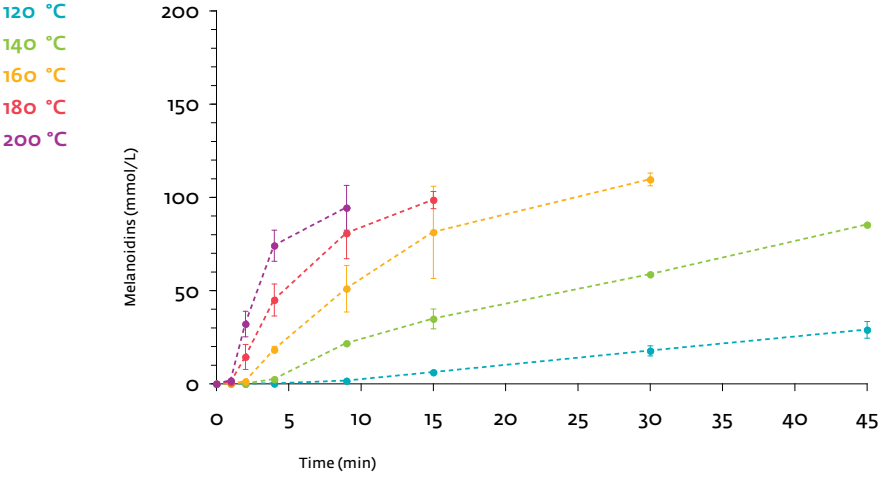


Figure 3.6 Experimental data of the formation of melanoidins in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

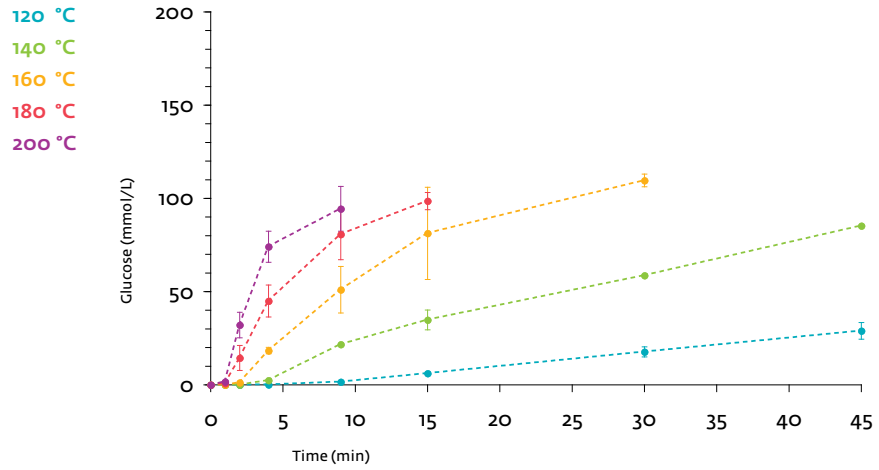


Figure 3.7 Model fit (lines) to experimental data (symbols) of the loss of glucose in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

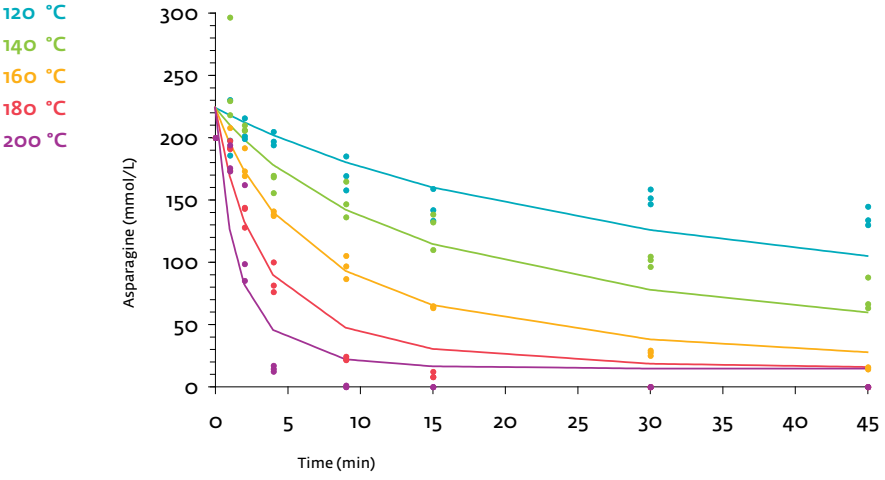


Figure 3.8 Model fit (lines) to experimental data (symbols) of the loss of asparagine in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

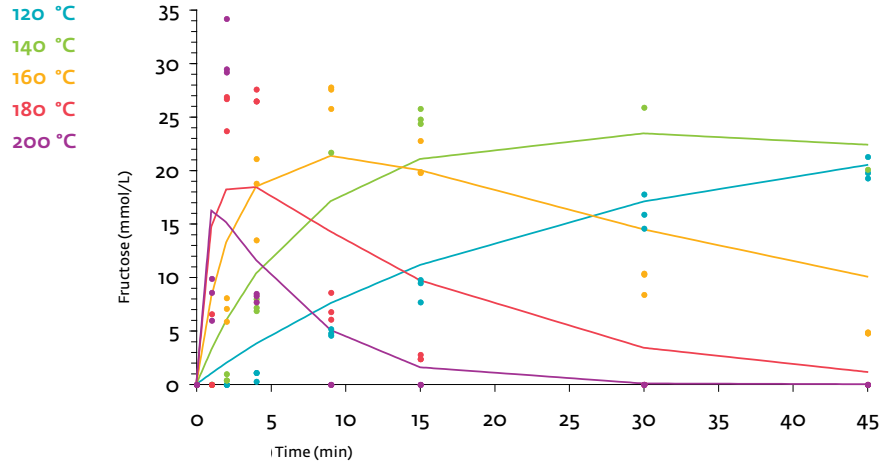


Figure 3.9 Model fit (lines) to experimental data (symbols) of the formation of fructose in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

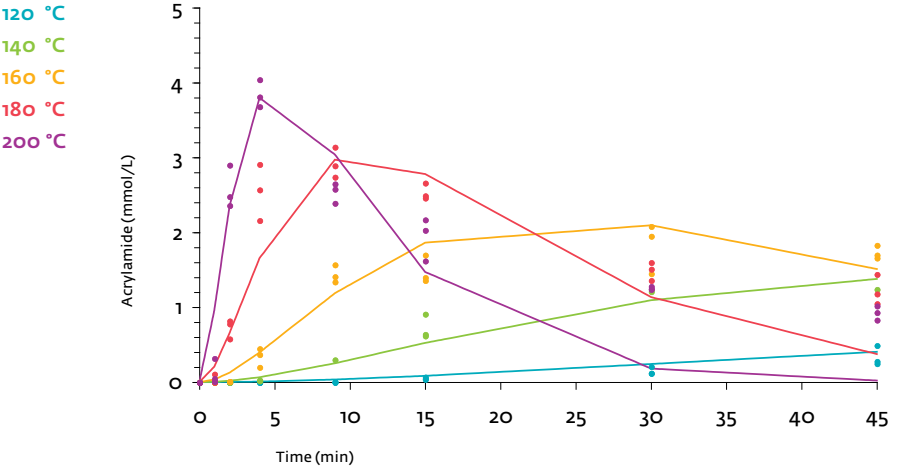


Figure 3.10 Model fit (lines) to experimental data (symbols) of the formation of acrylamide in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

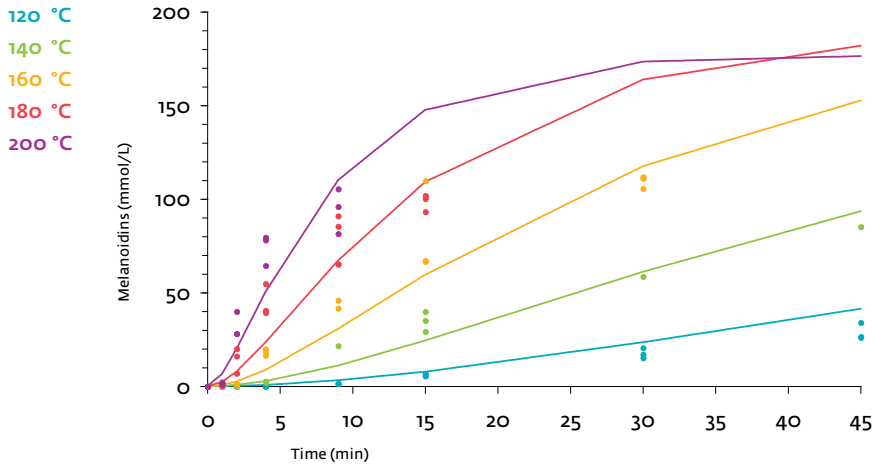


Figure 3.11 Model fit (lines) to experimental data (symbols) of the formation of melanoidins in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

Chapter 4

Unraveling the kinetics of the formation of acrylamide in the Maillard reaction by multiresponse modeling

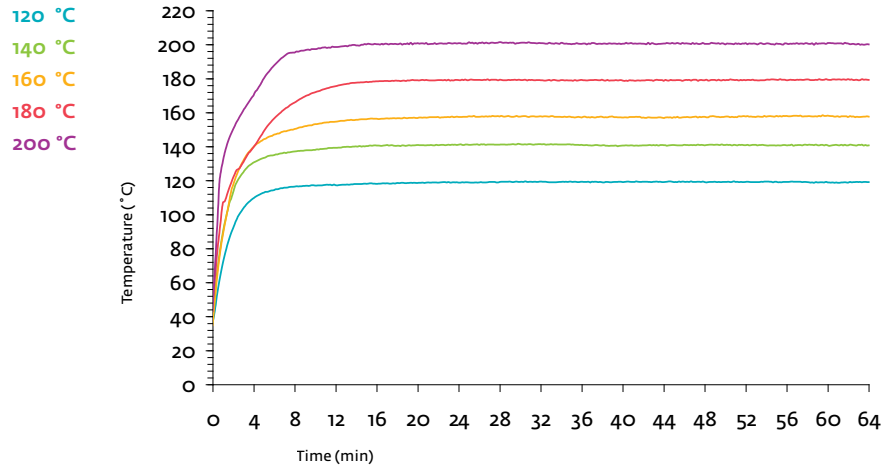


Figure 4.1 Temperature profile of the aqueous reaction system during heating of the stainless steel reaction tubes in a heating block at 120, 140, 160, 180 and 200 °C.

