

# **VARIATION IN ACRYLAMIDE CONCENTRATION IN FRENCH FRIES**

**Effects of control measures in food service establishments**

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# **VARIATION IN ACRYLAMIDE CONCENTRATION IN FRENCH FRIES**

**Effects of control measures in food service establishments**

**Maimunah Sanny**

## **Thesis**

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## **Abstract**

The aim of this thesis was first to identify the major technological and managerial factors and to investigate their contribution to variation in acrylamide concentrations. The second aim was to investigate the effect of technological and managerial control measures on the concentration and variation of acrylamide in the preparation of French fries in food service establishments (FSE). The variation in initial concentration of reducing sugars, variable frying conditions and food handler's inadequate control of these factors in their daily practice could lead to the large variation and high acrylamide concentrations. The least variation in acrylamide was found in French fries prepared in chain fast-food services (CFS) compared to institutional caterers (IC) and restaurants, although the mean concentration of acrylamide among the three FSE types was not significantly different. The variation in frying temperature contributed most to the variation in acrylamide, followed by the variation in frying time; no obvious effect of reducing sugars was found. The lack of standardised control of frying temperature and time (due to inadequate frying equipment) and variable frying practices of food handlers seem to contribute most to the large variation and high acrylamide concentrations in French fries prepared in restaurants. Lowering the concentration of reducing sugars in par-fried potato strips can be an effective control measure to reduce acrylamide concentrations in French fries, but only if prepared under standardised frying conditions in CFS and IC. Frying instructions were shown to support food handlers' decisions to start frying when the oil temperature reached 175°C, although an inconsistent effect of the instructions on the food handlers' decisions to end frying was observed. The mean concentration of acrylamide for the restaurants as a group was not significantly different, but data analysis for each restaurant showed that if the food handlers properly followed the instructions, the mean concentration of acrylamide was significantly lower compared to before instructions.

# Table of contents

<i>Chapter 1</i> General introduction	7
<i>Chapter 2</i> Impact of control behaviour on unacceptable variation in acrylamide concentration in French fries	29
<i>Chapter 3</i> Possible causes of variation in acrylamide concentration in French fries prepared in food service establishment: An observational study	61
<i>Chapter 4</i> Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments?	95
<i>Chapter 5</i> Effect of frying instructions to food handler on acrylamide concentration in French fries: An explorative study	123
<i>Chapter 6</i> General discussion	155
Summary	181
Samenvatting	187
Acknowledgements	193
Curriculum vitae	196
List of publications	197
Overview of completed training activities	199

**Chapter**

**1**

**General Introduction**





## **Chapter 1**

### **1.1 Presence of acrylamide in food and the need for control measures**

Foods have been heat-treated since ancient times to modify and to preserve its organoleptic and nutritional properties for consumption. However, heat treatment of foods can also generate some undesired compounds, such as food-borne toxicants that may exhibit mutagenic and carcinogenic properties. Well known examples of these compounds are heterocyclic amines, 5-hydroxymethylfurfural and polycyclic aromatic hydrocarbons (Capuano & Fogliano, 2011; Park & Penning, 2009; Turesky, 2009; van Boekel et al., 2010). Acrylamide has been added to the list of food-borne toxicants since 2002, after Swedish researchers reported the presence of acrylamide in a range of fried and oven-cooked foods (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000, 2002).

Acrylamide is used worldwide to synthesize polyacrylamide. Polyacrylamide has found numerous applications as a soil conditioner, in wastewater treatment, in cosmetics, paper, and textile industries (Friedman, 2003). Acrylamide is neurotoxic in animals and humans, and it has shown to be a reproductive toxicant, genotoxic, and carcinogen in rodents (Capuano & Fogliano, 2011; Friedman, 2003; LoPachin, 2004). Neurotoxicity of acrylamide results from high levels of exposure, and it concerns workers occupationally exposed to acrylamide through inhalation or dermal absorption and characterized by ataxia and skeletal muscle weakness (FAO/WHO, 2005; LoPachin, 2004). Acrylamide is classified by the International Agency for Research on Cancer (1994) as a probable human carcinogen on the basis of positive results with bioassays in rodents, and supported by evidence that acrylamide is metabolised in mammalian tissues to glycidamide (FAO/WHO, 2005). Glycidamide is reactive towards DNA (genotoxic) and thus can form

## General Introduction

several DNA adducts, which is believed to be the key process in the carcinogenicity of acrylamide (Capuano & Fogliano, 2011; Friedman, 2003; Rice, 2005).

After the discovery of the presence of acrylamide in foods, considerable research activities were started to gain knowledge about the occurrence of acrylamide in a wide range of foods and its intake. High concentrations of acrylamide have been detected in potato based products, such as French fries (Claeys et al., 2010; DiNovi, 2006; Friedman, 2003). French fries are a predominant source of dietary intake of acrylamide because they are widely consumed (Fiselier & Grob, 2005). However, many dietary intake studies observed significant variation in acrylamide concentrations (Dybing & Sanner, 2003; Konings et al., 2003; Matthys et al., 2005; Svensson et al., 2003). Capuano & Fogliano (2011) stated that the large variation in acrylamide concentrations complicates the estimation of actual dietary exposure to acrylamide. Furthermore, Grob (2007) showed that high acrylamide concentrations combined with high consumption could increase the dietary exposure for an individual by a factor of 5, from an average exposure of 2.8 µg/day.

Extensive epidemiological studies have been conducted in the attempt to assess cancer risk according to dietary acrylamide intake in human subjects. Most of these studies found no association between estimated dietary acrylamide intake and an increased risk of cancer (Mucci, Dickman, Steineck, Adami, & Augustsson, 2003; Mucci et al., 2005; Pelucchi et al., 2003; Pelucchi et al., 2006). However, some studies have recently reported an increased risk for some types of cancer. Hogervorst and co-authors (2008) reported the first statistical significant relation between the estimated dietary intake of acrylamide and the risk of endometrial and

## Chapter 1

ovarian cancer, particularly in non-smoking women. Wilson and co-authors (2010) confirmed the finding of Hogervorst and co-authors (2008) and reported the second positive association between the estimated dietary intake of acrylamide and the risk of endometrial and ovarian cancer. In addition, Olesen and co-authors (2008) reported a positive association between the risk for breast cancer and increased acrylamide hemoglobin adducts in red blood cells. The results of the above-mentioned studies suggest that the evidence of acrylamide posing a cancer risk for humans has been strengthened, at least for some target organs as well as for some populations.

The Joint FAO/WHO Expert Committee of Food Additives (2005) advised to put appropriate efforts to reduce acrylamide concentration in food to protect human health. In addition, the Scientific Committee on Food of the European Commission (2002) recommended to deal with acrylamide concentration in food with the 'ALARA' principle, i.e., that concentrations should be as low as reasonable achievable. However, the FAO/WHO (2007) identified the observed significant variation in acrylamide concentrations as one of the constraints in developing control measures. Different authors reported that significant variation in acrylamide concentrations is often observed even on repetition of a specific mitigation measures under controlled conditions, e.g. between different batches of a product made within the same manufacturing plant, or between manufacturing plants using the same process, ingredients and formulations (CIAA, 2006; FAO/WHO, 2007). The FAO/WHO (2007) emphasised that possible factors contributing to the significant variation need to be researched. In addition, various authors proposed control measures that mainly focused on the reducing sugars concentration and frying temperature-time regimes and tested under strictly controlled laboratory conditions (Biedermann-Brem et al.,

## **General Introduction**

2003; Fiselier & Grob, 2005; Ishihara, Matsunaga, Nakamura, Sakuma, & Koga, 2006; Palazoğlu & Gökmen, 2008). Different authors suggested that the results of these studies must be validated under actual food processing conditions (CIAA, 2006; Stadler, 2005). Studies have recently evaluated the effects of control measures of actual food processing conditions, but they were mainly performed in industrial production (Vinci, Mestdagh, de Muer, van Peteghem, & de Meulenaer, 2010; Vinci et al., 2011). Although French fries are widely prepared in food service establishments (FSE), data on the effects of control measures in a real preparation of French fries in FSE are limited compared to the data now available for industrial production (Stadler, 2005; Taeymans et al., 2004). Nevertheless, the number of meals prepared in a food service is increasing due to changes in lifestyles, such as preferences for leisure, convenience and eating out (Chang & Cottrell, 2010; Lee, 1991). Because French fries are widely prepared in FSE, the preparation of French fries in FSE needs to be controlled to reduce dietary exposure to acrylamide. Possible factors contributing to significant variation in acrylamide concentrations need to be firstly understood in order to develop an effective control measure. In that respect, this thesis aims to understand the underlying factors and practices contributing to significant variation and consequently to investigate the effects of control measures in the real preparation of French fries in-practice in FSE. These control measures can be used as a tool to reduce acrylamide exposure from French fries.

The formation of acrylamide is highly complex because various product properties and processing conditions affect its formation. In the following sections, an overview of the main mechanisms of acrylamide formation via the Maillard reaction is given to demonstrate its

## **Chapter 1**

complexity. In addition, the product properties and the processing conditions influencing the formation of acrylamide are discussed.

### **1.2 Mechanisms of acrylamide formation**

Shortly after the discovery of the presence of acrylamide in foods (Tareke, et al., 2000, 2002), it was clearly established that the major mechanistic pathway for acrylamide formation in foods is the Maillard reaction with free asparagine as main precursor (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Zyzak et al., 2003). The backbone of acrylamide originates from the amide group of asparagine (Stadler, et al., 2002). Asparagine can thermally decompose by deamination and decarboxylation, but the yield of acrylamide from asparagine is much higher when a carbonyl source is present (Mottram, et al., 2002; Weißhaar & Gutsche, 2002; Yaylayan, Wnorowski, & Locas, 2003). The high yield of acrylamide when a carbonyl source is present explains the high concentration of acrylamide detected in foods rich in reducing sugars and free asparagine such as fried potatoes (Mottram, et al., 2002; Weißhaar & Gutsche, 2002; Yaylayan, et al., 2003). In addition, acrylamide can be generated by deamination of 3-aminopropionamide (3-APA) (Granvogl & Schieberle, 2006). 3-APA is an intermediate in Maillard reaction, can also be formed by enzymatic decarboxylation of free asparagine and can yield acrylamide upon heating even in the absence of a carbonyl source (Granvogl, Jezussek, Koehler, & Schieberle, 2004; Granvogl & Schieberle, 2007).

The sugar-asparagine adduct, N-glycosylasparagine is a direct precursor of acrylamide (Stadler, et al., 2002). High temperature and reduction in water content in food-processing systems favour the formation of the N-glycoside (Stadler, et al., 2002). The ability to form an open-chain of N-

## General Introduction

glycosylasparagine favours the formation of the decarboxylated Amadori product of asparagine. The decarboxylated Amadori compound can be formed under mild conditions, however, it requires elevated temperatures to cleave the carbon-nitrogen covalent bond and to produce acrylamide (Yaylayan, et al., 2003). The above-mentioned studies demonstrated that high temperatures and water loss play a critical role in inducing the formation of acrylamide, especially at high temperature during frying and roasting.

Different authors showed that acrylamide formation is more sensitive to temperature changes than browning at low moisture contents (Amrein, Limacher, Conde-Petit, Amadó, & Escher, 2006; Eichner, Laible, & Wolf, 1985). Because of this, a reduction of the frying temperature toward the end of the frying process decreases acrylamide formation more strongly as compared to browning (Amrein, et al., 2006).

Acrylamide is an intermediate of the Maillard reaction rather than an end product because it is subjected to a degradation reaction (Knol et al., 2005). The concentration of acrylamide in heated foodstuffs, therefore, is the net result of complex reactions leading to formation and degradation of this compound (Claeys, De Vleeschouwer, & Hendrickx, 2005; De Vleeschouwer, Plancken, Loey, & Hendrickx, 2009). The complexity behind the formation of acrylamide has been illustrated above. Its formation appears to result not only from product properties (e.g. concentration of reducing sugars, amino acids, and moisture content), but also from processing conditions (e.g. frying temperature-time regimes).

## **Chapter 1**

### **1.3 The preparation of French fries in food service establishments**

In FSE, the typical steps in the preparation of French fries are receiving and storing of raw materials, thawing, frying, and holding (Glenn et al., 1993). The final preparation of French fries in FSE is considered as the key end point in the food production chain (Knight, Worosz, & Todd, 2007). It is also the most critical point (Sanny, Luning, Marcelis, Jinap, & van Boekel, 2010) because acrylamide is formed towards the end of the frying process (Amrein, Andres, Escher, & Amadó, 2007; Fiselier, Bazzocco, Gama-Baumgartner, & Grob, 2006), if specific measures to influence the concentration of acrylamide are not taken earlier in the chain. In addition, the preparation of French fries in FSE is highly relying on food handlers. Food handlers in FSE make decisions on out-of-tolerances situation of product properties and processing conditions, and on subsequent corrective actions. For example, food handlers are required to control the actual product features by inspecting (visually) the colour of French fries to aim for a golden yellow colour before ending the frying (CIAA, 2006). They may take a corrective action that includes sorting out fines or brown pieces of French fries (Grob et al., 2003). With respect to processing conditions, food handlers are required to set, measure and subsequently control the actual frying temperature to avoid frying above 175°C (CIAA, 2006). They may take corrective actions such as reducing frying time when frying a smaller portion of frozen par-fried potato strips (CIAA, 2006). However, daily decisions of food handlers to control product properties and processing conditions are expected to vary due to variable workforce, inadequate skills, lack of awareness, and non-compliance to procedures (Crowther, Herd, & Michels, 1993; Griffith, 2006; Sanny, et al., 2010). Furthermore, daily decisions of food handlers in this type of (often) small and medium enterprises are expected to be less controlled because of time constraint as food handlers need to prepare and supply food to order rather than from stock (Clayton, Griffith,

Price, & Peters, 2002; Griffith, 2006; Sanny, et al., 2010). In section 1.2, the product properties and the processing conditions influencing the formation of acrylamide are identified. Food handlers' daily decisions on these influencing factors during the preparation of French fries in FSE, therefore, can potentially influence the concentration of acrylamide.