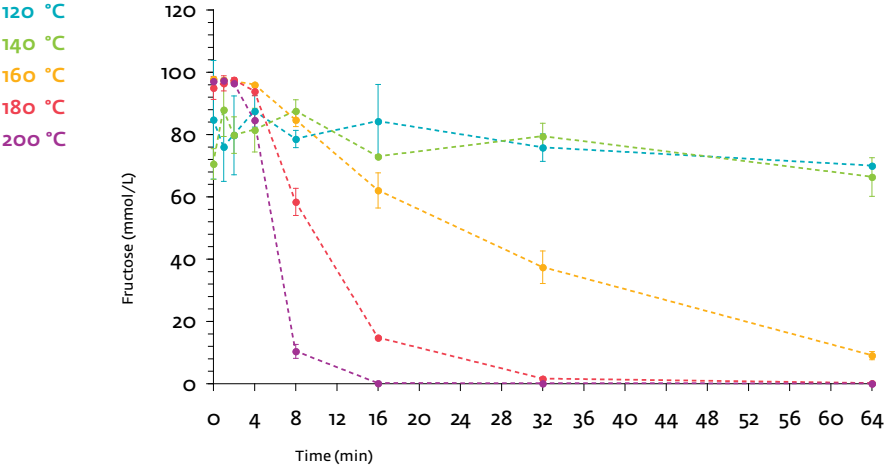


Figure 4.2 Experimental data of the loss of fructose in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

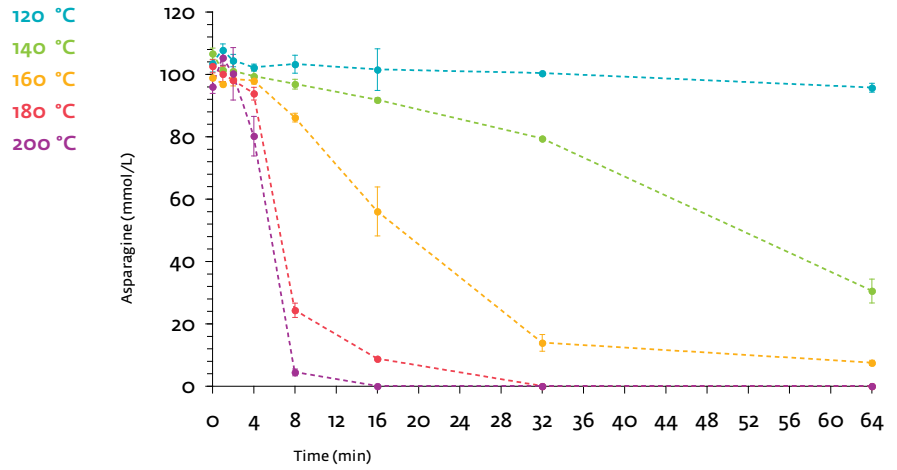


Figure 4.3 Experimental data of the loss of asparagine in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

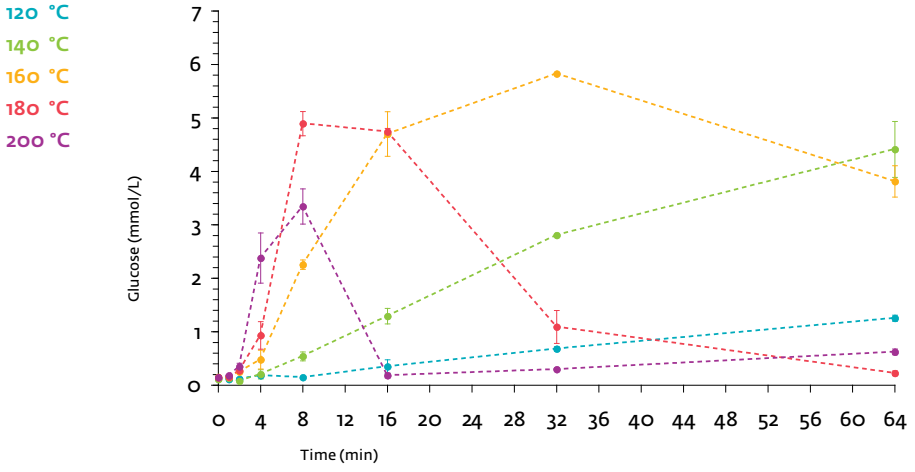


Figure 4.4 Experimental data of the formation of glucose in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

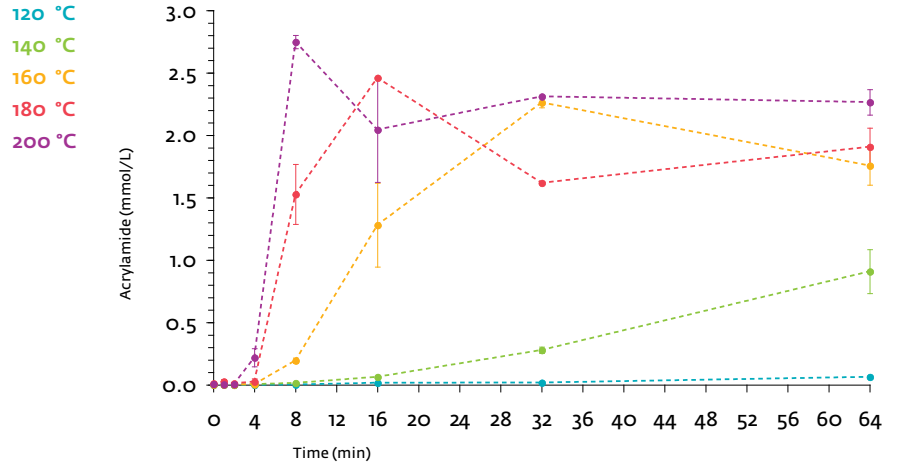


Figure 4.5 Experimental data of the formation of acrylamide in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120 , 140, 160, 180 and 200 °C.

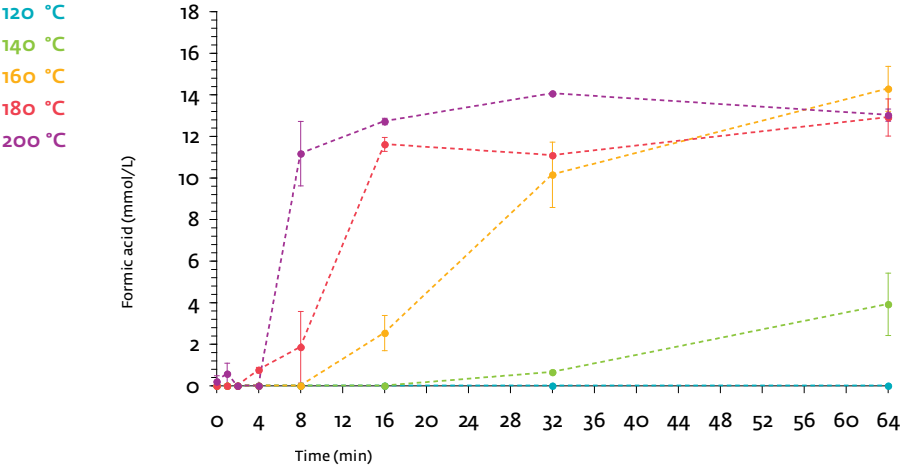


Figure 4.6 Experimental data of the formation of formic acid in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

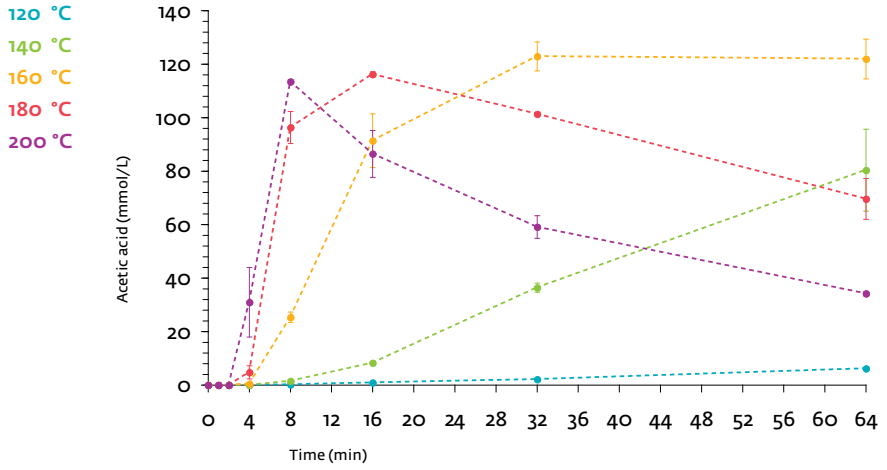


Figure 4.7 Experimental data of the formation of acetic acid in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120 , 140, 160, 180 and 200 °C.

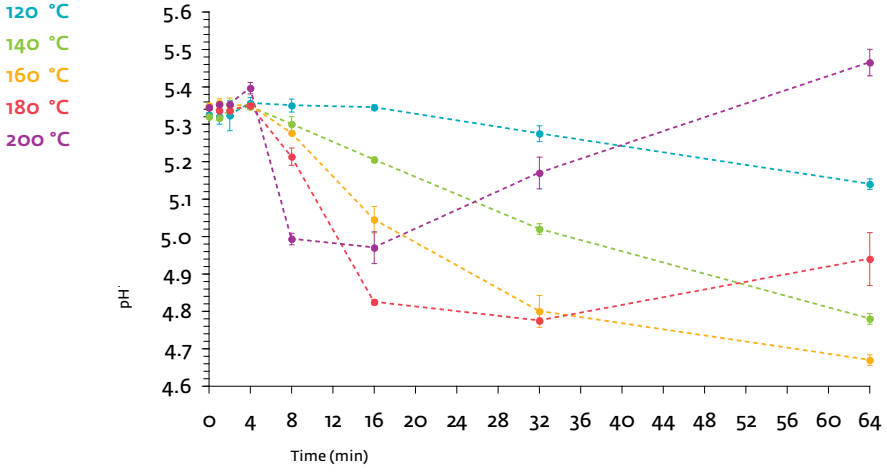


Figure 4.8 Experimental data of the pH in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

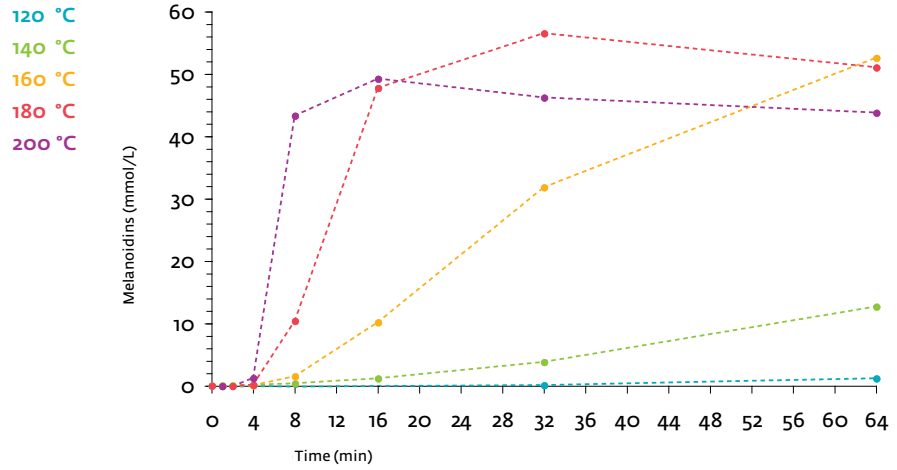


Figure 4.9 Experimental data of the formation of melanoidins in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

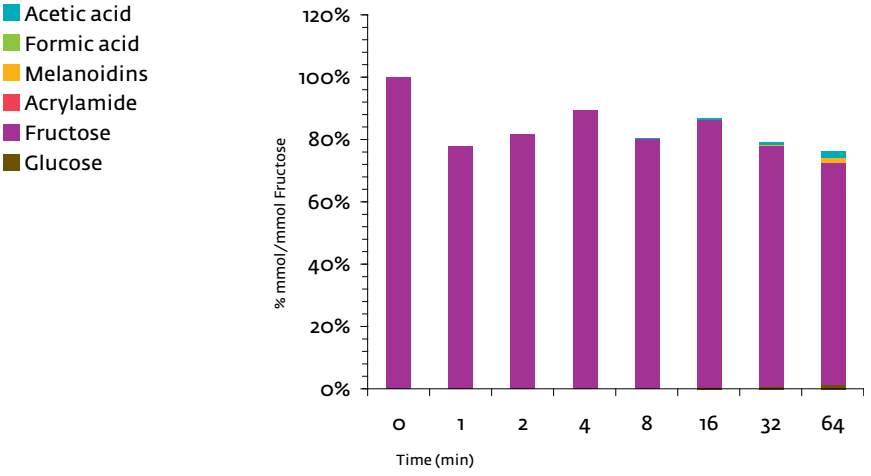


Figure 4.10 Mass balance of reactants and reaction products in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120 °C.

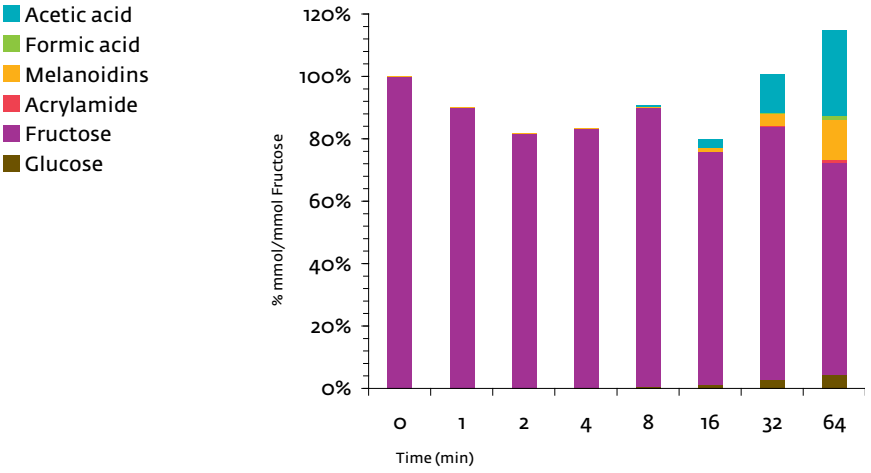


Figure 4.11 Mass balance of reactants and reaction products in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 140 ˚C.

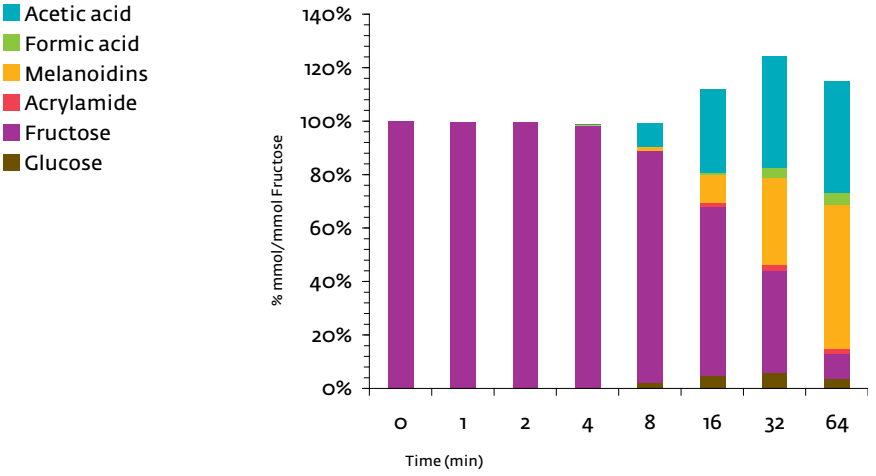


Figure 4.12 Mass balance of reactants and reaction products in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 160 ˚C.

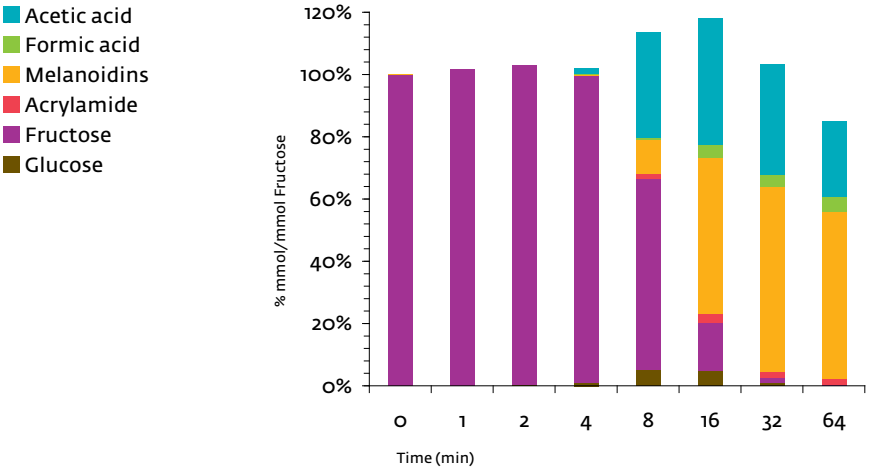


Figure 4.13 Mass balance of reactants and reaction products in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 180 °C.

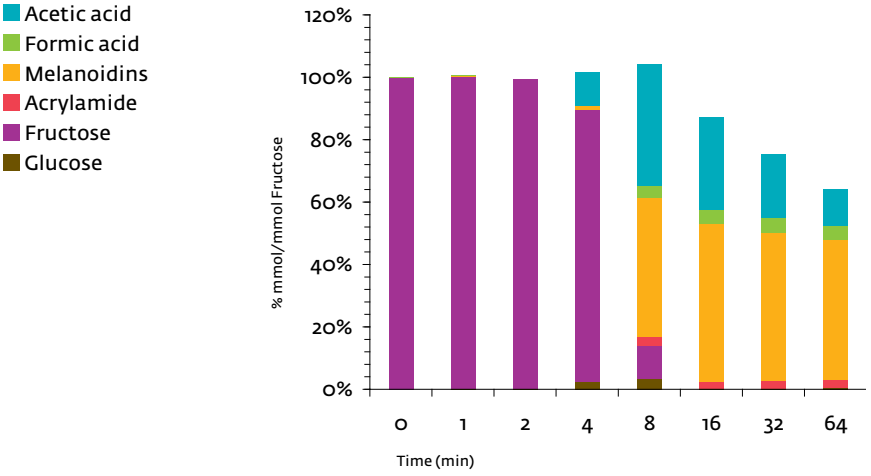


Figure 4.14 Mass balance of reactants and reaction products in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 200 °C.

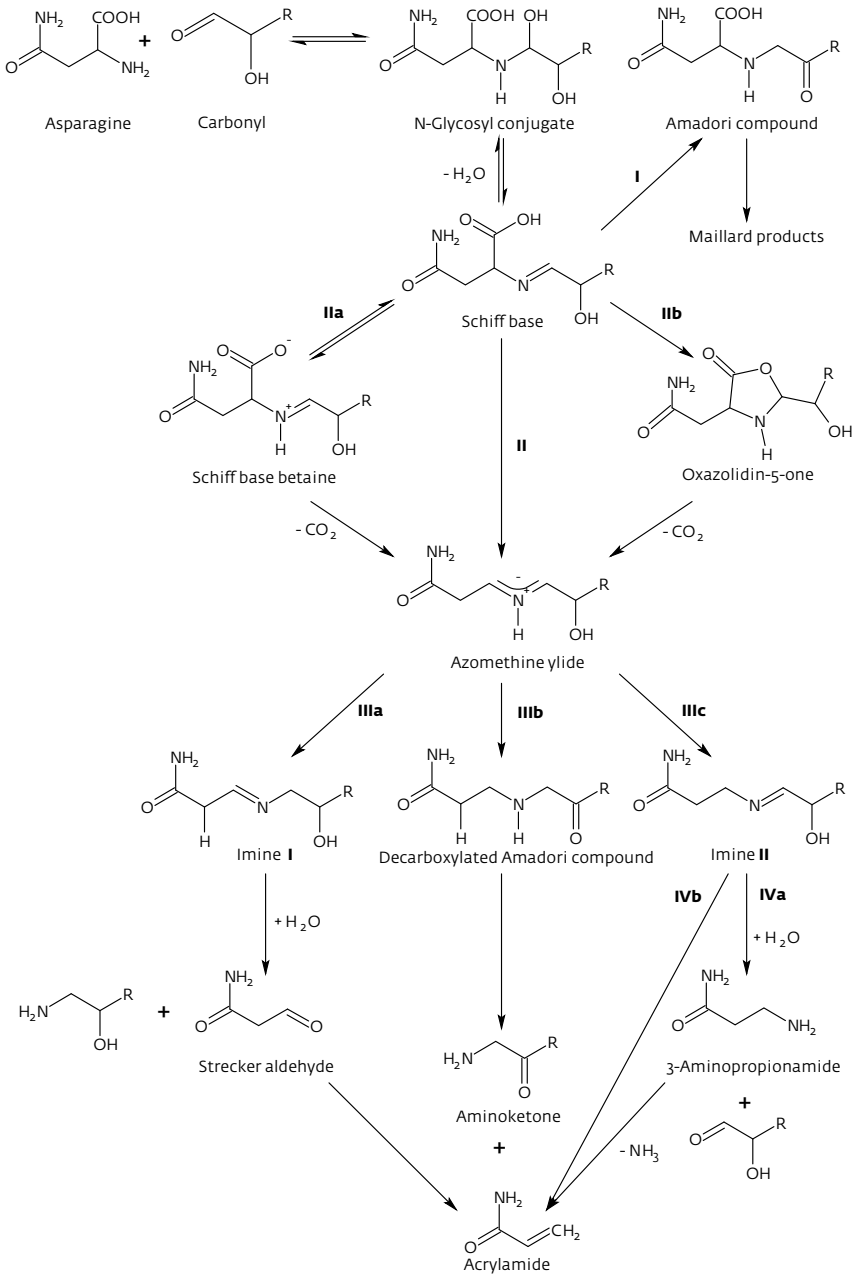


Figure 4.15 Proposed reaction network for the formation of acrylamide in the Maillard reaction adapted from Stadler *et al*. (34).