### 1.4 A techno-managerial research approach

Food safety is one aspect of food quality. High acrylamide concentrations in French fries, therefore, can be seen as a negative quality attribute (Luning & Marcelis, 2006). In realising food quality, both food systems and human systems are involved as shown above. Luning and Marcelis (2006) described food systems as the whole of product properties (ranging from raw materials, ingredients to final products) and profiles of reaction processes (i.e. microbial, chemical, biochemical and physical food processes). Human systems are defined as people with certain individual characteristics making decisions in order to reach goals (Luning & Marcelis, 2006). Food and human systems are highly complex and not fully predictable because each behaviour varies in time (Luning & Marcelis, 2006). In section 1.2, the complexity of the formation of acrylamide is illustrated and the product properties and the processing conditions (food systems) influencing the formation of acrylamide are identified. In section 1.3, it is discussed how people make decisions on these influencing factors in daily operating conditions and practices in FSE that affect the concentration of acrylamide (human systems). Food handler's inadequate decisions on the influencing factors are expected to result in high and variable concentrations of acrylamide. The involvement of food systems and human systems in realising food quality has been illustrated above. Understanding the underlying factors and practices contributing to significant variation in acrylamide concentrations are crucial to develop



## Chapter 1

effective control measures in complex systems. Depending on procedures and control circles as mechanisms to control and assure quality, the intended results are often not obtained (Luning & Marcelis, 2007). Therefore, understanding the major factors contributing to variation in acrylamide concentrations in French fries and investigating the effect of control measures in FSE requires a multidisciplinary research approach, including the technological and the managerial aspects.

Such a multidisciplinary research approach is realised in the techno-managerial approach that was proposed to deal with the complex and dynamic behaviour of food and human systems (Luning & Marcelis, 2006). The core element of this approach is the integrative use of technological and managerial theories and models, wherein technological theories are explaining food behaviour and managerial theories are explaining human behaviour, and integrative use refers to integrating these theories due to the mutual influence of food and human behaviour on each other (Luning & Marcelis, 2006). Using the techno-managerial approach, a food quality relationship model was developed to support a systematic analysis to research food quality management issues (Luning & Marcelis, 2006, 2009). The food quality relationship model reflects that food quality is dependent on food and human behaviour (Luning & Marcelis, 2006). The relationship shows that food behaviour is dependent on food dynamics (i.e., variability in properties due to, e.g., variable compositions, enzyme activities, or level of pathogens) and applied technological conditions (like process conditions, equipment and building circumstances). Likewise, human behaviour is dependent on human dynamics (i.e., variability in decisions due to, e.g., variable perceptions, attitudes, or choice intentions) and administrative conditions (like information systems and procedures) (Luning & Marcelis, 2007). In addition, *a*

## General Introduction

*food quality decision model* (Figure 1.1), which is derived from the food quality relationship model, was also proposed to show that human systems make decisions to influence food quality on these four factors; food dynamics (1a), human dynamics (1b), technological conditions (2a) and administrative conditions (2b) (Luning & Marcelis, 2007). The food quality decision model was used to systematically analyse the decisions of manager and food handler to control the influencing factors that affect the variation in acrylamide concentration between certain acceptable tolerances.

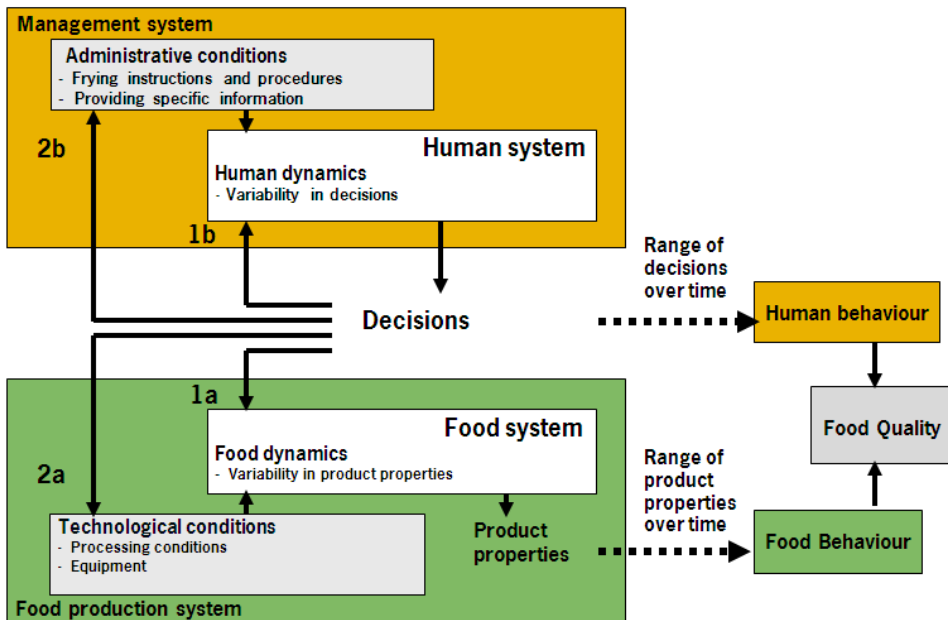


Figure 1.1. A food quality decision model adapted from Luning & Marcelis, 2007

## Chapter 1

### 1.5 Research aims and objectives

The aim of the research described in this thesis is first to identify the major technological and people related factors and to investigate their contribution to variation in acrylamide concentrations. The second aim is to evaluate the effect of control measures in FSE, which focused on lowering reducing sugars concentration in par-fried potato strips (a technological intervention) and on introducing frying instructions (a managerial intervention).

The following objectives were put forward in order to achieve the above-mentioned aims:

1. To identify the major product properties and processing conditions that affect the formation of acrylamide.
2. To analyse literature that provides quantitative data on the possible contribution of product properties and processing conditions to variation in acrylamide concentrations.
3. To use the 'food quality decision model' to systematically analyse the decisions of manager and food handler at the critical points of control in the preparation of French fries.
4. To obtain insight into the actual variation in acrylamide concentrations in French Fries prepared under typical FSE-conditions and to investigate the contribution of technological and people related factors to variation in acrylamide concentrations.
5. To evaluate the effect of lowering reducing sugars concentration in par-fried potato strips (a technological intervention) on the concentration and variation of acrylamide in French fries prepared under experimental conditions in FSE.

6. To evaluate the effect of frying instructions (a managerial intervention) on food handlers' control decisions on the concentration and variation of acrylamide in French fries prepared in the restaurant type of FSE.

### 1.6 Thesis outline

The content of this thesis is as follows:

**Chapter 2** is a qualitative study to obtain insight into the technological as well as the people related factors that could contribute to significant variation in acrylamide concentrations. An overview of the preparation of French fries in FSE is given. The major product properties and processing conditions, i.e., basically technological factors affecting the formation of acrylamide were identified, followed by literature analysis that provided insights into their possible contribution to significant variation in acrylamide concentrations. Subsequently, the critical points of control in the preparation of French fries were identified. Also in this chapter, *a food quality decision model* was used to systematically analyse the decisions of manager and food handler, i.e., basically managerial factors, at the critical points.

**Chapter 3** is an observational study to obtain insight into the actual variation in acrylamide concentrations in French Fries prepared under typical FSE-conditions. Three types of FSE, i.e., chain fast-food services (CFS), institutional caterers (IC), and restaurants (R) were selected to reflect the common types of FSE. The actual practices at receiving, thawing and frying during the preparation of French fries were observed to get an insight into the typical frying practices in

## **Chapter 1**

these FSE types. Influencing factors and practices that contributed most to the variation in acrylamide concentrations were investigated.

**Chapter 4** is an intervention study to evaluate the effect of lowering reducing sugars concentration in par-fried potato strips (a technological intervention) on the concentration and variation of acrylamide in French fries. French fries using par-fried potato strips with lower and higher concentration of reducing sugars were prepared under experimental conditions in FSE. They were compared with French fries made from commonly used par-fried potato strips.

**Chapter 5** is also an intervention study to evaluate the effect of frying instructions (a managerial intervention) on food handlers' control decisions on the concentration and variation of acrylamide in French fries prepared in restaurants. Frying instructions were provided to influence the decisions of the food handlers to control frying conditions and compliance to the frying instructions was observed. The obtained results before and after instructions were compared for restaurants as a group as well as for each restaurant.

**Chapter 6** is the general discussion that discusses the main findings of the research described in this thesis and positions them in a broader perspective. It also critically discusses both the techno-managerial approach and the proposed control measures in term of their advantages and challenges and consequently recommends future research efforts.

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# Chapter 2

## **Impact of control behaviour on unacceptable variation in acrylamide concentration in French fries**

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## **Chapter 2**

### **Abstract**

Various studies have identified high concentrations of acrylamide in French fries and they emphasised that there is much uncertainty about actual concentrations in French fries upon consumption. The aim of this study was to get insight into technological as well as people related factors that contribute to variable and high acrylamide concentrations in French fries prepared under typical food service establishment circumstances. The literature study indicated that glucose and fructose, and actual frying conditions (time-temperature) are the dominant technological factors in acrylamide formation and inadequate control of these factors can result in variable and high concentrations in French fries upon consumption. Analysis of the common control situation in FSE revealed that control decisions on resources by management (i.e. appropriate suppliers, specified raw materials, adequate frying equipment, and competent personnel) can be effective in lowering acrylamide formation, but they are restricted in practice. It is expected, however, that food handlers' control decisions (at receipt and during frying) may be still a major factor contributing to variable concentrations in acrylamide, even under appropriate technological (advanced equipment, low reducing sugars in raw materials) and managerial conditions (competent people, appropriate procedures, etc.).

## **Identification of technological and managerial causes of variation**

### **2.1 Introduction**

Reports on the presence of acrylamide in a range of fried and oven-cooked foods have raised considerable health concern world-wide (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000, 2002). Evaluation of the health risk has resulted in the recommendation that intake concentrations of acrylamide should be reduced in order to protect human health (FAO/WHO, 2005), starting with products that contain high concentrations. Up to now, the highest concentrations have been identified in potato-based products such as French fries (DiNovi, 2006; Friedman, 2003). This product is a predominant source of dietary intake of acrylamide because they are widely consumed (Fiselier & Grob, 2005). However, dietary intake studies, conducted by various researchers, observed large differences in acrylamide concentrations both between single foodstuffs (different brands) within particular food categories and within batches of products processed under the same conditions (Dybing & Sanner, 2003; Konings et al., 2003; Matthys et al., 2005; Svensson et al., 2003). For example, in French fries production, detected concentrations varied several-fold, even within a restaurant chain where starting ingredients and frying times tend to be consistent and regimented (FDA/CFSAN, 2006). Similarly, considerable differences have been observed even on repetition of a specific mitigation measure under controlled conditions, e.g. between different batches of a product made within the same manufacturing plant, or between manufacturing plants using the same process, ingredients and formulations (CIAA, 2006; FAO/WHO, 2007).

The above-mentioned findings underpin that there is much uncertainty about actual concentrations of acrylamide in French fries. This variation is the result of the sum of variations of all individual factors (Kasper, 2007; Lewontin, 2006) that influence acrylamide formation.



## Chapter 2

Reducing too high concentrations and large variation in acrylamide in French fries therefore requires insight into the contribution of individual sources of variation to final variation. Previously, it has been discussed that variation in food quality and safety is due to variation in product properties and technological conditions as well as variation in decision-making behaviour of people involved in the food production system (Luning & Marcelis, 2006, 2007). Many technological studies have provided profound insight into which product properties and processing conditions influence the formation of acrylamide, like frying time and temperature (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Taeymans et al., 2004). However, many of these influencing factors are also affected by how people deal with them in daily practice. Variable and or inadequate decision-making on these factors may result in large variation in the actual concentrations of acrylamide in French fries at the time of consumption. For example, a competent food handler at a frying station who exercised control to avoid over-frying was capable to reduce acrylamide concentration in French fries by a factor of 2-3 (Fiselier et al., 2004). It shows that the way people exercise control of the technological conditions can affect the variation in acrylamide concentration. Differences in decision-making behaviour of people may thus contribute to uncertainty about the actual concentrations of acrylamide in French fries at the time of consumption.

French fries are widely prepared in Food Service Establishments (FSE), and their preparation is highly relying on the food handler. Especially in this type of (often) small and medium enterprises, we expect a significant contribution of variable decision-making behaviour to the variation in acrylamide concentration. Moreover, we expect that the sum of the individual variations of all influencing factors may result in such a large variation that unacceptable high

## **Identification of technological and managerial causes of variation**

acrylamide concentrations in French fries upon consumption are possible. Mills and co-authors (2009) reported acrylamide concentrations in French fries as high as 3500 µg/kg from approximately 710 samples of French fries (data were taken from the European Union acrylamide monitoring database), which illustrates that high concentrations may occur.

The aim of this study is to get insight into the technological as well as people related factors that contribute to variable and high acrylamide concentrations in French fries prepared under typical FSE circumstances. First, the major product properties and technological conditions involved in the formation of acrylamide were ascertained, followed by an analysis of studies that provided insight into their possible contribution to large variation in acrylamide concentrations. We used these insights to assess the critical points of control in French fries preparation in FSE and used a ‘food quality decision model’ to describe which type of control decisions can effectively lower acrylamide concentrations and reduce its variation at the critical points. Subsequently, the typical control situation in FSE in practice has been examined and the consequences for acrylamide concentrations have been discussed.