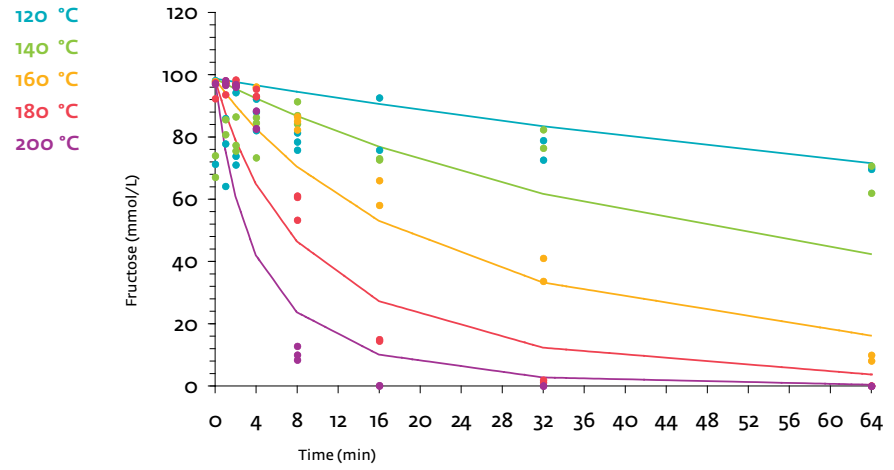


Figure 4.16 Model fit (lines) to experimental data (symbols) of the loss of fructose in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

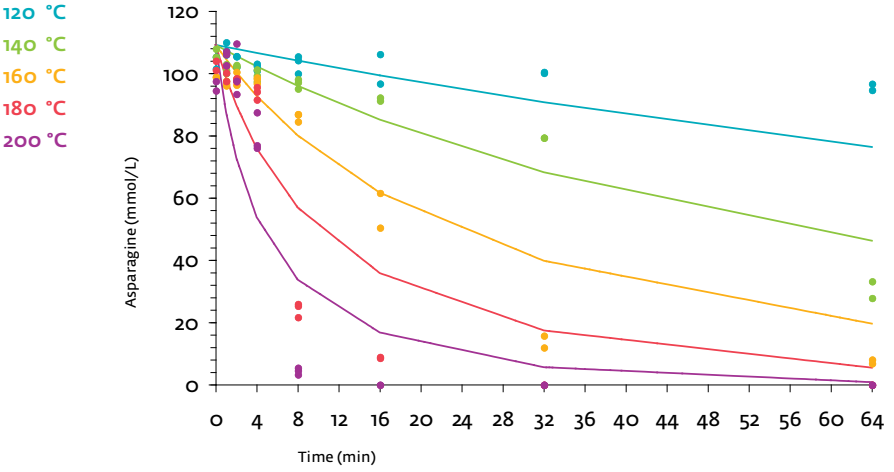


Figure 4.17 Model fit (lines) to experimental data (symbols) of the loss of asparagine in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

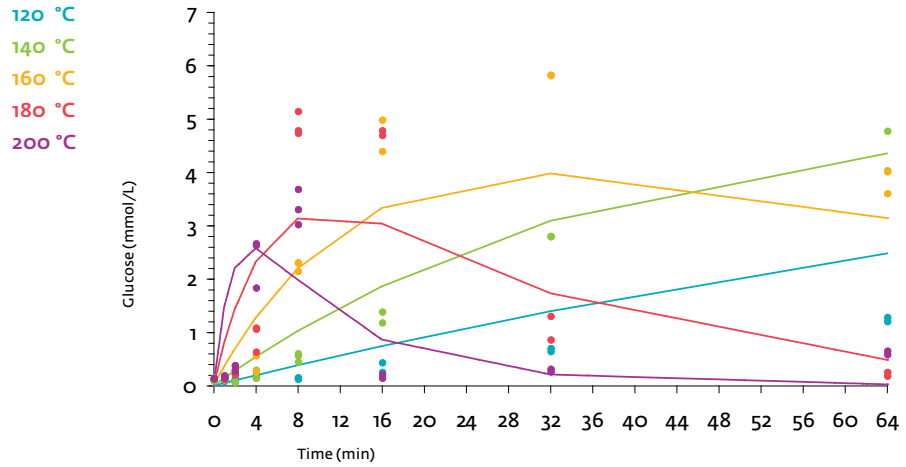


Figure 4.18 Model fit (lines) to experimental data (symbols) of the formation of glucose in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

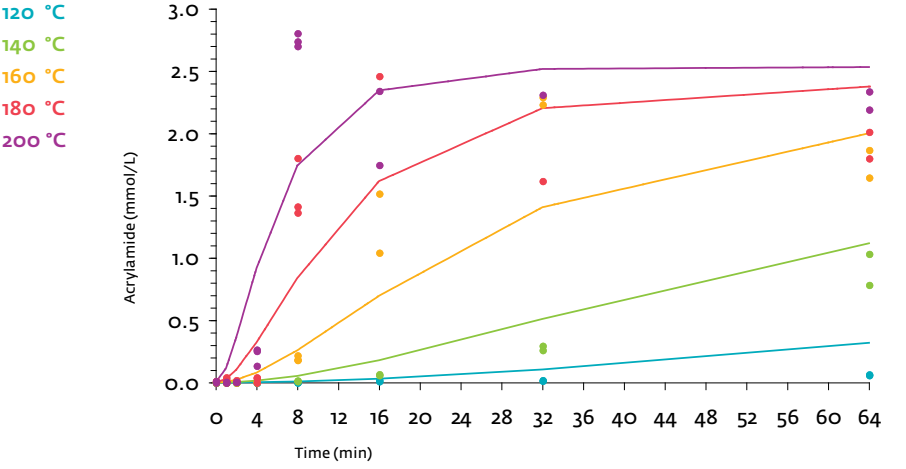


Figure 4.19 Model fit (lines) to experimental data (symbols) of the formation of acrylamide in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

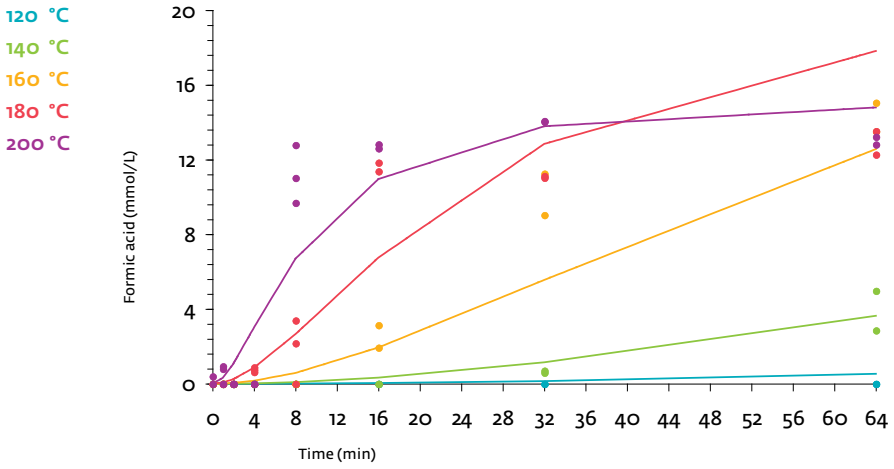


Figure 4.20 Model fit (lines) to experimental data (symbols) of the formation of formic acid in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

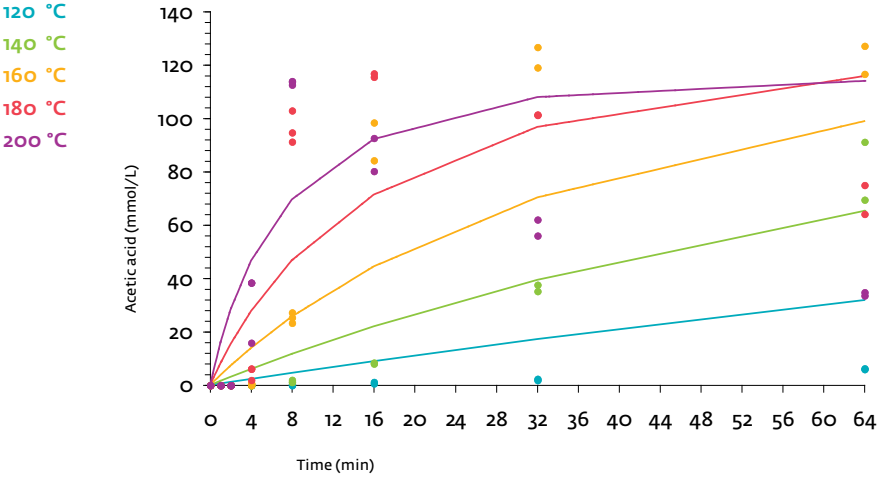


Figure 4.21 Model fit (lines) to experimental data (symbols) of the formation of acetic acid in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

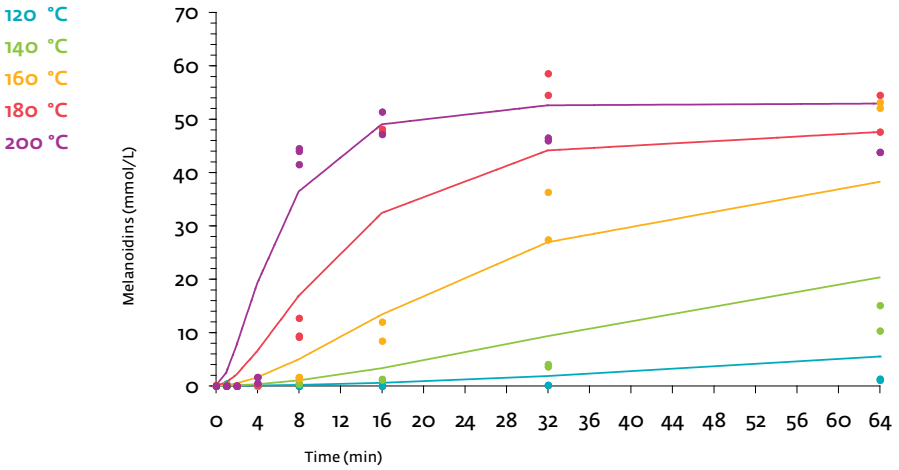
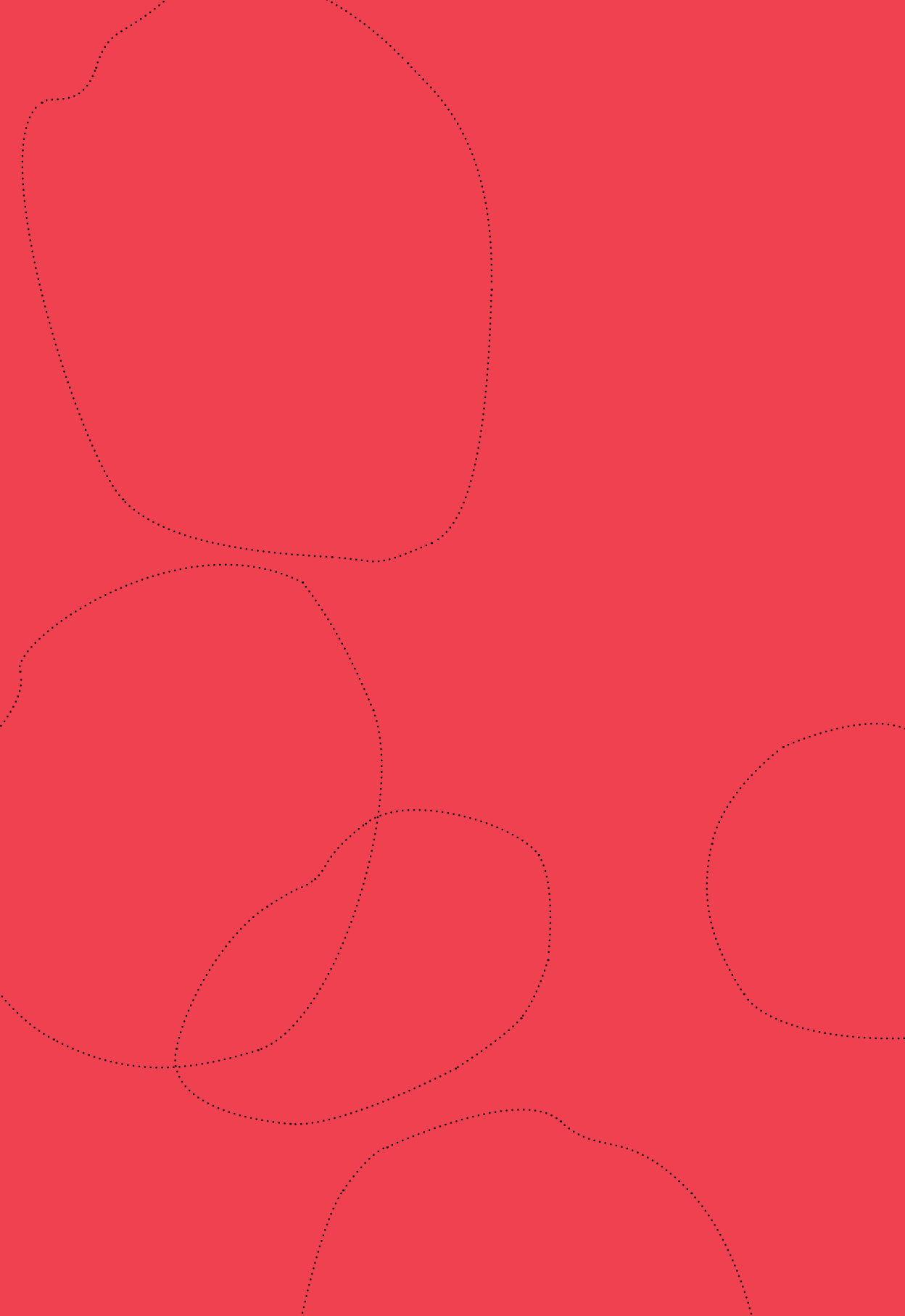


Figure 4.22 Model fit (lines) to experimental data (symbols) of the formation of melanoidins in a fructose/asparagine (0.1 mol/L, pH 5.5) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

Chapter 5

A study on the use of empirical models to predict the formation of acrylamide in potato crisps



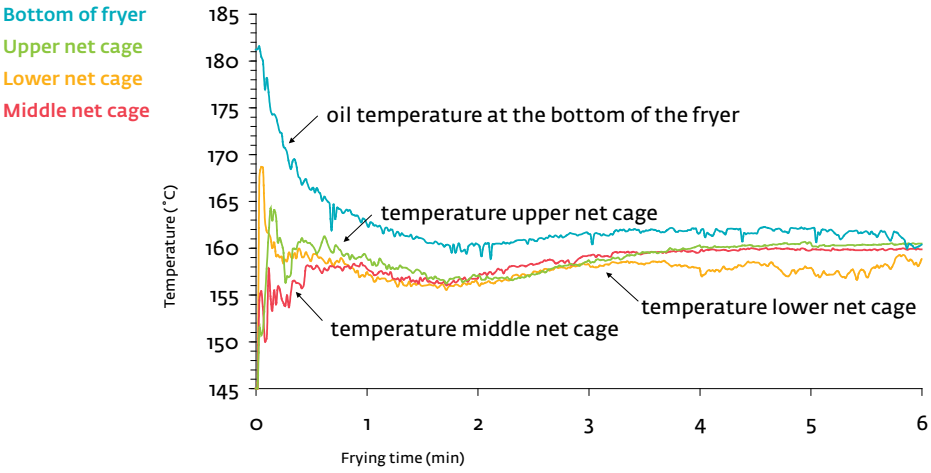


Figure 5.1 Temperature profiles during frying of potato crisps of the oil at the bottom of the fryer, the lower, middle and upper net cage.

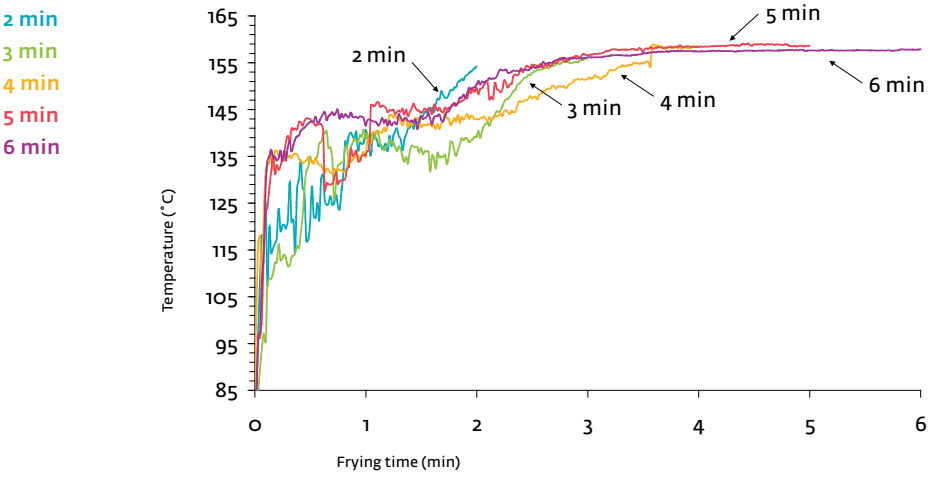


Figure 5.2 Temperature profiles during frying of potato crisps on or close to the surface of a potato crisp during frying for the different frying experiments.

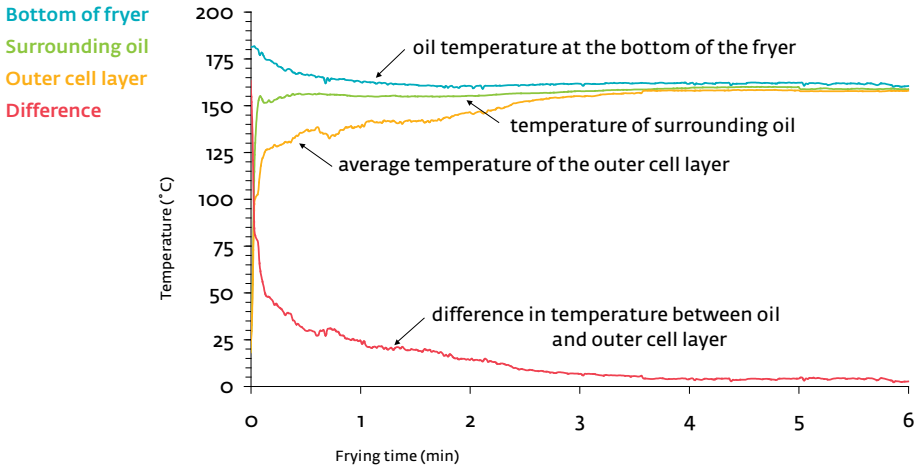


Figure 5.3 Average temperature profile during frying of potato crisps of the outer cell layer of a potato crisp during frying compared to the oil temperature in the oil bath, the oil surrounding the crisp and the difference between the oil and the outer cell layer.

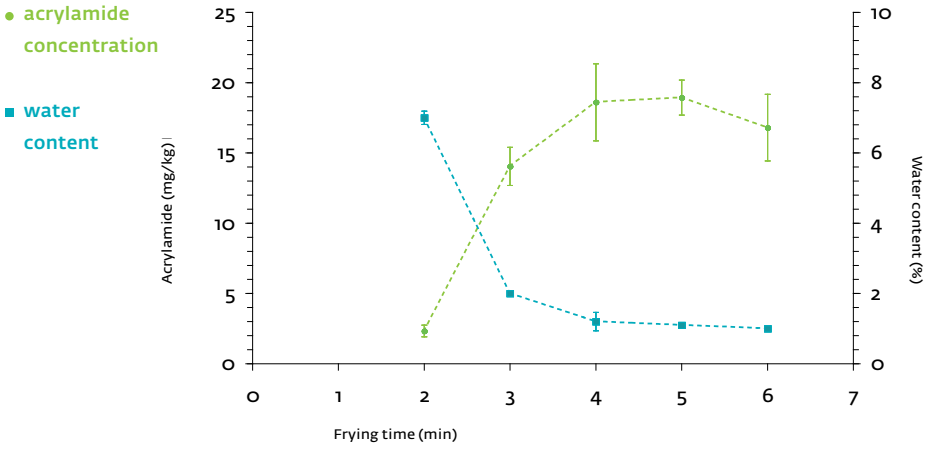


Figure 5.4 Average acrylamide concentration and water content *versus* frying time in potato slices (1.5 mm) fried for 2–6 min. Error bars indicate SD (acrylamide concentration: $n = 4$; water content: $n = 2$). Initial water content at t = 0 was around 75%.