### **2.2 Understanding the major product and process factors involved in acrylamide formation**

Numerous studies have been performed to understand the complex mechanisms of acrylamide formation (Claeys, De Vleeschouwer, & Hendrickx, 2005; De Vleeschouwer, Plancken, Loey, & Hendrickx, 2009; De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2009; Knol et al., 2005). The major mechanistic pathway established for acrylamide is via the Maillard reaction. In a model system, it has been demonstrated, that asparagine in combination with

## Chapter 2

reducing sugars (glucose and fructose, directly or via hydrolysis of sucrose) generated acrylamide (Amrein et al., 2003; Becalski, et al., 2003; De Vleeschouwer, Plancken, et al., 2009; Mottram, et al., 2002; Stadler, et al., 2002). High temperature and water loss in food-processing systems favour the formation of acrylamide (Stadler, et al., 2002; Yaylayan, Wnorowski, & Locas, 2003). Acrylamide formation however is subjected to a degradation reaction and it is an intermediate of the Maillard reaction rather than an end product (De Vleeschouwer, Plancken, Van Loey, & Hendrickx, 2008; De Vleeschouwer, Van Der Plancken, Van Loey, & Hendrickx, 2008; Knol, et al., 2005). So, the mechanisms behind the formation of acrylamide are affected by both product properties (like reducing sugars, amino acids and moisture content), and processing condition (like temperature). In addition, acrylamide is generated parallel with aroma and colour compounds, because reducing sugars and free amino acids are also the precursors of aroma components and of browning formed in the Maillard reaction (Amrein, Limacher, Conde-Petit, Amadó, & Escher, 2006).

### 2.3 Technological factors contributing to variation in acrylamide concentration

Table 2.1 shows the compiled data from our literature search on studies providing quantitative data on the contribution of product properties and process conditions to variation in acrylamide concentrations, at different steps in French fries production.

## Identification of technological and managerial causes of variation

Table 2.1. Technological factors and conditions contribute to variation in acrylamide concentration

Steps in French fries production	Factors and conditions affecting variation in acrylamide concentration	Actual product properties and process conditions	Observed acrylamide concentrations	Reference(s)
Receiving supplied materials.	Initial concentration of glucose and fructose in par fried potato strips.	<ul style="list-style-type: none"> <li>Glucose concentration ranged from 0.04 - 2.7 g/kg, a 68-fold difference between 17 potato cultivars (the lowest value in Lady Claire and Marlene, and the highest in Naturella and Nicola).</li> <li>Fructose concentration ranged from 0.033 - 1.537 g/kg, a 47-fold difference between 17 potato cultivars (the lowest value in Markies and Marlene, and the highest values in Naturella and Nicola).</li> <li>Glucose concentration ranged from 1.1 - 34.7 mmol/kg fresh weight, a 31.3-fold difference between 31 potato cultivars (the lowest value in Jelli and the highest in Yukon Gold B)</li> <li>Fructose concentration ranged from 1.73 - 33.6 mmol/kg fresh weight, a 19.5-fold difference between 31 cultivars (the lowest value in Fingerling Ozette and the highest in Red).</li> </ul>	Grated potato (placed in a preheated oven at 120°C for 40 min) of cultivar Nicola had the highest potential of acrylamide formation (maximum, 2020 µg/kg), followed by Charlotte (maximum, 1700 µg/kg), while the cultivar Panda exhibited the lowest mean potential (80 µg/kg). The difference between the extremes corresponds to a factor of 25.	Amrein et al., 2003
		<ul style="list-style-type: none"> <li>Glucose concentration ranged from 1.8 - 9.8 g/kg, a 5.4-fold difference between 5 potato cultivars (the lowest value in Saturna in year 2005 and the highest in Binjtje in year 2004)</li> <li>Fructose concentration ranged from 1.1 - 6.2 g/kg, a 5.6-fold difference between 5 potato cultivars (the lowest value in Saturna in year 2005 and the highest in Saturna in year 2006).</li> <li>Glucose concentration ranged from 2.22 - 48.33 mol/kg, a 21.8-fold difference between 5 potato cultivars (the lowest value in Saturna and the highest in Estima)</li> <li>Fructose concentration ranged from 2.22 - 37.78 mol/kg, a 17-fold difference between 5 potato cultivars (similar to glucose, the lowest value in Saturna, and the highest in Estima).</li> </ul>	No acrylamide formation was analysed	Vivanti et al., 2006
		<ul style="list-style-type: none"> <li>Glucose concentration ranged from 1.8 - 9.8 g/kg, a 5.4-fold difference between 5 potato cultivars (the lowest value in Saturna in year 2005 and the highest in Binjtje in year 2004)</li> <li>Fructose concentration ranged from 1.1 - 6.2 g/kg, a 5.6-fold difference between 5 potato cultivars (the lowest value in Saturna in year 2005 and the highest in Saturna in year 2006).</li> <li>Glucose concentration ranged from 2.22 - 48.33 mol/kg, a 21.8-fold difference between 5 potato cultivars (the lowest value in Saturna and the highest in Estima)</li> <li>Fructose concentration ranged from 2.22 - 37.78 mol/kg, a 17-fold difference between 5 potato cultivars (similar to glucose, the lowest value in Saturna, and the highest in Estima).</li> </ul>	Potato chips of cultivar Binjtje had the highest potential of acrylamide formation (maximum, 7600 µg/kg), while the cultivar SW 91 102 exhibited the lowest mean potential (1800 µg/kg). The difference between the extremes corresponds to a factor of 4.2.	Viklund et al., 2008
		<ul style="list-style-type: none"> <li>Glucose concentration ranged from 2.22 - 48.33 mol/kg, a 21.8-fold difference between 5 potato cultivars (the lowest value in Saturna and the highest in Estima)</li> <li>Fructose concentration ranged from 2.22 - 37.78 mol/kg, a 17-fold difference between 5 potato cultivars (similar to glucose, the lowest value in Saturna, and the highest in Estima).</li> </ul>	Potato chips of cultivar Estima had the highest potential of acrylamide formation (4.12 log(acrylamide)), while the cultivar Saturna exhibited the lowest mean potential (3.3 log(acrylamide)). The difference between the extremes corresponds to a factor of 6.6.	Williams, 2005

## Chapter 2

Steps in French fries production	Factors and conditions affecting variation in acrylamide concentration	Actual product properties and process conditions	Observed acrylamide concentrations	Reference(s)
Storage and thawing.	Not expected.	No studies.	No studies.	
Frying.	Frying time-temperature condition.	Frying time-temperature conditions range: 7 minutes at 150°C and 3.5 minutes at 190°C. Frying time-temperature conditions range: 10 minutes at respectively 170°C and 190°C. Time-temperature conditions range: 22 min at 140°C and 17 minutes at 180°C.	Acrylamide concentration of potato slices increased from 500 - 4500 µg/kg, a 9-fold difference. Acrylamide concentration in French fries increased from 800 - 3700 µg/kg, a 5-fold difference. Acrylamide concentration in a glucose-asparagine model system increased 450 - 2900 µg/kg a 6.4 fold difference	Pedreschi et al., 2004 Matthäus et al., 2004 Claeys et al., 2005
		<ul style="list-style-type: none"> <li>Time-temperature conditions range: 15 and 45 minutes at 140°C.</li> <li>Time-temperature conditions range: respectively 2, 4, 9 and 45 minutes at 180°C.</li> </ul>	<ul style="list-style-type: none"> <li>Acrylamide concentration in a glucose-asparagine ranged from 0.8-1.4 mmol/l.</li> <li>Acrylamide concentration in a glucose-asparagine model system increased from 0.85, 2.5 to 3, and decreased to 1.4 mmol/l (after 45 minutes).</li> </ul>	Knol et al., 2005
		Time-temperature conditions range: respectively 1.5, 4 and 12 minutes at 160°C (temperature constant after about 1.5-2.0 min frying at initial oil temperature of 180°C)	Acrylamide concentration in the Lady Rosetta potato crisps increased from 0.2 to 12 and decreased to 10 mg/kg (after 12 minutes).	Knol et al., 2009
		Frying time-temperature conditions range: 3 minutes at 150°C and 5 minutes at 190°C.	Acrylamide concentration French fries increased from 1995 - 7079 µg/kg (3.3 - 3.85 log((acrylamide))), a 3.5-fold difference.	Williams, 2005
		Frying time-temperature conditions range: respectively 4, 5, 7 and 9 minutes at 120°C.	Acrylamide concentration in French fries increased from 50, 68, 208 and 830 µg/kg.	Romani et al., 2008
		Frying time-temperature conditions range: from 5 to 60 min at 180°C, a rapid increase between 10 and 20 min, reaching a	Maximum concentrations for acrylamide in potato, rye, and whole wheat cakes were 6800, 3200, and	Elmore et al., 2005

## Identification of technological and managerial causes of variation

Steps in French fries production	Factors and conditions affecting variation in acrylamide concentration	Actual product properties and process conditions	Observed acrylamide concentrations	Reference(s)
	Portion size.	<p>maximum value between 20 and 35 min, followed by a slow linear decrease.</p> <p>Portion-frying time-temperature drop conditions range: 30 g potato strips to 2 l of oil and a temperature drop to 168°C (from an initial temperature of 170°C) required frying time of 3:30 min, 100 g potato strips and a temperature drop to 158°C required frying time of 5:15 min, 150 g potato strips and a temperature drop to 144°C required frying time of 6:30 min, and 200 g potato strips and a temperature drop to 136°C required frying time of 8 min.</p> <p>Portion size-temperature drop conditions range (at a constant frying time and an initial temperature of 180°C): 50 g potato strips to 1 l of oil (5%) and 100 g potato strips (10%) and a temperature drops by 20–35°C.</p>	<p>1100 µg/kg, respectively.</p> <p>Acrylamide concentration in French fries decreased from 75, 45, 35 and 25 µg/kg.</p>	Fiselier et al., 2006
Holding.	Not expected.	No studies.	No studies.	Grob et al., 2003

## Chapter 2

### *Receiving supplied materials*

The first step of French fries production is the receipt of raw materials, which is usually in the form of frozen par-fried potato strips (Zhang, Guenther, Dwelle, & Foltz, 1999). In French fries, reducing sugars are the limiting factor in the formation of acrylamide (Amrein, et al., 2003; Amrein et al., 2004) and various researchers observed a strong correlation between acrylamide formation and the reducing sugar available in potatoes (Amrein, et al., 2003; Becalski et al., 2004; Viklund, Olsson, Sjöholm, & Skog, 2008; Williams, 2005). One recent study however indicated that the effect of its initial concentration and ratio on the kinetic parameters of acrylamide formation in an asparagine-glucose model system was negligible (De Vleeschouwer, Plancken, et al., 2008). Besides, being the limiting factor, the concentrations of reducing sugars vary widely among potato varieties, which may result in variation in the actual formation of acrylamide. To illustrate, Amrein and co-authors (2003) found that the concentration of glucose ranged from 0.04 to 2.7 g/kg, with the lowest values found in samples of variety Lady Claire and Marlene, and the highest in Naturella and Nicola. Concentrations of fructose varied similarly but were generally lower than of glucose, again with the highest values in Naturella and Nicola.

Similarly, Vivanti and co-authors (2006) observed widely varying concentrations of reducing sugars among varieties. Fructose concentration ranged from 0.31 (1.73, Fingerling Ozette) to 6.05 g/kg (33.6 mmol/kg, Red), a 19.5-fold difference from the lowest to the highest value. For glucose, the concentration ranged from 0.20 (1.11, Jelli) to 6.25 g/kg (34.7 mmol/kg, Yukon Gold B), a 31.3-fold difference from the lowest to the highest value. Some varieties are inherently more prone to higher concentrations of reducing sugars than others, and should be avoided if at all possible for high temperature cooking processes because they are a potential

### **Identification of technological and managerial causes of variation**

source of high and variable acrylamide concentrations (De Wilde et al., 2005). However, considerable differences in reducing sugars among potatoes of the same cultivar suggested that other factors, such as storage, may have an even stronger influence on variation (Biedermann, Noti, Biedermann Brem, Mozzetti, & Grob, 2002). Cooling of potato tubers below 8-10°C caused a large increase in reducing sugars that are released from starch, i.e. low temperature sweetening (Foot, Haase, Grob, & Gondé, 2007; Granda, Moreira, & Castell-Perez, 2005). This effect can be minimised by reconditioning after storage (Vivanti, Finotti, & Friedman, 2006). A significant decrease of reducing sugars (from 7.5 g/kg (0.75%) to 1.8 g/kg (0.18%) on dry matter) can be achieved if potatoes are reconditioned for 3 weeks at 15°C (De Wilde, et al., 2005). Moreover, also climatic conditions can result in variable concentrations of reducing sugars within cultivars. For example, higher reducing sugar concentrations were found in cultivars Eba (+165%), Bintje (+146%), and Agria (+113%) tested in 2003 as compared to the samples from 2002, due to the extraordinary hot and dry conditions in that year (Amrein, et al., 2004). Cultivar and climate have been shown to be more important to reducing sugar concentration than fertilisation (Amrein, et al., 2004), although one study indicated that decreasing fertilisation increased concentrations of reducing sugars from 60% up to 100%, on dry matter, for all varieties studied (De Wilde et al., 2006).

The concentrations of reducing sugars may vary over storage time as well, and its concentration at a specific time may not always reflect concentrations present at earlier or later time periods (Vivanti, et al., 2006). For example, a mean value of 0.82 g/kg Fresh Weight (FW) was found after harvesting and may increase to 1.26 g/kg FW after storage at 8°C (Matthäus, Haase, & Vosmann, 2004). Based upon above studies we may conclude that frozen par-fried potato strips



## Chapter 2

potentially can have high concentrations in reducing sugars with a considerable variation between potatoes, which can contribute to high and variable acrylamide concentrations if no specific measures are taken earlier in the chain.

### *Storage of frozen par-fried potato strips*

The frozen par-fried potato strips are subsequently stored at -18°C until use. At frozen state, the molecular mobility strongly reduces and rates of all chemical and biochemical reactions slow down. In fact, almost no chemical activity was observed (Archer, 2004). Therefore, this step does not seem to contribute to variation.