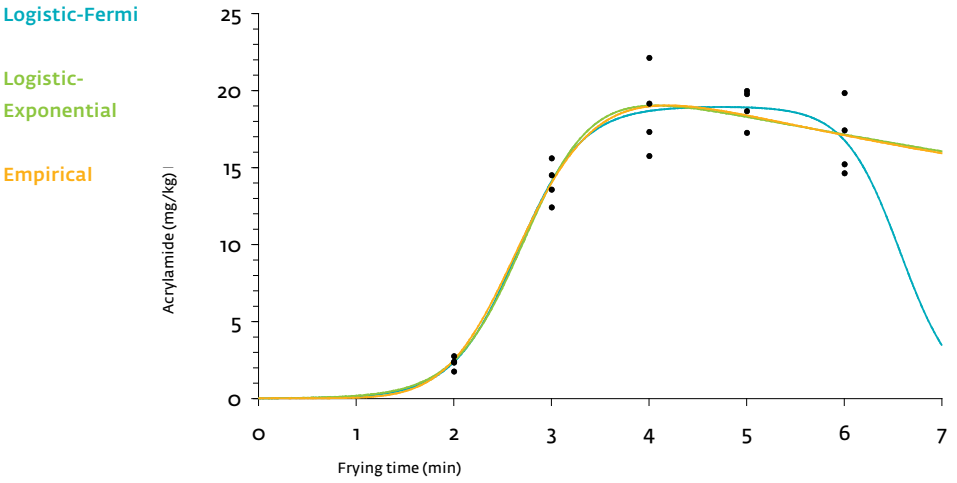


Figure 5.5 Acrylamide concentration (●) *versus* time in potato slices (1.5 mm) fried for 2–6 min, fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines).

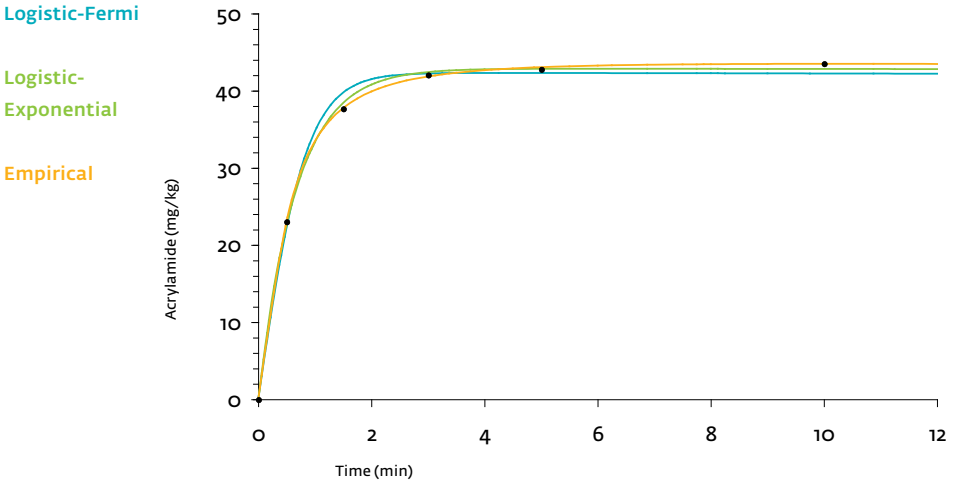


Figure 5.6 Average acrylamide concentration (●) *versus* time in potato model samples (1 mm) fried for 0.5–10 min with an initial temperature of 180 °C, fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines). Original data are from Kim *et al*. (14).

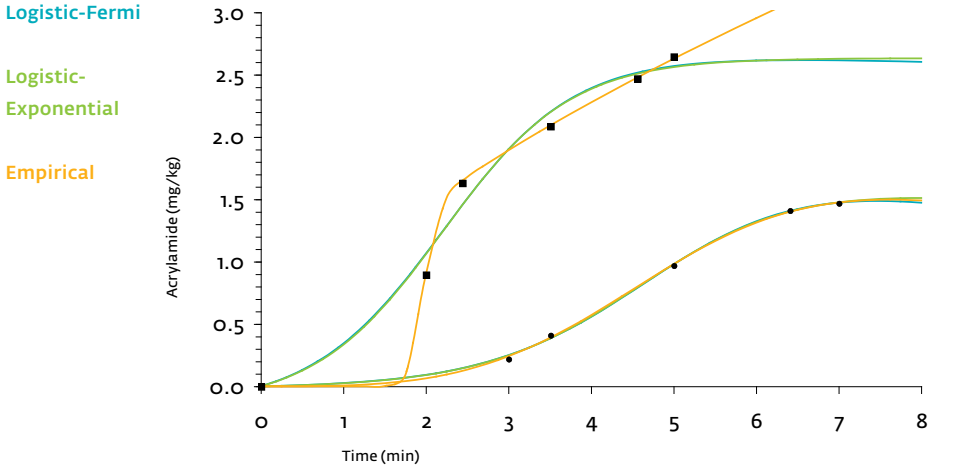


Figure 5.7 Average acrylamide concentration (symbols) *versus* time in potato slices (1.5 mm) fried for 2–7 min with an initial temperature of 160 °C (●) and 185 °C (■), fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines). Original data are from Kita *et al*. (31).

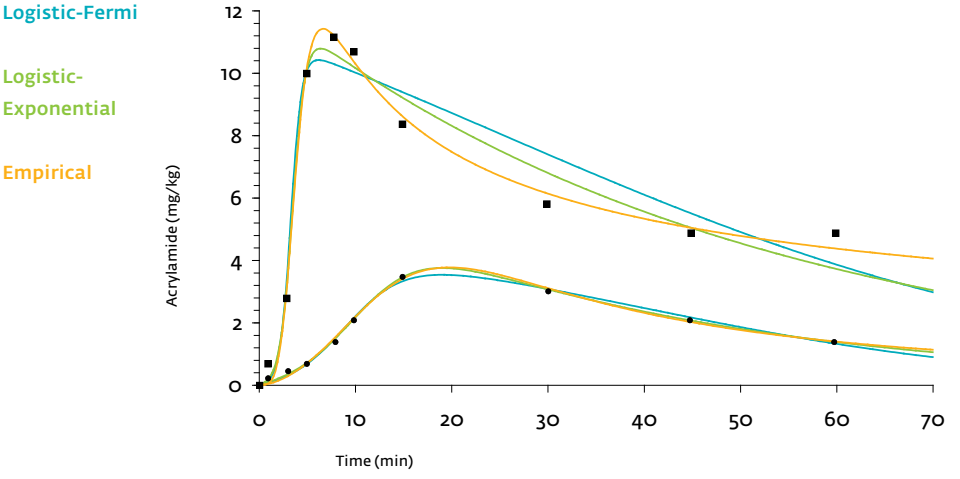


Figure 5.8 Average acrylamide concentration (symbols) *versus* time in potato slices (2 mm) fried for 0 – 60 min with an initial temperature of 150 °C (●) and 170 °C (■), fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines). Original data are from Gökmen *et al*. (27).

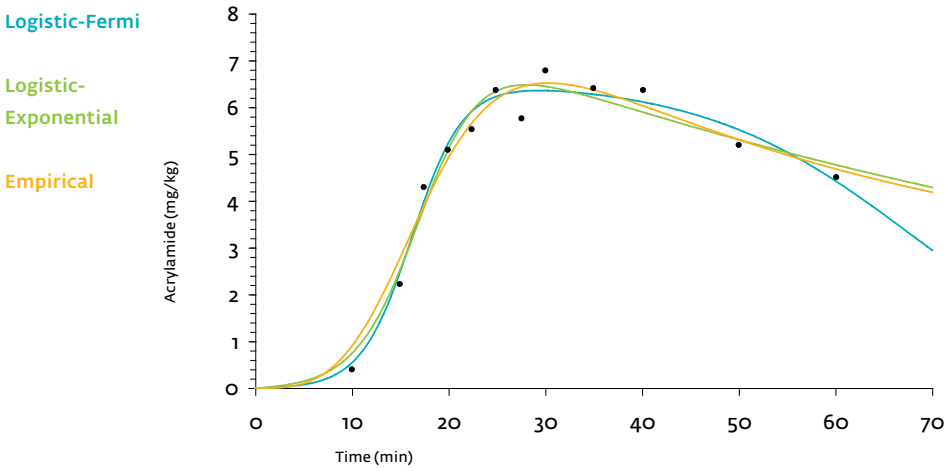


Figure 5.9 Average acrylamide concentration (●) *versus* time in potato cakes (3.0 mm) fried for 10–60 min with an initial temperature of 180 °C, fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines). Original data are from Elmore *et al*. (30).

Chapter 6

Kinetic modeling: a tool to predict the formation of acrylamide in potato crisps

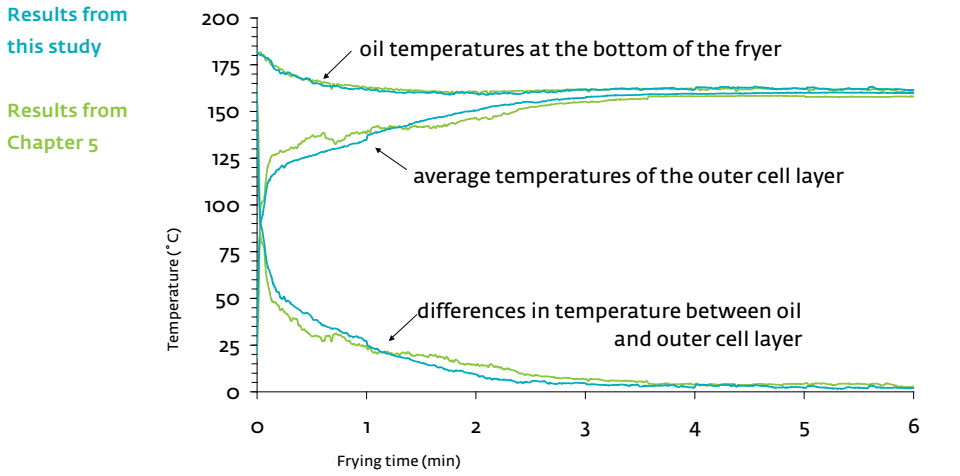


Figure 6.1 Average temperature profiles during frying of potato crisps of the oil temperature in the oil bath and the outer cell layer of a potato crisp and the difference between the temperature of the oil and the outer cell layer. The blue lines represent the results from this study, the green lines the results from **Chapter 5.**

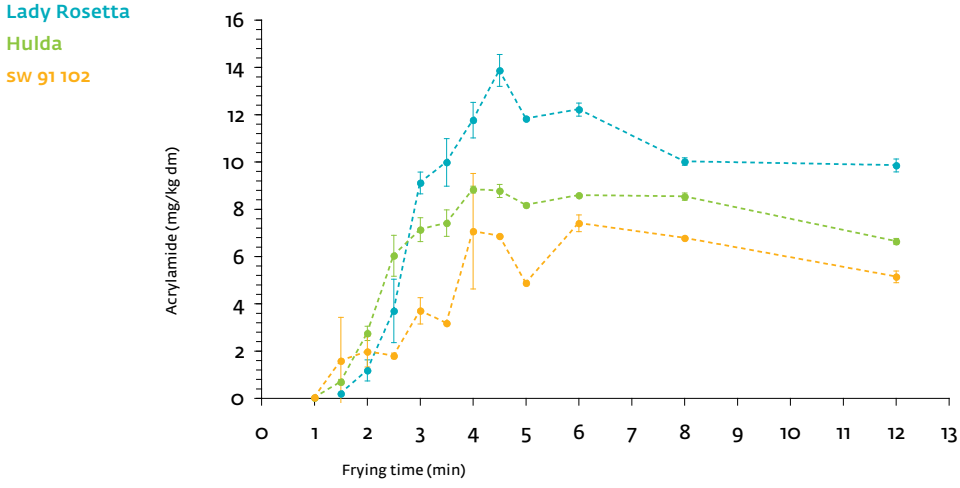


Figure 6.2 Average acrylamide concentration *versus* frying time in potato slices (1.5 mm) from the potato genotypes Lady Rosetta, Hulda and SW 91 102 fried for 1-12 min. Error bars indicate SD (n = 4 for 2, 2.5, 3 and 4 min; n = 2 for 1, 1.5, 3.5, 4.5, 5, 6, 8 and 12 min).

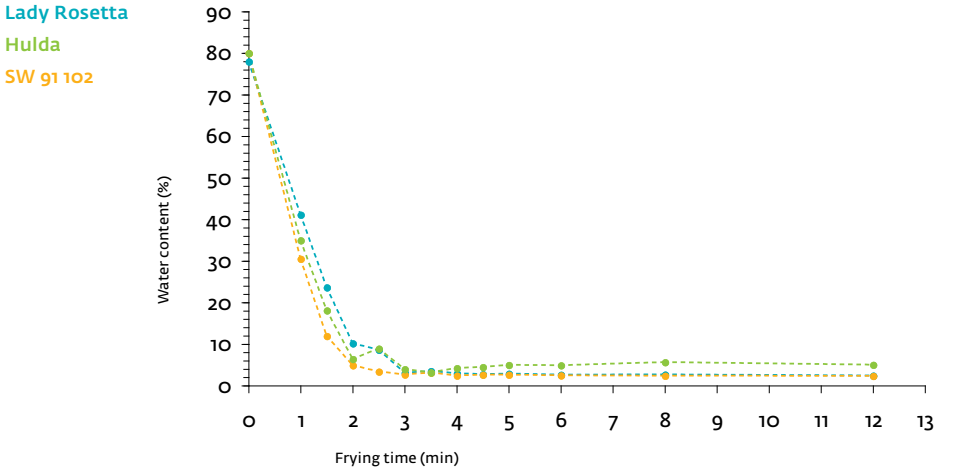


Figure 6.3 Water content *versus* frying time in potato slices (1.5 mm) from the potato genotypes Lady Rosetta, Hulda and sw 91 102 fried for 0-12 min.

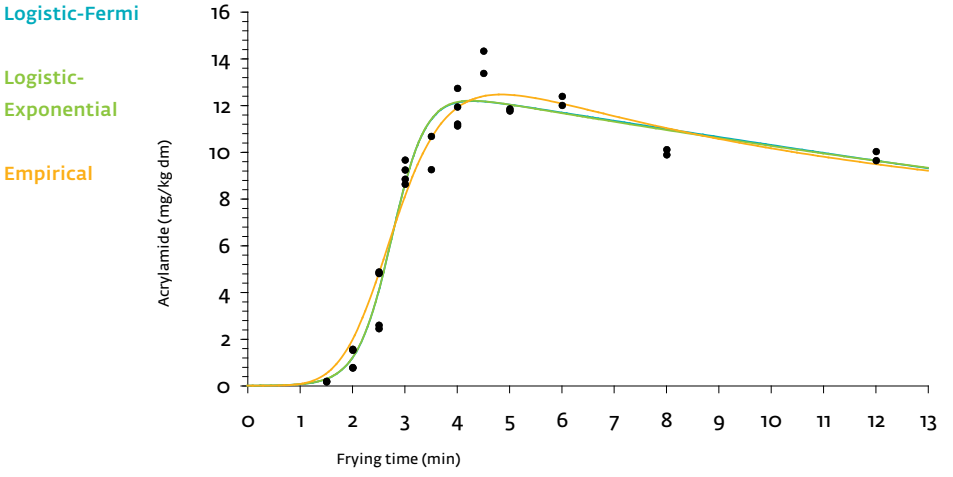


Figure 6.4 Acrylamide concentration (●) *versus* time in potato slices (1.5 mm) made from Lady Rosetta and fried for 1-12 min, fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines).

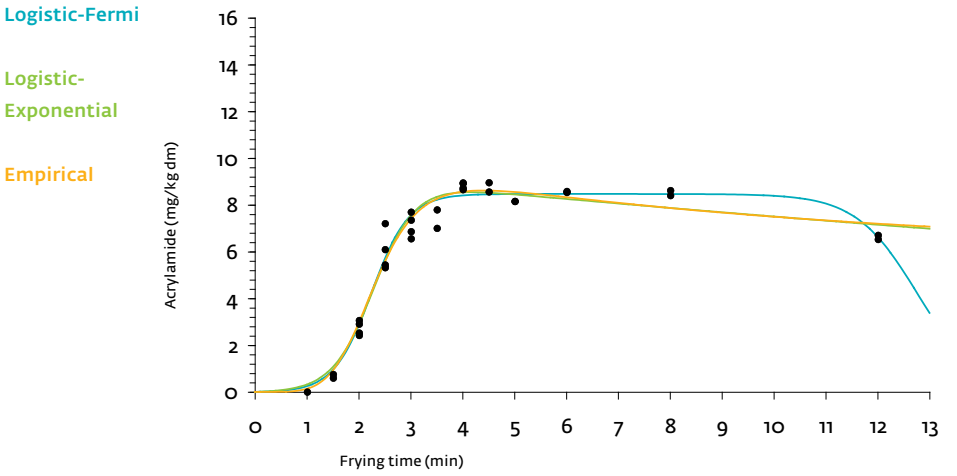


Figure 6.5 Acrylamide concentration (●) *versus* time in potato slices (1.5 mm) made from Hulda and fried for 1-12 min, fitted with Logistic-Fermi, Logistic-Exponential and Empirical models (lines).

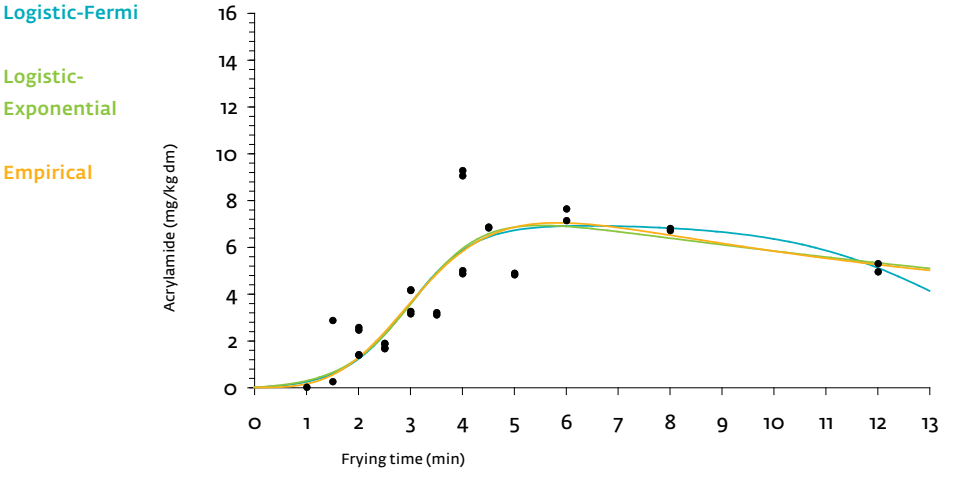


Figure 6.6 Acrylamide concentration (●) *versus* time in potato slices (1.5 mm) made from SW 91102 and fried for 1-12 min, fitted with Logistic-Ferm, Logistic-Exponential and Empirical models (lines).

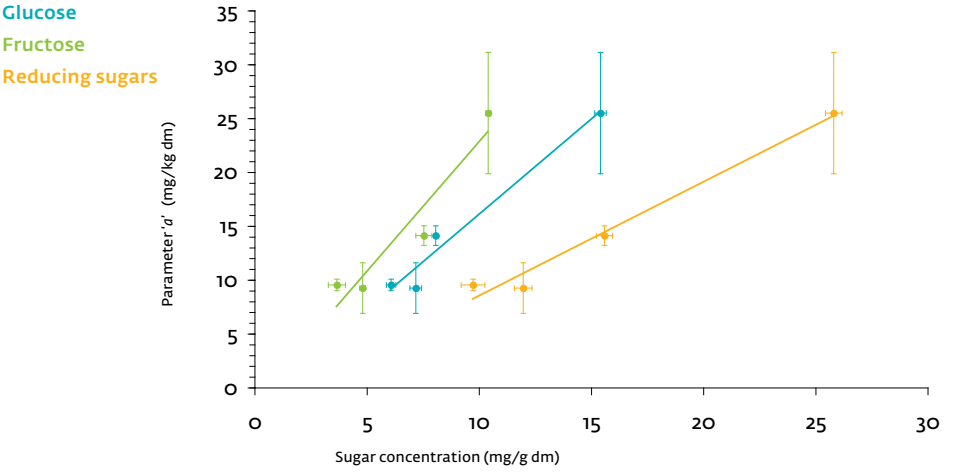


Figure 6.7 Relationship between the model parameter '*a*' of the Logistic-Exponential models for the crisps made with four different genotypes and the concentration of glucose, fructose and reducing sugars.