### *Thawing*

The sacks with frozen French fries are removed from the freezer storage, prior to the deep-frying process. The sacks of French fries usually stand for two or more hours at room temperature, until the French fries have thawed to a point where they can be deep fried (Glenn et al., 1993). To our knowledge, no studies have been performed on the effect of thawing at room temperature for duration of minimum 2 hours on changes in glucose and fructose, and or the formation of acrylamide.

### *Frying*

Deep-frying of par-fried potato strips is the most important processing step. The formation of acrylamide starts at temperatures slightly above 120°C, and reaches a maximum around 170-180°C, depending on the model system studied and the duration of heating (Claeys, et al., 2005; Mottram, et al., 2002; Tareke, et al., 2002). Pedreschi and co-authors (2004) reported that the

### **Identification of technological and managerial causes of variation**

acrylamide concentration of potato slices was about 500  $\mu\text{g}/\text{kg}$  after frying for 7 min at 150°C as opposed to about 4500  $\mu\text{g}/\text{kg}$  after frying for 3.5 min at 190°C. Matthaus and co-authors (2004) found that the concentration of acrylamide in French fries was about 800  $\mu\text{g}/\text{kg}$  after frying for 10 min at 170°C and it increased to about 3700  $\mu\text{g}/\text{kg}$  after 10 min at 190°C. However, at higher temperatures (180°C and 200°C), the drastic increase was followed by a fast decrease due to degradation of acrylamide (De Vleeschouwer, Plancken, et al., 2008; De Vleeschouwer, Van Der Plancken, et al., 2008; Knol, et al., 2005). The increase in acrylamide concentration with increasing frying temperature followed an exponential function (Amrein, et al., 2006; Gökmen, Palazoğlu, & Şenyuva, 2006; Grob et al., 2003; Matthäus, et al., 2004), which implies that small deviations in frying temperature can have large consequences for acrylamide formation. Therefore, frying temperature is expected to be an important factor that determines the extent of variation in acrylamide concentrations.

However, not only temperature is crucial also the time of frying is important, because both determine the kinetics of acrylamide formation (Knol et al., 2009). Williams (2005) found that most acrylamide formation occurs during the early stages of frying at the higher temperatures (175°C), but sufficient quantities of precursors still remained for acrylamide formation at the early stage when frying at lower temperature (150°C for 3 minutes). Romani and co-authors (2008) observed that the increase of time became a key factor in acrylamide formation in French fries after around 4 minutes of frying when the temperature of potato surface reached 120°C and the temperature of oil bath reached 140°C. Elmore and co-authors (2005) cooked potato flake, wholemeal wheat, and wholemeal rye at 180°C, from 5 to 60 min, and observed an exponential increase in acrylamide formation between 10 and 20 min, reaching a maximum value between 20

## Chapter 2

and 35 min, followed by a slow linear decrease. Above studies clearly demonstrated the profound effect of time as an important factor to variation, because minor deviations in frying time can result in large variation (and high values) of acrylamide concentrations in French fries.

Portion size is another factor that may contribute to variation, because it affects the actual temperature and time that is necessary to make French fries with optimal quality attributes (like crispness, flavour) and low acrylamide concentration. According to Fiselier and co-authors (2006), an isothermal frying at 160°C is required, which can be achieved after a temperature drop from an initial frying temperature of 170–175°C. However, bigger portion sizes will result in larger temperature drops and require longer frying times to achieve an optimum frying quality. For example, Fiselier and co-authors (2006) demonstrated that adding 30g potato strips to 2L of oil, dropped the temperature to 168°C from an initial temperature of 170°C. It required 3:30 minutes (210s) before optimal quality of French fries was achieved and the fries contained 75 µg/kg acrylamide. When 150g potato strips were added, temperature dropped to 144°C, and it took 6:30 min (390s) to obtain French fries with optimal quality, but acrylamide concentration was 35 µg/kg. Acrylamide concentration was reduced more than half, whereas the culinary quality was still good. In another study, it was found that the temperature dropped by 20–35°C from an initial temperature of 180°C when adding 100g potato strips to 1L of oil (10%). The optimum quality of French fries was reached after 2:45 minutes (165s) of frying and contained 530 µg/kg. When 50g of potato strips (5%) was added, and the frying time was kept constant (a temperature drop was not mentioned), products of similar quality contained a higher amount of acrylamide, i.e. 710 µg/kg (Grob, et al., 2003). Portion size determines temperature drop and the required frying time and consequently the extent of acrylamide formation. Generally,

### **Identification of technological and managerial causes of variation**

acrylamide formation decreased as the portion size increased, but portion size should be limited to achieve a temperature drop to around 140°C to obtain a fully satisfactory product (Fiselier, et al., 2006). These studies indicate that small deviations in portion size result in variable acrylamide concentrations. Portion size seems therefore an important factor to variation.

Different authors have observed an increased acrylamide formation rate at low water content, whereas with increasing water content the reaction rate decreased (Amrein, et al., 2006; De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2007). This can possibly be explained by the temperature: moisture gradient within the food, which is dependent on reaction time and on distance from the centre of the French fries (De Vleeschouwer, et al., 2007; De Vleeschouwer, Van Der Plancken, et al., 2008). As a result, the formation of acrylamide is concentrated at the crust region rather than core region (Franke, Sell, & Reimerdes, 2005; Gökmen, et al., 2006).

Recent studies demonstrated that the type of oil used in the frying process has no influence on acrylamide formation (Matthäus, et al., 2004; Williams, 2005), although it was thought earlier that palm oil (Gertz & Klostermann, 2002) and olive oil (Becalski, et al., 2003) induced higher acrylamide formation compared to other types of oils. Acrylamide formation, is further proven to be independent not only of oil oxidation but also of oil hydrolysis, even in sequential frying (Mestdagh, De Meulenaer, & Van Peteghem, 2007)

## **Chapter 2**

### *Holding*

Once the French fries are in the holding bin, they will be distributed to the consumers as per their orders (Glenn, et al., 1993). French fries are consumed hot and no systematic study on the effect of holding step influencing the formation of acrylamide has been reported (Castle, 2006). It is unlikely that the holding step has an effect because the temperature is much below the temperature at which formation and degradation takes place.

Overall, we can conclude that the variable concentrations of reducing sugars in the par-fried potato strips at receipt can be expected to be a major source of variation of acrylamide formation, if no specific measures are taken. Moreover, mainly the deviations in actual frying regimes (due to set temperature, time, and portion size) will contribute to variable and high acrylamide concentrations, if no specific measures are taken during frying. Similarly, Cummins and co-authors (2009) concluded, based on Monte Carlo simulations, that low reducing sugar concentrations as well as strict control of frying conditions in French fries production are most crucial.

### **2.4 Control decisions to reduce acrylamide in French fries preparation**

The next step in our study was to analyse the major decisions that can be taken in FSE to control the variation and concentration of acrylamide. Control decisions are aimed at keeping product properties, production processes, and human processes between certain acceptable tolerances and when necessary take corrective action (Evans & Lindsay, 2004; Luning & Marcelis, 2007). The major acrylamide control points in FSE include the receipt of raw materials and the frying practices. To systematically analyse the control decisions, we used a ‘food quality decision

### **Identification of technological and managerial causes of variation**

model' that distinguishes decisions on technological and administrative (organisational) *conditions* to create the circumstances to prevent undesirable product properties and or people actions, and decisions on *dynamics* of the food and human systems to reduce actual variation (Luning & Marcelis, 2007).

Management typically takes decisions on technological and administrative *conditions* by selecting appropriate (technological and people) *resources* to create an adequate technological and managerial infrastructure for the production of desired products. These are often mid or long-term decisions. In addition, management takes *short-term decisions* on food *dynamics* by putting requirements on product properties, to ensure adequate materials/products, and on the control of human *dynamics* by directing their actions through providing specific information, gaining commitment, and/or by giving detailed direct instructions. Additionally, food handlers typically take daily control decisions on out of tolerance situations of product properties and process conditions, and on subsequent corrective actions (Luning and Marcelis, 2007, 2009). Table 2.2 shows the distinct decisions that can be taken at receipt and frying in order to control acrylamide concentrations in FSE's.

## Chapter 2

Table 2.2. Four types of control decision at the critical points (supplied materials and frying) that can reduce variation in acrylamide concentration

Decisions on	Critical control decisions affecting acrylamide concentrations in supplied materials	Critical control decisions affecting acrylamide concentrations in frying	Reference(s)
Product properties.	<ul style="list-style-type: none"> <li>Specify requirements on acceptable initial concentration of reducing sugars in materials specifications.</li> <li>Potato variety selection.</li> <li>Supplier selection.</li> </ul>	<p>Specify requirements for a lighter golden colour of French fries in product specifications.</p> <ul style="list-style-type: none"> <li>Investment in technological infrastructure, such as frying equipment.</li> <li>Determine acceptable frying practices such as the setting of process parameters (temperature-time regimes) and other frying practices (how to deal with oil conditions, portion size, thawing time, sort out fines, and remaining portions).</li> <li>Instruct food handlers on frying behaviour.</li> <li>Assign competent food handler at the frying station.</li> </ul>	<ul style="list-style-type: none"> <li>Grob, 2007</li> <li>Fiselier &amp; Grob, 2005</li> <li>Fiselier et al., 2006</li> <li>Grob, 2005</li> <li>Grob, 2007</li> <li>Fiselier, et al., 2006</li> <li>Palazozglu &amp; Gökmen 2008</li> </ul>
Technological conditions.			
Food handler behaviour.	<ul style="list-style-type: none"> <li>Instruct food handler on purchasing and receiving behaviour.</li> <li>Assign competent food handler to purchasing and receiving incoming materials.</li> </ul>	<ul style="list-style-type: none"> <li>Latham &amp; Ernst, 2006</li> <li>Teigland &amp; Wasko, 2009</li> <li>Gauci &amp; Gauci, 2005</li> <li>Jevšnik et al., 2008</li> <li>Luning et al., 2008</li> <li>Walker et al., 2003</li> <li>Fiselier et al., 2004</li> </ul>	
Administrative conditions.	<ul style="list-style-type: none"> <li>Recruit people with appropriate competencies and skills.</li> <li>Develop procedures that include prescriptions on purchasing and incoming material inspection.</li> </ul>	<ul style="list-style-type: none"> <li>Recruit people with appropriate competencies and skills.</li> <li>Develop frying procedures that include prescriptions of thawing time before frying, oil temperature, frying time, portion size etc.</li> <li>Determine competencies of food handlers and training on frying procedures.</li> </ul>	<ul style="list-style-type: none"> <li>Naing et al., 2007</li> <li>Seaman &amp; Eves, 2006</li> <li>Crowther, et al., 1993</li> <li>Uen, et al., 2009</li> <li>Latham &amp; Ernst, 2006</li> <li>Teigland &amp; Wasko, 2009</li> <li>Gauci &amp; Gauci, 2005</li> <li>Jevšnik et al., 2008</li> <li>Luning et al., 2008</li> <li>Walker et al., 2003</li> <li>Griffith, 2006</li> </ul>

## Identification of technological and managerial causes of variation

### *Control decisions at receipt affecting acrylamide concentration*

At receipt, the initial concentration and variation in reducing sugars in the raw materials can be controlled via management decisions on *incoming materials* (i.e. setting strict product specifications on reducing sugars), and decisions on *supply sources* (i.e. selecting preferred suppliers). Various researchers considered the selection of appropriate varieties (with lower concentrations of reducing sugars) as a simple and efficient measure to reduce the extent of variation of acrylamide concentrations in French fries production (Fiselier & Grob, 2005; Grob, et al., 2003; Lindsay & Jang, 2005).

Management decisions on the *organisation* are crucial for adequate control of raw materials. They concern, first of all, acquiring food handlers with appropriate competences and skills, providing training to maintain and or improve existing competences and skills, and providing procedures for homogenous decision-making on purchasing and receiving (Luning & Marcelis, 2007, 2009; Naing et al., 2007; Seaman & Eves, 2006)

In addition to these resource-type decisions, also the *daily control decisions* are important for the raw material quality. These are assigning competent people for the purchasing and receiving tasks, and instructing them on how to purchase and check raw materials, and correction of inadequate behaviour. In this way, it is controlled that only appropriate raw materials are accepted for French fries preparation.



## Chapter 2

### *Control decisions at frying affecting acrylamide concentration*

As analysed in the technological section, control should be focused on the frying time-temperature regime (CIAA, 2006; Romani, et al., 2008; Williams, 2005) and the actual portion size (Fiselier, et al., 2006; Grob, et al., 2003). Actual frying can be controlled via different types of decisions. Control includes a clear specification of *product properties*, in this case the colour of the French fries (CIAA, 2006; Grob, et al., 2003), and management decisions on the *frying resources*, i.e. selecting frying equipment (e.g. with advanced automated frying temperature regimes that allow a lower temperature at the end of frying), and setting process parameters (e.g. stipulating correct temperature-time regimes). To illustrate, the actual frying temperature mainly depends on the capability of the heating power of the fryer to remain near the adjusted frying temperature (Fiselier, et al., 2006). Palazoglu and Gokmen (2008) showed that advanced equipment with controlled temperature programs can reduce acrylamide concentrations in French fries more than half (58%).

Decisions on the *organisation* concern minimally required competences, skills, and training of food handlers and procedures on frying practices (i.e. prescribing how to deal with thawing time, remaining portions, sort out fines, oil conditions, etc.). *Daily control decisions* include assigning competent people to frying tasks, and giving them instructions about the exact final colour, frying time, and portion size, and correcting inadequate behaviour. Various studies have provided evidence for certain optimal frying regimes realising French fries with good culinary properties while being low in acrylamide (Fiselier, et al., 2004; Fiselier & Grob, 2005; Grob, et al., 2003). It has been recommended to keep acrylamide concentrations low by ending the frying process before the on-set of browning (Fiselier, et al., 2006). However, the appropriateness of the

## **Identification of technological and managerial causes of variation**

selected frying regime should be validated for the actual circumstances in the specific FSE (Luning et al., 2009) to assure that good quality and low acrylamide concentration can be really met.

### *Control situation in FSE in practice*

Examining the current situation in FSE revealed that they have restricted possibilities to control their raw materials. The selection of potato varieties specifically for production of frozen par-fried potato strips for French fries preparation is not yet widely practiced (Grob, 2007). Although manufacturers of frozen par-fried potato strips usually select potato varieties with lower concentrations of reducing sugars (long before acrylamide became a subject) by a frying test to avoid strong browning of the finished product (Grob, 2005), frozen par-fried potato strips still can contain high reducing sugars. For example, in a survey, Fiselier and Grob (2005) found still samples with high concentrations of reducing sugars (2 out of 49 samples contained 2.8 g/kg and 3.5g/kg, respectively) although the average concentration amounted to 0.67 g/kg. The possibilities for selecting appropriate suppliers seem to be still limited. Moreover, small and medium FSE's commonly purchase frozen par-fried potato strips from different sources on local markets even without knowing what reducing sugars are. This means that they just deal with the given concentrations and variation in reducing sugars in their purchased frozen par-fried potato strips, which put demands on the capability of their frying equipment and the appropriateness of frying protocols and compliance to them.

However, fryers that are commonly used in small and medium sized FSE's are not so sophisticated. They often have insufficient heating sources (causing temperature drops in an

## Chapter 2

uncontrolled manner dependent on portion size) or have (too) strong heaters causing oil temperature returning to the initial value in the most critical time for acrylamide formation (Grob, 2007). Although industry uses automated temperature program fryers, which allows maintenance of exact temperature profiles (PPM-Technologies, 2008), such automated temperature program fryers suitable for use in FSE's are not (yet) available in practice.

Procedures on standardised control of frying practices are however available. They require food handlers to control the actual product features by visually inspecting the colour of French fries against the product specification provided (CIAA, 2006). Food handlers may take a corrective action that includes sorting out fines or brown pieces of French fries (Grob, et al., 2003). With respect to process features, food handlers are required to set, measure and subsequently control the temperature and time of actual frying to avoid frying above 175°C (CIAA, 2006). They should take corrective actions such as reducing frying time when frying smaller portion of frozen par-fried potato strips (CIAA, 2006). Nevertheless, the successful implementation of standardised control of frying practices depends on the workforce quality and on the actual compliance to procedures by the food handlers in their daily practice (Crowther, Herd, & Michels, 1993; Griffith, 2006; Uen, Wu, & Huang, 2009). To what extent food handlers actually control product and process parameters depends on how they are instructed, trained, and motivated (Latham & Ernst, 2006; Teigland & Wasko, 2009), how their frying practices are controlled (by whom, how often, etc.), and their specific knowledge and awareness (Gauci & Gauci, 2005; Jevšnik, Hlebec, & Raspor, 2008; Luning, Bango, Kussaga, Rovira, & Marcelis, 2008; Walker, Pritchard, & Forsythe, 2003). In the FSE context, however, food handlers often work under stress conditions because they need to prepare and supply food to order rather than

## **Identification of technological and managerial causes of variation**

from stock, which can limit the actual compliance to safety practices (Chinchilla Lee, 2009). Moreover, FSE's often have to deal with high turnover, lack of competent and motivated personnel and under capacity, which also complicates appropriate and consistent safety practices (Chinchilla Lee, 2009; S. L. Jones, Parry, O'Brien, & Palmer, 2008; T. F. Jones & Angulo, 2006; Rodgers, 2005). For example, Chinchilla Lee (2009) studied actual hygiene practices in FSE and found that required hygiene practices were not followed or wrongly executed in 80% of the handlings (out of 120).

### **2.5 Conclusions and further research**

The large variation of acrylamide concentrations found in, amongst others, French fries have raised the question, which factors contribute most to it. Our study aimed first at gaining qualitative insight into major technological and people related factors contributing to variable concentrations of acrylamide concentration in French fries, prepared under FSE conditions. Our literature analysis revealed that glucose and fructose concentration (in the par-fried potato strips), and the actual frying conditions (as affected by frying equipment capability, portion size, setting of frying parameters) are the major technological factors in acrylamide formation, and inadequate control of these factors can result in high concentrations of acrylamide. The current situation in small and medium FSE's indicates that management control of resources (i.e. decisions on appropriate supplies and suppliers, adequate frying facilities, and adequate personnel) is yet restricted, which creates conditions for variable acrylamide formation. However, the question remains if even under appropriate technological and managerial conditions for production of French fries with lower acrylamide concentrations, variable daily decisions may be still a considerable source of variation. To our knowledge, little is known about

## **Chapter 2**

the quantitative contribution of variable decision-making of food handlers to acrylamide concentrations in French fries.

Further research includes a study on the actual variation profiles of acrylamide concentrations in French fries prepared in different types of FSE, having different technological and managerial infrastructures for French fries production. Subsequent studies aim at investigating the effects of technological (focused on raw material properties) and managerial control interventions (focused on food handlers) on the variation of acrylamide concentrations prepared under FSE circumstances. Moreover, a Bayesian belief network model will be developed based on expert knowledge and data from the experimental studies, indicating the actual contribution of the individual technological and people related factors to the final variation of acrylamide concentrations in French fries for consumption. The results of these quantitative studies will be published in due course as a follow-up of the present qualitative study.

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# Chapter

# 3

## **Possible causes of variation in acrylamide concentration in French fries prepared in food service establishment: An observational study**

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## Chapter 3

### Abstract

Acrylamide is a probable human carcinogen, and its presence in a range of fried and oven-cooked foods has raised considerable health concern world-wide. Dietary intake studies observed significant variations in acrylamide concentrations, which complicate risk assessment and establishment of effective control measures. The objective of this study was to obtain an insight into the actual variation in acrylamide concentrations in French fries prepared under typical conditions in food service establishment (FSE). Besides acrylamide, frying time, frying temperature, and reducing sugars were measured and the actual practices at receiving, thawing and frying during French fries preparation were observed and recorded. Although the mean concentration of acrylamide among the three FSE types was not significantly different, the least variation in acrylamide concentration was found in French fries prepared in the CFS compared to the IC and the restaurants. The variation in actual frying temperature contributed most to the variation in acrylamide concentrations, followed by the variation in actual frying time; no obvious effect of reducing sugars was found. The lack of standardised control of frying temperature and frying time (due to inadequate frying equipment) and the variable practices of food handlers seem to contribute most to the large variation and high acrylamide concentrations in French fries prepared in restaurant type of FSE as compared to chain fast-food services, and institutional caterers. The obtained insights in this study can be used to develop dedicated control measures in FSE, which may contribute to a sustainable reduction in acrylamide intake.

## Contribution of technological and managerial factors to variation

### 3.1 Introduction

Acrylamide is a probable human carcinogen (IARC, 1994) and its presence in a range of fried and oven-cooked foods (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000, 2002) has raised considerable health concern world-wide (FAO/WHO, 2002, 2005). Highest concentrations have been identified in potato based products such as French fries (Claeys et al., 2010; DiNovi, 2006; Friedman, 2003). In addition, significant variations in acrylamide concentrations were observed in many dietary intake studies (Dybing & Sanner, 2003; Konings et al., 2003; Matthys et al., 2005; Svensson et al., 2003), which complicate risk assessment. Judging the actual risk of acrylamide and developing appropriate control measures, therefore requires insight into the distribution profiles of acrylamide concentrations (FAO/WHO, 2007).

French fries are considered as a predominant source of acrylamide because they are extensively consumed (Fiselier & Grob, 2005). French fries are widely prepared in food service establishments (FSE) and their preparation is apparently a crucial factor because acrylamide is formed towards the end of the frying process (Amrein, Andres, Escher, & Amadó, 2007; Fiselier, Bazzocco, Gama-Baumgartner, & Grob, 2006). **Chapter 2** revealed that besides the variation in initial concentration of reducing sugars, the variable frying conditions (time-temperature) seem a major factor influencing the variation in acrylamide concentration. It was proposed that food handler's inadequate control on these influencing factors in their daily practise in FSE could lead to the large variation and high acrylamide concentration in French fries (Sanny, Luning, Marcelis, Jinap, & van Boekel, 2010). Al-Kahtani (1991) showed that frying practices among various types of FSE differed considerably in terms of frying conditions (temperature-time regime), frying equipment selection (design, capacity, heating system and material of



### Chapter 3

fabrication), and quality control during frying. Many studies that were done under controlled laboratory conditions established the relationship between various food properties (e.g., concentration of reducing sugars and amino acids) and processing conditions (e.g., frying temperature-time regimes and pH adjustment with organic acids) on acrylamide formation (Jung, Choi, & Ju, 2003; Knol et al., 2005; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). However, there is a restricted insight into the contribution of actual frying practices in FSE (e.g., using different raw materials, applying different frying temperature-time regimes and using different frying equipment) to the variation in acrylamide concentration in French fries for consumption.

The objective of this study was to obtain an insight into the actual variation in acrylamide concentrations in French Fries prepared under typical FSE-conditions. Three types of FSE, i.e., chain fast-food services (CFS), institutional caterers (IC), and restaurants (R) were selected in this observational study to reflect the common types of FSE in Malaysia. The actual practices at receiving, thawing and frying during the preparation of French fries were observed to get an insight into the typical frying practices in these FSE types. The set and the actual frying temperature and frying time were recorded and acrylamide and reducing sugars concentrations were analysed. We hypothesised that the mean concentration as well as the extent of variation in acrylamide concentrations in French fries prepared by the three FSE types would differ.

## **Contribution of technological and managerial factors to variation**

### **3.2 Materials and methods**

#### *3.2.1 Characteristics of Food Service Establishments*

The study was focused on FSE that located within a 25 km radius of Serdang, Selangor, Malaysia. This city was selected to represent a typical urban area. Three different establishments with more than five workers were selected from each FSE type. The establishments consisted of local or international chain fast-food services for CFS type, caterers in a college or in a university for IC type, and family style restaurants for R type.

#### *3.2.2 Sampling and data collection*

##### *3.2.2.1 French fries samples*

The study was focused on straight-cut French fries samples that were 8 mm x 8 mm in cross section and 60-70 mm long. Frozen par-fried potato strips were used as raw materials. For each establishment, samples were collected over three days of production. Each day, samples were taken from five different frying batches. In each frying batch, a total of four servings of the French fries was collected. Of these servings, three were used to determine acrylamide concentration whereas the last serving was served to a customer. The determination of acrylamide concentration was done in triplicate, one measurement of acrylamide concentration for each serving. Regardless of the serving size of the collected French fries, each serving weighed approximately 67 gram, the recommended serving size (Shahar et al., 2002). Each serving was coded and stored in polyethylene bags at -18°C prior to analysis.

## Chapter 3

### 3.2.2.2 *Frozen par-fried potato strips*

Frozen par-fried potato strips samples were also collected over three days of French fries production. Samples were collected before they were used in frying. Each day, samples were taken from five different frying batches. In each frying batch, a total of three servings of the frozen par-fried potato strips were collected. These servings were used to determine reducing sugars concentration in triplicate, one measurement of reducing sugars for each serving. Each serving weighed approximately 67 gram. Each serving was coded and stored in polyethylene bags at -18°C prior to analysis.

### 3.2.2.3 *Measurements and observations*

The food handler was asked to fry four servings of French fries as he normally does. During frying, the following frying practices were measured: frying temperature and time, serving size and volume of oil. In addition, receiving supplied materials, brand of frozen par-fried potato strips, type of fryer, mode of heating, oil type, thawing practise and presence of procedures and guidelines were also observed.

The CFS and the IC used a commercial fryer whereas the R used a frying pan to fry French fries. The actual frying temperature once the fryer reached the set temperature (thermostat light off) was monitored and measured by a digital thermometer 51 series II coupled with a temperature probe (Fluke Corporation, Everett, USA). The temperature probe was placed into the oil bath at the same location between different measurements and away from the fryer's/frying pan's wall. The set frying temperature as indicated by the thermostat setting was also recorded. For a frying

## **Contribution of technological and managerial factors to variation**

pan, the actual frying temperature was monitored and measured by the same device just before par-fried potato strips were inserted into the oil.

The actual frying time once the fryer reached the set frying time (timer goes off) was monitored and measured using a built-in timer. The set frying time as indicated by a built-in timer was also recorded. For a frying pan, the actual frying time was recorded using a stopwatch SW01 (ALBA, Japan) just after the removal of French fries from the oil.

### *3.2.3 Analysis methods*

#### *3.2.3.1 Chemicals and reagents*

D-(+)-glucose (99.5%) and D-(-)-fructose (99%) were obtained from Sigma (St Louis, USA). <sup>13</sup>C<sub>3</sub>-labelled acrylamide (99% isotopic purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile, methanol, ethyl-acetate, hexane, hydrobromic acid (47% w/v), bromine (99.99%), potassium bromide, sodium sulphate anhydrous were purchased from Merck (Darmstadt, Germany). Acrylamide (99%), D-(+)-maltose monohydrate (99%), triethylamine, and sodium thiosulphate were purchased from Fluka Chemie AG (Buchs, Switzerland). Ultra pure water was used throughout the experiments (Purelab Classic UV, Elga Labwater, Lane End, UK). Solid phase extraction cartridges (Oasis Hydrophilic-Lipophilic Balance (HLB), 6cc and Oasis Mixed-mode Cation-eXchange (MCX), 3cc) were supplied by Waters Corp (Milford, Massachusetts, USA).