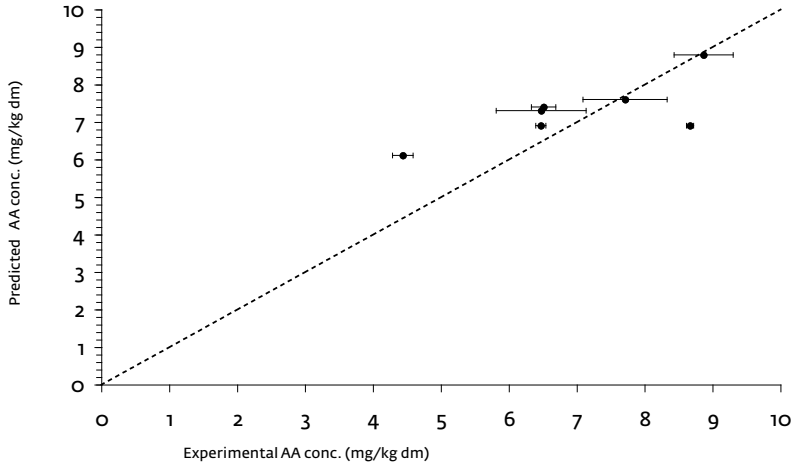


Figure 6.8 Acrylamide (AA) concentrations predicted by the Logistic-Exponential model plotted against experimental acrylamide concentrations from Viklund *et al.* (23) for potato crisps made with Hulda, stored for 6-24 weeks and fried for 4 min.

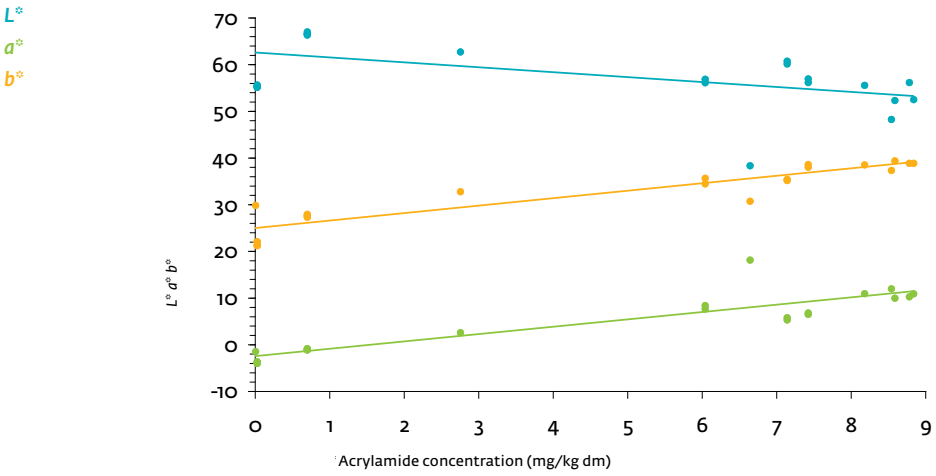
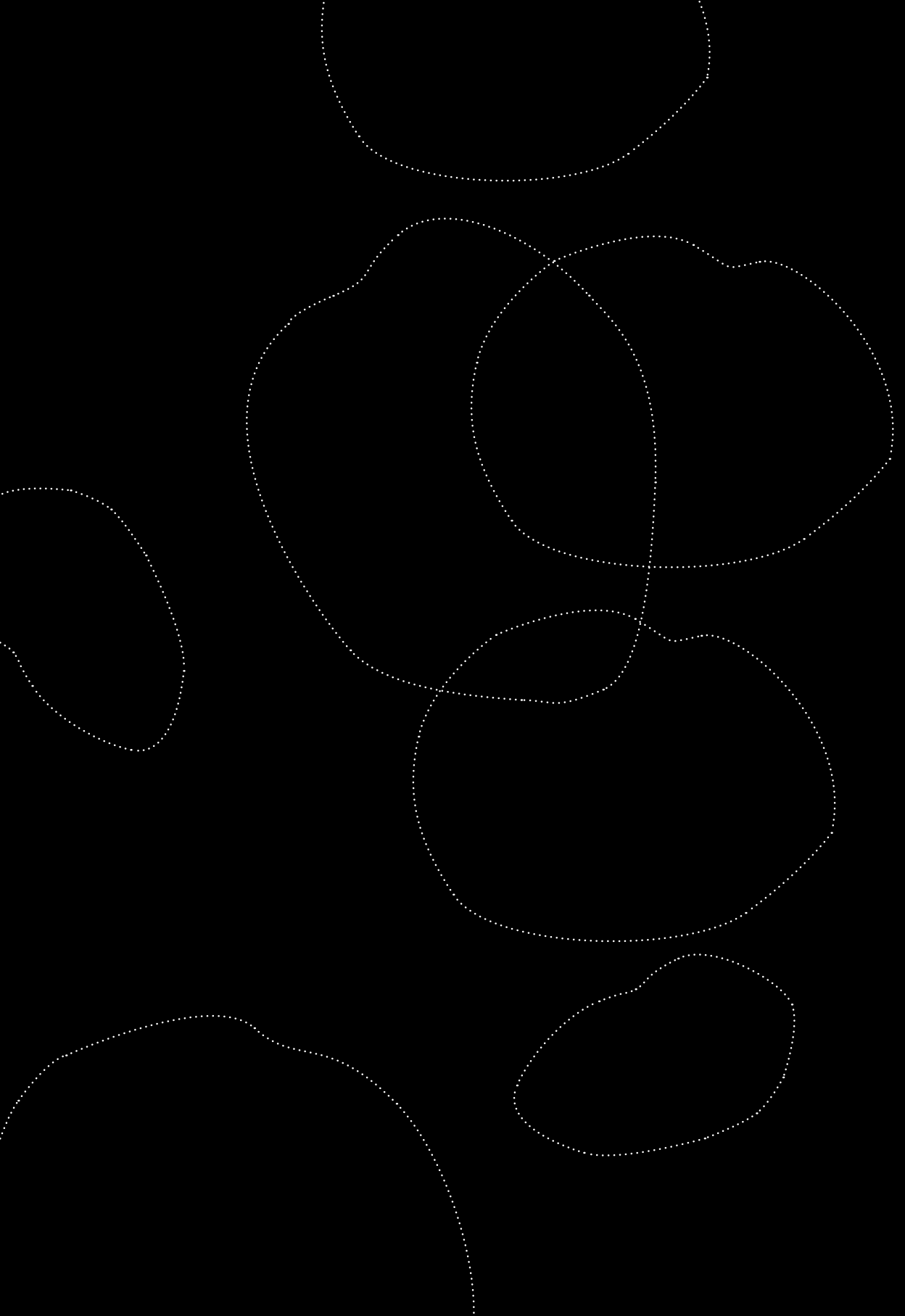


Figure 6.9 Color parameters *L**, *a** and *b** *versus* acrylamide concentration in potato crisps (1.5 mm) made from Hulda.

Chapter 7
General discussion



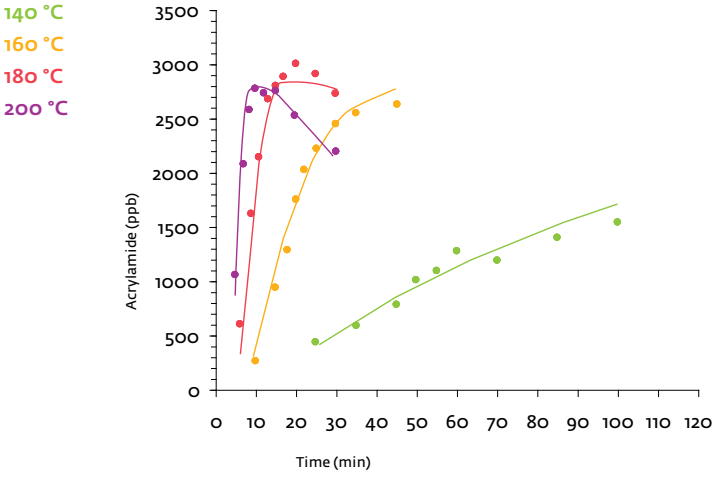


Figure 7.1 Formation of acrylamide as a function of heating time at 140, 160, 180 and 200 °C in an equimolar asparagine-glucose model system (0.01 M, pH 6) taken from Claeys *et al*. (21). The full lines represent acrylamide formation/elimination predicted by the kinetic model.

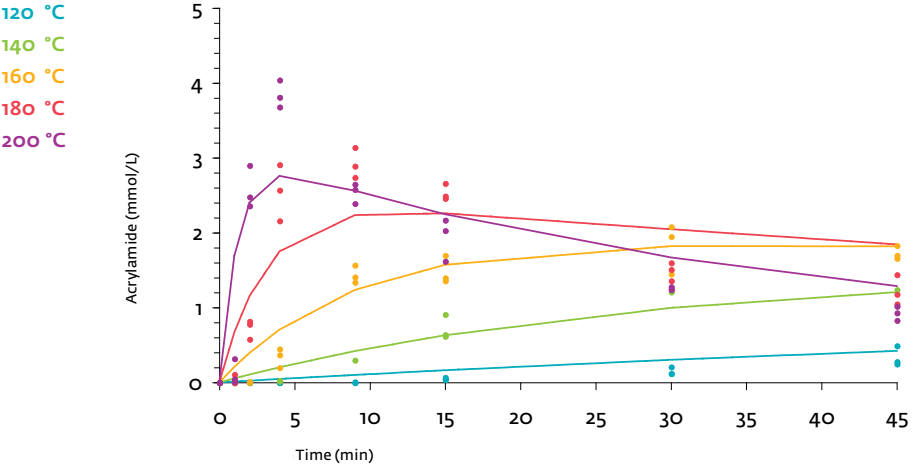


Figure 7.2 Model fit (lines) from single response model to experimental data (symbols) of the formation of acrylamide in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.

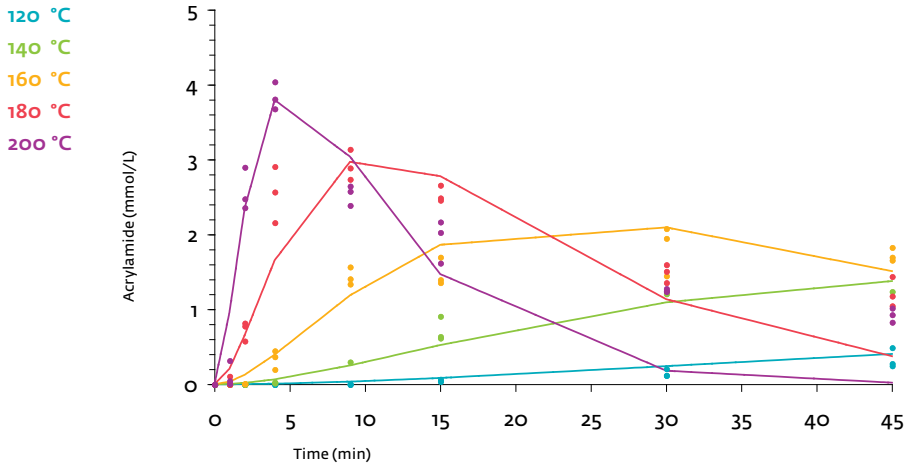


Figure 7.3 Model fit (lines) from multiresponse model to experimental data (symbols) of the formation of acrylamide in a glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120, 140, 160, 180 and 200 °C.