A bromination reagent was prepared from potassium bromide (152 g), hydrobromic acid (8 mL), and bromine-saturated water (saturated at 4°C, 50 mL) and 600 mL distilled water. This reagent

## Chapter 3

was stored at 4°C. Stock solutions of acrylamide (0.2 mg/mL) and <sup>13</sup>C<sub>3</sub>-labelled acrylamide (4 µg/mL) were prepared by dissolving the compound in distilled water. Working standards were prepared by diluting the stock solution of acrylamide to concentrations of 1, 5, and 10 µg/mL with water. All stock solution and working standards were stored in a refrigerator at 4°C for a maximum of 3 months.

### 3.2.3.2 *Analysis of fructose, glucose, and sucrose*

The procedure was adapted from Vivanti and co-authors (2006). The sample was grinded in a blender (Braun multiquik ZK3, Frankfurt, Germany). A sub-sample (2 gram) was weighed in a 50 mL centrifuge tube and 10 mL of acetonitrile/water (80:20 v/v) were added. The suspension was then centrifuged at 1643 RCF (g) for 10 min and the supernatant was passed through a 0.45 µm nylon syringe filter (Sartorius AG, Goettingen, Germany). Aliquots (50 µL) of the filtrate were injected into a Waters high-performance liquid chromatography (HPLC) instrument equipped with a refractive index (R.I.) detector and an µBondapack NH<sub>2</sub>, 3.9 × 300 mm column (Waters Corp, Milford, Massachusetts, USA). An isocratic mode of elution was used with the mobile phase consisting of acetonitrile/water (80:20, v/v) at a flow rate of 0.8 mL/min. Maltose was used as an internal standard. Calibration curves were linear ( $r^2 > 0.999$ ). The detection limit for fructose, glucose and sucrose was 5 mg/kg. The recoveries for fructose ranged between 91.3-100.3%, for glucose ranged between 92.9-96.7% and for sucrose ranged between 98.8-106.6%. The intra-day and inter-day precisions, expressed as the relative standard deviation, were 4.6 and 6.7% (fructose), 11.7 and 13.2% (glucose) and 12.2 and 13.5% (sucrose), respectively.

## **Contribution of technological and managerial factors to variation**

### *3.2.3.3 Analysis of acrylamide*

#### *3.2.3.3.1 Extraction*

The scheme described by Becalski and co-authors (2005) was followed. The sample was grinded in a blender. A sub-sample (2 gram) was weighed in a 50 mL centrifuge tube and 10 mL of water containing 1000 ng  $^{13}\text{C}_3$ -labelled acrylamide as the internal standard (final concentration = 100 ng/mL) were added. The mixture was shaken at medium speed level (ca. 256 pulses / minute) on a vertical shaker (RS-1, Jeio Tech Co., Gyeonggi-do, Korea) for 30 minutes and centrifuged in a refrigerated centrifuge (3-18K, Sigma, Gillingham Dorset, United Kingdom) at 10956 RCF (g) for 30 minutes. Oasis HLB were conditioned with 3 mL of methanol and equilibrated with 3 mL of water. The filtrate from the centrifuge (5 mL) was promptly transferred through an Oasis HLB cartridge connected in tandem to an Oasis MCX cartridge, and the eluate was collected.

Standards contained acrylamide at concentrations of 10, 25, 50, 100, 250, 500, 1000 ng/mL and isotopically labelled acrylamide at 100 ng/mL. Eluates or standards (5 mL) was treated with bromination reagent (15 mL) overnight at 4°C. Excess bromide was decomposed by adding 0.7M sodium thiosulphate solution drop wise until the yellow colour disappeared. Calcined sodium sulphate anhydrous (4 gram) was added and the mixture was stirred using a magnetic stirrer for 5 minutes. The mixture was transferred to a 125 mL separatory funnel and was extracted twice with 15 mL of ethyl acetate/hexane (4:1, v/v) by shaking using a vertical shaker for 1 minute. After phase separation, the lower aqueous layer was discarded. The organic phase was transferred into a 50 ml centrifuge tube containing calcined sodium sulphate anhydrous (approximately 4 gram) and centrifuged at 10956 RCF (g) for 10 min. The liquid phase was

## Chapter 3

decanted through glass wool, into a 50 ml round-bottom flask. Pooled fraction was evaporated to dryness under vacuum at 40°C using a rotary evaporator (Rotavapor R-210, Buchi Labortechnik AG, Flawil, Switzerland). The residue was re-dissolved in 450 µL of ethyl acetate, and 50 µL of triethylamine was added to convert 2,3-dibromopropionamide to 2-bromopropenamide. The resulting solution was transferred into an insert in an amber vial and stored in a freezer at -18°C until analysis by Gas Chromatograph-Time-of-Flight-Mass Spectrometry (GC-TOF-MS).

### 3.2.3.3.2 GC-TOF-MS analysis

Brominated sample extracts and calibration standards were analysed on an Agilent Technologies 6890N gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) coupled to a LECO Pegasus III Time-of-Flight (TOF) Mass Spectrometry detector (MSD) with positive electron impact (EI) ionisation (LECO Corporation, Lakeview Avenue, St. Joseph, MI, USA). The GC column was a HP-Innowax capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Palo Alto, CA, USA) and the carrier gas was helium at 1.6 ml/min. Following injection, the column was held at 65°C for 1 min, then programmed at 15°C/min to 170°C, 5°C/min to 200°C, followed by 40°C/min to 250°C, and held for 15 min at 250°C. Injections by an Agilent Technologies 7683 auto-sampler (2 µl) were made in split less mode (split flow 60 ml/min) with a purge activation time of 1.0 min and an injection temperature of 250°C. The GC-TOF-MS interface transfer line was held at 260°C. Ions monitored were  $m/z$  70, 149, and 151 for 2-bromopropenamide, and  $m/z$  110 and 154 for 2-bromo(<sup>13</sup>C<sub>3</sub>)propenamide.

## Contribution of technological and managerial factors to variation

### 3.2.3.3.3. Quantification

Acrylamide in French fries samples was quantified using the ion at  $m/z$  149 for 2-bromopropenamide, and the ion at  $m/z$  154 for 2-bromo( $^{13}\text{C}_3$ )propenamide. Other ions at  $m/z$  70, 110, and 151 were considered only for confirmation purposes. A calibration graph was constructed by plotting peak area ratios (149/154) against the corresponding ratios of analyte amounts. Acrylamide concentrations in sample extracts were calculated from the calibration slope and intercept value. Calibration curves were linear ( $r^2 > 0.999$ ). The limit of detection was 10  $\mu\text{g}/\text{kg}$  and the recoveries were in the range between 102-110%. The intra-day and inter-day precisions (expressed as the RSD) were 6.4 and 3.1%, respectively.

### 3.2.4. Statistical analysis

The original data of acrylamide concentration in each serving of French fries samples were  $\log_e$  transformed to construct histograms. The  $\log_e$  transformation was done to approximate a normal distribution of the data while retaining information about the degree of variation in the distribution (Dallal, 2009; Garner, 2010; Hopkins, 2003).

An analysis of variance (ANOVA) was used to explore the mean differences in the  $\log_e$  transformed acrylamide concentration among the three FSE types while also investigating the variation due to different establishments, sampling days and frying batches. A mixed model was used with FSE type as fixed factor and establishment, sampling day and frying batch as random factors. The mixed model was selected because FSE type was used as a control factor in the present study whereas the establishments, sampling days and frying batches were not fixed in advance and their effects were therefore considered as random. All random factors are nested



### Chapter 3

within the pre-mentioned sampling level. The significances of the differences among the three ranges of reducing sugars as well as between the two FSE types were determined using Tukey's HSD test. The obtained means of  $\log_e$  transformed acrylamide concentration were back-transformed to an original scale of measurement at which they then are the geometric means (Bland & Altman, 1996; Petrie & Sabin, 2009). The coefficient of variation (coded as CV) was calculated using the formula of  $100(e^{SD} - 1)$ , where SD is the standard deviation of the  $\log_e$  transformed acrylamide concentration (Dallal, 2009; Hopkins, 2003). The CV is used to compare the degree of variation for each FSE type to another. To analyse the reducing sugars concentrations, the same mixed ANOVA model with nested factors was used. The CV was calculated as the standard deviation divided by the mean and is reported as a percentage.

Bivariate correlations were used to investigate the strength and direction of the relationship of acrylamide formation to a set of influencing factors (frying temperature, frying time, reducing sugars, sucrose and thawing). Results were expressed as Pearson correlations.

Multiple linear regression analysis was used to investigate the predictive ability of a set of influencing factors (frying temperature, frying time, reducing sugars, sucrose and thawing) as well as to assess the relative contribution of each influencing factor on acrylamide formation. These influencing factors, considered as possible predictors for acrylamide formation, were put into the model. Multiple regression analysis was checked for possible violations from the model assumptions during analysis. In tests, a p-value of 0.05 or less was considered significant. Statistical analyses were performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL.).

## Contribution of technological and managerial factors to variation

### 3.3 Results

Initial data analysis indicated that the frequency distribution of acrylamide concentration was slightly skewed to the right (data not shown). This is due to a small number of samples with very high acrylamide concentrations in the data set. Therefore, a  $\log_e$  transformation was performed to approach more the normal distribution of data set. Figure 3.1 shows that the mean of  $\log_e$  transformed acrylamide concentration of French fries differed among the three FSE types; the mean was lowest in the chain fast-food services (CFS) (5.44), followed by the institutional caterers (IC) (5.54), and was highest in the restaurants (R) (5.87). The figure also reveals that the standard deviation of the (mean  $\log_e$  transformed) acrylamide concentration was lower in the CFS (0.19) than in the IC (0.27), and in the R (0.44).

### Chapter 3

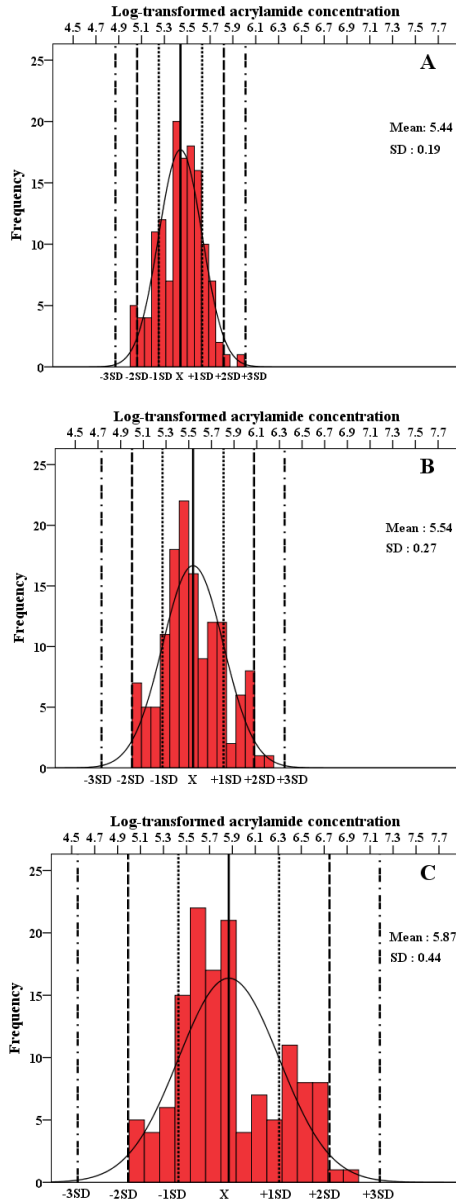


Figure 3.1. Histograms of  $\log_e$  transformed acrylamide concentration for the three FSE types (A) Chain fast-food service (CFS), (B) Institutional caterer (IC), and (C) Restaurant (R).

### **Contribution of technological and managerial factors to variation**

Observations and recorded data of various practices in the FSE are shown in Table 3.1. It reveals that at receipt, all FSE have no insight into the initial concentrations of reducing sugars of frozen par-fried potato strips. Moreover, none of the FSE purchased specific potato varieties and or selected specific suppliers to obtain par-fried potato strips with low concentrations of reducing sugars. In fact, selection of frozen par-fried potato strips was mainly determined by price as well as by simple evaluation on the taste and colour of French fries. They also had no instructions (or procedures) in place for purchasing and inspection of the incoming raw materials. The CFS type purchased frozen par-fried potato strips from preferred suppliers whereas the IC and the R types purchased frozen par-fried potato strips from different sources in the market such as wholesale traders, hypermarkets, and local supermarkets. Table 3.1 reveals that at thawing, the CFS type systematically thawed the frozen par-fried potato strips before frying, which was not the case in the IC and the R types. With respect to the frying practices, Table 3.1 shows that none of the FSE had a documented frying procedure, but the requirements on setting of frying temperature and time in the CFS and the IC types were orally communicated. The CFS and the IC types used fryers that were equipped with a temperature controller, a timer, and a basket whereas the R type used frying pans. Moreover, the baskets of fryers in the CFS were automatically lifted from the oil once the fryer reached the set frying time.

Table 3.1. Observation and recorded data of various practices in the preparation of French fries among the three FSE types.

	Chain fast-food service (CFS)			Institutional caterer (IC)			Restaurant (R)		
	A <sup>1</sup>	B	C	D	E	F	G	H	I
Practices at different steps in French fries production									
<b>Receiving</b>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
• Initial concentration of reducing sugars unknown	No	No	No	No	No	No	No	No	No
• Potato varieties selected	Yes	Yes	Yes	No	No	No	No	No	No
• Supplier selected	PS1 <sup>2</sup>	PS2 <sup>3</sup>	ND <sup>4</sup>	PS2	PS1	PS2	PS2	Pomme Frites	Pomme Frites
• Description or brand of frozen par-fried potato strips used									Pomme Frites Golden and Simplot
• Presence of (documented) procedures:	No	No	No	No	No	No	No	No	No
- Instructions on purchasing raw materials that contain acceptable initial concentration of reducing sugars	No	No	No	No	No	No	No	No	No
- Instructions of incoming material inspection									
<b>Thawing</b>	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Frozen par-fried potato strips were thawed before frying									
<b>Frying</b>	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
• Fryer equipped with a temperature controller, timer and basket									
• Fryer	EF1 <sup>5</sup>	EF1	EF1	EF2 <sup>6</sup>	GF1 <sup>7</sup>	EF3 <sup>8</sup>	GF2 <sup>9</sup>	GF2	GF2
- Type of fryer	Digital	Digital	Digital	Analogue	Digital	Digital	NA <sup>10</sup>	NA	NA
- Type of temperature and time controller	20 <sup>11</sup>	15	15	6	15	15	8	5	2
- Volume of oil, L									
• Presence of (documented) frying procedures, i.e. instruction on requirement for thawing time before frying, oil temperature, frying time, portion size, remaining portions, sort out fines etc.	No	No	No	No	No	No	No	No	No

## Contribution of technological and managerial factors to variation

	Chain fast-food service (CFS)				Institutional caterer (IC)				Restaurant (R)			
	A	B	C	D	E	F	G	H	I			
• Oral instruction to set frying temperature and time of the fryer	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
• Used palm oil in frying	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
• Guideline of portion size (10% potato referring to the oil) followed	No	No	No	No	No	No	No	No	No	No	No	No
• Estimated maximum portion size	4-6 servings	4-6 servings	4-6 servings	4-5 servings	4-6 servings	4-6 servings	4-6 servings	4-5 servings	4-5 servings	4-5 servings	4-5 servings	4-5 servings
- Mean serving size, gram/serve (SD)	119(4.8)	103(12.2)	102(15.9)	95(11.1)	131(14.4)	96(12.0)	135(14.9)	180(28.8)	180(28.8)	180(28.8)	180(28.8)	90(10.9)
- Maximum	127	127	128	119	161	123	262	109	109	109	262	262
- Minimum	101	82	67	69	89	58	83	67	67	67	65	65
- Number, n	45	45	45	45	45	45	30	30	30	30	30	30
• Assigned a dedicated personnel at the frying station	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes
<b>Holding</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Practice in holding is not expected as source of variation

<sup>1</sup>Data from an establishment of each FSE type.

<sup>2</sup>PS1 = frozen par-fried potato strips type 1 (no brand, expiry date printed on the package).

<sup>3</sup>PS2 = frozen par-fried potato strips type 2 (no brand, green colour printing on package with expiry date).

<sup>4</sup>ND = no data.

<sup>5</sup>EF1 = Electric fryer, floor model FM II (Frymaster L.L.C, Shreveport, L.A., USA). This fryer has a temperature range of 60°C to 195°C and heating power capability of no more than ± 2°C.

<sup>6</sup>EF2 = Electric fryer, countertop model DFT 2000 (MSM Equipment Manufacturer Sdn. Bhd., Cheras, Malaysia).

<sup>7</sup>GF1 = Gas fryer, floor model BIH 152-2 CSD (Frymaster L.L.C, Shreveport, L.A., USA).

<sup>8</sup>EF3 = Electric fryer, floor model HEF 77 (Hobart, London, United Kingdom).

<sup>9</sup>GF2 = conventional gas-fried steel pan.

<sup>10</sup>NA = not applicable.

<sup>11</sup>This fryer has a bowl of 20L oil capacity, equipped with two static baskets (of which only one was used in the study) and a regulating thermostat.

### Chapter 3

Besides the above observations, both the set and the actual frying temperature and frying time were recorded (data not shown). For the CFS type, the setting of the frying temperature was identical (177°C). The actual frying temperature ranged between 170-177°C and the coefficient of variation (coded as CV) in actual frying temperature was 1.1%. In the IC type, the setting of frying temperature ranged from 168°C to 200°C. The actual frying temperature ranged from 160 to 186°C, and the CV was 3.0%. In the R type, the setting of frying temperature was not possible due to the fact that they use frying pans. The actual frying temperature ranged from 148 to 215°C, which was reflected in the highest CV (8.9%).

In the CFS type, the setting of frying time ranged from 150 to 165 seconds and it corresponded with the actual frying time (150-165 seconds). In the IC type, the setting of frying time ranged from 180 seconds to 300 seconds. However, the actual frying time ranged from 180 seconds to 390 seconds, resulting in a CV of 26.5%. In the R type, the setting of frying time was also not possible due to their simple frying equipment. The actual frying time ranged from 199 seconds to 694 seconds, which was reflected in the highest CV (39.2%).

Table 3.2 shows that the mean concentration of acrylamide in French fries among the three FSE types was not significantly different. The mean concentration of acrylamide was 231 µg/kg for the CFS, 254 µg/kg for the IC, and 354 µg/kg for the R. The CV in acrylamide concentration was lower in the CFS (21%) than in the IC (31%), and in the R (55%). The concentration of acrylamide in French fries ranged from 150 to 392 µg/kg (a 2.6-fold difference) for the CFS, 151 to 505 µg/kg (a 3.3-fold difference) for the IC, and 152 to 1023 µg/kg (a 6.7-fold difference) for the R.

### **Contribution of technological and managerial factors to variation**

Table 3.2 also shows that the mean concentration of reducing sugars in the frozen par-fried potato strips among the three FSE types was not significantly different. The mean concentration of reducing sugars was 0.66 g/kg for the CFS, 1.51 g/kg for the IC, and 1.10 g/kg for the R. The CV in reducing sugars concentration was 45.5% for the CFS, 26.5% for the IC, and 18.2% for the R. The concentration of reducing sugars ranged from 0.25 to 1.24 g/kg (a 5.0-fold difference) for the CFS, from 0.88 to 2.22 g/kg (a 2.5-fold difference) for the IC, and from 0.72 to 1.62 g/kg (a 2.3-fold difference) for the R.



Table 3.2. Concentration of acrylamide, reducing sugars, and sucrose in samples from the three FSE types.

	Chain fast-food service			Institutional caterer			Restaurant					
	A <sup>1</sup>	B	C	Overall <sup>2</sup>	D	E	F	Overall	G	H	I	Overall
Acrylamide, µg/kg <sup>3</sup>												
Mean <sup>4,5</sup> (SD <sup>6</sup> )	249(0.10)	203(0.17)	243(0.21)	231(0.19)	224(0.28)	234(0.11)	313(0.25)	254(0.27)	281(0.17)	292(0.37)	540(0.38)	354(0.44)
CV <sup>7</sup> , %	11	19	23	21	32	12	28	31	19	45	46	55
Median <sup>8</sup>	246	202	246	236	217	238	320	244	280	302	603	330
Minimum	197	150	156	150	151	178	177	151	193	575	251	152
Maximum	312	302	392	392	414	285	505	505	389	424	1023	1023
Range	115	152	236	242	263	107	328	354	197	151	772	871
Reducing sugars, g/kg <sup>3</sup>												
Mean <sup>9</sup> (SD <sup>10</sup> )	0.33(0.04)	0.99(0.1)	ND <sup>11</sup>	0.66(0.3)	1.15(0.1)	1.53(0.3)	1.85(0.2)	1.51(0.4)	0.97(0.1)	1.14(0.2)	1.20(0.2)	1.10(0.2)
CV <sup>12</sup> , %	12.1	10.1		45.5	8.7	19.6	10.8	26.5	10.3	17.5	16.7	18.2
Minimum	0.25	0.82		0.25	0.88	1.18	1.33	0.88	0.72	0.81	0.86	0.72
Maximum	0.45	1.24		1.24	1.45	2.14	2.22	2.22	1.16	1.62	1.54	1.62
Range	0.2	0.42		0.99	0.57	0.96	0.89	1.34	0.44	0.81	0.68	0.9
Sucrose, g/kg <sup>3</sup>												
Mean <sup>9</sup> (SD <sup>10</sup> )	2.57(0.2)	0.60(0.03)	ND	1.58(1.0)	1.64(0.2)	1.06(0.2)	1.31(0.1)	1.34(0.3)	1.67(0.1)	1.75(0.3)	0.53(0.2)	1.32(0.6)
CV <sup>12</sup> , %	7.8	5.0		63.3	12.2	18.9	7.6	22.4	6.0	17.1	37.7	45.5
Minimum	2.27	0.52		0.52	1.15	0.79	1.12	0.79	1.46	1.25	0.21	0.21
Maximum	3.14	0.66		3.14	1.84	1.55	1.58	1.84	1.93	2.58	1.12	2.58
Range	0.87	0.14		2.62	0.69	0.76	0.46	1.05	0.47	1.33	0.91	2.37

<sup>1</sup>Data from an establishment of each FSE type.

<sup>2</sup>Mean of data from three or two (where applicable) different establishments of each FSE type.

<sup>3</sup>Referring to fresh weight.

<sup>4</sup>The mean of log<sub>e</sub> transformed acrylamide concentration in each establishment was back transformed to an original scale using an anti-logarithmic and expressed as geometric mean.

<sup>5</sup>Mean value based on N = 45.

<sup>6</sup>Standard Deviation (SD) of the log<sub>e</sub> transformed acrylamide concentration.

<sup>7</sup>The exact coefficient of variation (CV) was calculated using a formula of 100(e<sup>SD</sup> - 1), where SD is the standard deviation of the log<sub>e</sub> transformed acrylamide concentration.

<sup>8</sup>The median of log<sub>e</sub> transformed acrylamide concentration in each establishment was back transformed to an original scale using an anti-logarithmic and expressed as geometric median.

<sup>9</sup>Arithmetic mean (mean of untransformed data).

<sup>10</sup>Arithmetic standard deviation (standard deviation of untransformed data).

<sup>11</sup>ND = no data.

<sup>12</sup>The CV was defined as the standard deviation divided by the mean, the result of which is reported as a percentage.

### Contribution of technological and managerial factors to variation

Bivariate correlations and multiple linear regression analyses were performed to investigate the strength and direction of the relationship of the influencing factors (frying temperature, frying time, reducing sugars, sucrose and thawing) and their possible contribution to the formation of acrylamide. Table 3.3 shows a strong correlation between frying temperature and acrylamide concentration ( $r=0.596$ ,  $n=360$ ,  $p<0.05$ ). There was a moderate negative correlation between thawing practise and acrylamide concentration ( $r=-0.482$ ,  $n=360$ ,  $p<0.05$ ). Frying time showed a small but significant correlation with acrylamide concentration ( $r=0.104$ ,  $n=360$ ,  $p<0.05$ ). Reducing sugars showed no significant correlation with acrylamide concentration ( $r=0.102$ ,  $n=360$ ,  $p= 0.053$ ). The multiple linear regression model revealed that all factors (except reducing sugars and sucrose) significantly contributed to the prediction of acrylamide formation ( $r^2=0.483$ ,  $n=360$ ,  $p<0.05$ ). The order of contributions, from highest to lowest are frying temperature ( $\beta=0.513$ ), thawing practise ( $\beta=-0.359$ ), and frying time ( $\beta=0.143$ ).

Table 3.3. Bivariate correlations and multiple linear regression analyses on the influencing factors (frying temperature, frying time, reducing sugars, sucrose and thawing).

Influencing factors	Bivariate correlations analysis		Multiple linear regression analysis	
	Pearson correlation coefficient, r	p-Value	Beta <sup>1</sup>	p-Value
Frying time, seconds	0.104	<0.05	0.141	<0.05
Frying temperature, °C	0.596	<0.05	0.513	<0.05
Reducing sugars, g/kg <sup>2</sup>	0.102	0.053	-0.083	0.078
Sucrose, g/kg <sup>2</sup>	-0.231	<0.05	-0.044	0.315
Thawing	-0.482	<0.05	-0.359	<0.05

<sup>1</sup>Beta values are standardised coefficients.

<sup>2</sup>Referring to fresh weight.

N = 360.

## Chapter 3

### 3.4 Discussions

The present study shows that although the mean concentration of acrylamide among the three FSE types was not significantly different, the least variation in acrylamide concentration was found in French fries prepared in the chain fast-food service (CFS) as compared to the institutional caterers (IC), and the restaurants (R). In addition, the highest concentration of acrylamide (1023  $\mu\text{g}/\text{kg}$ ) was found in the R type of FSE. Our study showed that the actual frying temperature was strongly correlated with the acrylamide concentration ( $r=0.596$ ,  $n=360$ ,  $p<0.05$ , Table 3.3) and contributed the most to the prediction of acrylamide formation ( $\beta=0.513$ ,  $p<0.05$ , Table 3.3). The finding confirms the pronounced effect of frying temperature on the formation of acrylamide as reported in studies that were done under carefully controlled laboratory conditions (Amrein, Limacher, Conde-Petit, Amadó, & Escher, 2006; Grob et al., 2003; Matthäus, Haase, & Vosmann, 2004). Further, we demonstrated its pronounced effect under typical FSE-conditions. Comparing the frying practices among the three FSE types revealed that the setting of a low and identical frying temperature (177°C) of digitally controlled fryers (i.e. sophisticated frying equipment) in the CFS type apparently resulted in a small range of actual frying temperature (170-177°C). Correspondingly, the lowest variation in acrylamide concentration (i.e. 150 to 392  $\mu\text{g}/\text{kg}$ , a 2.6-fold difference) was found in the CFS type. In the R type, frying pans were used in which the temperature cannot be regulated. The food handlers in the R type, visually inspected the oil to estimate its temperature, which apparently resulted in a large range of actual frying temperatures (148-215°C). Correspondingly, the largest variation in acrylamide concentrations (i.e. 152 to 1023  $\mu\text{g}/\text{kg}$ , a 6.7-fold difference) was found in the R type. Food handler's lack of control on frying temperature seems to contribute to the large variation and high acrylamide concentration in French fries prepared in the R type as compared

### **Contribution of technological and managerial factors to variation**

to the other FSE types. Our findings on the frying practices are consistent with other studies that surveyed the actual frying conditions in FSE. Al-Kahtani (1991) earlier reported that CFS in Saudi Arabia also typically set frying temperatures at 177°C. Morley-John and co-authors (2002) reported that the setting of frying temperature in the FSE in New Zealand ranged from 175°C to 190°C and the actual frying temperature ranged from 136°C to 233°C. Furthermore, Gere (1985) also found that frying temperature in nearly 70 % of the FSE kitchens in Budapest was not controlled at all.

In our study, also the frying time showed a significant (but small) correlation with acrylamide concentration ( $r=0.104$ ,  $n=360$ ,  $p<0.05$ , Table 3.3). Similarly, a significant (but little) contribution of frying time to the prediction of acrylamide formation was observed ( $\beta=0.141$ ,  $p<0.05$ , Table 3.3). The finding seems in contrast to studies that reported a linear relationship between frying time and acrylamide concentration (Gökmen & Şenyuva, 2006; Matthäus, et al., 2004). However, Romani and co-authors (2008) discussed that the increase of time only becomes a key factor in the formation of acrylamide in French fries after circa 240 seconds of frying (at 180°C). The actual frying times recorded in the CFS and IC types ranged from 150 to 240 seconds, which are shorter than the identified critical time as mentioned by Romani et al (2008). Different authors reported that after immersion of potato strips, the frying temperature drops and slowly recover to the initial setting (Fiselier, et al., 2006; Grob, 2007). The short frying time seems to limit the formation of acrylamide, because frying ends before the frying temperature recovers to the initial setting and before the acrylamide concentration starts to increase linearly with time. Conversely, the actual frying times recorded in the R type ranged from 199 to 694 seconds, which indicate that longer frying times than 240 seconds were applied.

### Chapter 3

It appears that longer frying times applied in the R type as compared to the other FSE types also contributed to the large variation and high acrylamide concentration in French fries. The observed differences in actual frying times among the CFS (150-165 seconds), the IC (180-390 seconds), and the R (199-694 seconds) are possibly due to the differences in frying equipment and frying instructions. More in detail, the difference in frying equipment refers to the lifting of basket that was digitally controlled in the CFS versus simple frying pans in the R, and the difference in frying instructions refers to the oral instructions in the CFS and the CI versus no instructions at all in the R. The finding supports earlier suggestions that fryer type influenced the concentrations of acrylamide (Gertz, Klostermann, & Kochhar, 2003; Romani et al., 2006; Sanny, et al., 2010). Moreover, Haase (2006) observed that food handlers in restaurants tend to have different preferences regarding discoloration which resulted in individual preparation techniques to meet their specific requirements.

In our study, no significant correlation between reducing sugars and acrylamide concentration was found ( $r=0.102$ ,  $n=360$ ,  $p=0.053$ , Table 3.3) and reducing sugars also did not significantly contribute to the prediction of acrylamide formation ( $\beta=-0.083$ ,  $p=0.078$ , Table 3.3). Our data showed that a considerable variation in reducing sugars concentration was not corresponding to the variation in acrylamide concentration. To illustrate, the most variation in reducing sugars concentration in the CFS (0.25 to 1.24 g/kg, a 5.0-fold difference) was not reflected in the least variation in acrylamide concentration in French fries for the CFS (150 to 392  $\mu\text{g}/\text{kg}$ , a 2.6-fold difference). Similarly, the least variation in reducing sugars concentration in the R (0.72 to 1.62 g/kg, a 2.3-fold difference) was also not reflected in the most variation in acrylamide concentration in French fries for the R (152 to 1023  $\mu\text{g}/\text{kg}$ , a 6.7-fold difference). The data

### **Contribution of technological and managerial factors to variation**

seems in contrast to the established literature. Reducing sugars have been frequently mentioned as the limiting factor in the formation of acrylamide (Amrein et al., 2003; Amrein et al., 2004). Other studies established the strong correlation between acrylamide formation and reducing sugars available in potatoes (Amrein, et al., 2003; Becalski et al., 2004). However, these cited studies used potato tubers to prepare French fries and Amrein and co-authors (2003), as an example, reported the reducing sugars concentration in tubers ranged from 0.1 to 9 g/kg. In contrast, we used commercial par fried potato strips in the present study and reducing sugars concentration in potato strips ranged from 0.25 to 2.22 g/kg. Possibly, a lower range of reducing sugars concentration of the present study than the study of Amrein and co-authors (2003) contributed to the obtained insignificant correlation between reducing sugars and acrylamide concentration. The lower reducing sugars concentration in par fried potato strips than in potato tubers is resulted from the effects of blanching and pre-frying during the production of commercial potato strips (Fiselier & Grob, 2005; Grob, 2007). Moreover, various authors proposed that the selection of potato varieties or supplier (for low concentrations of reducing sugars) could be a simple and efficient measure to reduce the extent of variation in acrylamide concentration (Fiselier & Grob, 2005; Grob, et al., 2003; Lindsay & Jang, 2005; Sanny, et al., 2010). However, De Vleeschouwer and co-authors (2008) acknowledged the wide range of sugar concentrations as well as the highly variable sugar-asparagine ratios in real products, such as potatoes. They studied the effect of initial reactant concentrations and ratio on acrylamide formation, and reported a negligible effect of initial reactant concentration and ratio on the kinetic parameters of acrylamide formation in an asparagine-glucose model system. The above cited studies were mostly carried out under strictly controlled laboratory conditions. In our observational study, conditions such as reducing sugars, frying temperature, frying time and

### Chapter 3

frying equipment were not controlled. Possibly, the effect of reducing sugars is overshadowed by the effects of frying temperature and (less by) frying time. Although the finding of De Vleeschouwer and co-authors (2008) supported our observations, further research is needed to investigate the effect of initial concentration of reducing sugars on the variation in acrylamide concentration in French fries prepared under carefully controlled conditions in FSE.

Interestingly, in our study, thawing practise also showed a moderate (negative) correlation with acrylamide concentration ( $r=-0.482$ ,  $n=360$ ,  $p<0.05$ , Table 3.3) and it significantly contributed to the prediction of acrylamide formation ( $\beta=-0.359$ ,  $p<0.05$ , Table 3.3). Studies showed that thawing of frozen par-fried potatoes resulted in more oil absorption and greater moisture loss (Burr, 1971; O'Connor, Fisk, Smith, & Melton, 2001), which consequently associated with temperature drops during frying (Mehta & Swinburn, 2001). Various authors observed a decrease in acrylamide formation related with temperature drops due to an increase in portion size (Fiselier, et al., 2006; Grob, et al., 2003). However, few studies have been conducted on the effect of thawing practices on acrylamide formation. Tuta and co-authors (2010) have recently shown that microwave thawing of par-fried potato strips reduced the acrylamide formation by 89% (frying temperature of 180°C), but a shorter frying time was used for the thawed par-fried potato strips than for the unthawed potato strips. Nevertheless, drawing a conclusion on the contribution of thawing practices to the concentration of acrylamide has to be done with care. In our observational study, we only measured the (initial) frying temperatures, but not the temperature drop over the frying time. Furthermore, it is interesting to note that frying instructions on the packaging commonly recommended not to thaw the frozen par-fried potatoes before frying, to obtain crispier French fries (Lou, 2005; Products, 2009). Further research might

## **Contribution of technological and managerial factors to variation**

be necessary to obtain insight into the effect of thawing practices and corresponding frying temperature profiles on the formation of acrylamide. These insights, may have implications on how frying instructions on the packaging should be designed (with regard to thawing, besides frying temperature and time) to prevent high acrylamide concentrations in French.

When considering our data to reflect FSE as a whole, a 6.8-fold difference in magnitude (from 150 to 1023  $\mu\text{g}/\text{kg}$ ) in acrylamide concentrations was found with median at 256  $\mu\text{g}/\text{kg}$ . The median reported in this study is consistent with Mills and co-authors (2009) who found the median at 250  $\mu\text{g}/\text{kg}$  from approximately 710 samples of French fries (data were taken from the European Union acrylamide monitoring database). Mills and co-authors (2009) however reported a higher, i.e. a 35-fold difference in magnitude (from 100 to 3500  $\mu\text{g}/\text{kg}$ ) in acrylamide concentrations than our results.

### **3.5 Conclusion**

In this study, we found that the variation in actual frying temperature contributed most to the variation in acrylamide concentrations, followed by the variation in actual frying time (to a lesser extent). We found no obvious effect of reducing sugars. The lack of standardised control of frying temperature and frying time (due to inadequate frying equipment) and the variable practices of food handlers seem to contribute most to the large variation and high acrylamide concentrations in French fries prepared in the R type as compared to the other FSE types. The finding supports our previous discussions about the potential impact of food handler behaviour on the variation in food safety and quality (Luning & Marcelis, 2006, 2007; Sanny, et al., 2010). Acrylamide concentration in French fries as high as 1023  $\mu\text{g}/\text{kg}$  was obtained in the restaurant



## **Chapter 3**

type of FSE. Our study confirms that high acrylamide concentrations may occur if no specific controls on the influencing factors and people practices are taken in the preparation of French fries. The obtained insights in this study can be used to develop dedicated quality control measures in FSE, which may contribute to a sustainable reduction in acrylamide intake. Subsequent studies will focus on investigating the effects of technological (focused on raw material properties) and managerial control interventions (focused on food handlers) on the variation of acrylamide concentrations in French fries prepared under FSE circumstances.

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## Contribution of technological and managerial factors to variation

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### Chapter 3

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# Chapter 4

## **Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments?**

This chapter is submitted as Sanny, M., Jinap, S., Bakker, E. J., van Boekel, M.A.J.S., and Luning, P.A. Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments?





## Chapter 4

### Abstract

Control measures to reduce acrylamide exposure should be focused on potato-based products such as French fries because they are widely consumed and can contain high and variable concentrations of acrylamide. Although various control measures have been tested in controlled laboratory conditions, there is a need to validate them under actual food processing conditions. The objective of this study was to obtain insight into the actual effectiveness of lowering reducing sugars concentration in par-fried potato strips on the concentration and variation of acrylamide in French fries prepared in real life situation in food service establishments. Acrylamide, frying time, frying temperature, and reducing sugars were measured and characteristics of fryers were recorded. Data showed that the use of par-fried potato strips with lower concentrations of reducing sugars than the commonly used potato strips is indeed an effective measure to reduce acrylamide concentrations in French fries prepared under standardised frying conditions in chain fast-food services and institutional caterers. The lowest mean acrylamide concentration in French fries was found for the par-fried potato strips with the low concentration of reducing sugars (322 µg/kg) and was significantly lower ( $p < 0.05$ ) than from the strips with normal (491 µg/kg) and high concentration of reducing sugars (928 µg/kg). However, there was still large variation in the acrylamide concentrations in French fries, although the variation in reducing sugars concentrations in low and normal types of par-fried potato strips was very small and the frying conditions were similar. Additional factors that could affect the temperature-time profile of frying oil were discussed such as setting a lower frying temperature at the final than at the start of frying, product/oil ratio and thawing practice. They need to be controlled in daily practice to reduce the variation in acrylamide.

### 4.1 Introduction

The FAO/WHO (2005) recommended to put substantial efforts in identifying effective measures to reduce acrylamide concentrations in food to protect human health. High concentrations of acrylamide have been extensively detected in potato based products, such as French fries (Claeys et al., 2010; DiNovi, 2006; Friedman, 2003). French fries are a predominant source of dietary intake of acrylamide because they are widely consumed (Fiselier & Grob, 2005). However, many dietary intake studies observed significant variations in acrylamide concentrations (Dybing & Sanner, 2003; Konings et al., 2003; Matthys et al., 2005; Svensson et al., 2003). Capuano & Fogliano (2011) stated that the large variations in acrylamide concentration complicate the estimation of actual dietary exposure to acrylamide. Furthermore, Grob (2007) showed that high concentrations of acrylamide in French fries combined with high consumption could increase the dietary exposure for an individual by a factor of 5, from an average exposure of 2.8 µg/day.

Many studies established relationships between the various product properties (e.g., concentration of reducing sugars, amino acids, and moisture contents) and processing conditions (e.g., frying temperature-time regimes) that affect the acrylamide formation (De Vleeschouwer, Van Der Plancken, Van Loey, & Hendrickx, 2008; Knol et al., 2005; Mottram, Wedzicha, & Dodson, 2002). Because reducing sugars are identified as the limiting factors in the formation of acrylamide (Amrein et al., 2003; Amrein et al., 2004), the selection of potato varieties with low concentrations of reducing sugars was proposed as a simple and effective control measure to reduce acrylamide concentration (Fiselier & Grob, 2005; Grob et al., 2003; Lindsay & Jang, 2005). However, most of the above-cited studies were executed under strictly controlled laboratory conditions, whereas different authors stressed that the results of these studies could differ from field studies (De Graaf et al., 2005; Snyder & Matthews, 1983) and they must be

## Chapter 4

validated under actual food processing conditions (CIAA, 2006; Stadler, 2005). Studies have recently evaluated the effects of some control measures under actual food processing conditions systems (Vinci, Mestdagh, de Muer, van Peteghem, & de Meulenaer, 2010; Vinci et al., 2011). These studies evaluated various additives on their acrylamide mitigation potential and quality control of incoming potatoes in real industrial production systems. However, data on the effectiveness of control measures in real food service establishments (FSE) is very limited although French fries are widely prepared in FSE. The final preparation of French fries in FSE is considered as the key end point in the food production chain (Knight, Worosz, & Todd, 2007) and also the most critical point (Sanny, Luning, Marcelis, Jinap, & van Boekel, 2010), because acrylamide is formed only towards the end of the frying process (Amrein, Andres, Escher, & Amadó, 2007; Fiselier, Bazzocco, Gama-Baumgartner, & Grob, 2006).

Grob (2005) reported that recommendations on how to prepare French fries with less acrylamide were given to restaurants in Switzerland and found that 91% of the 157 French fries samples remained below 200 µg/kg with a median at 76 µg/kg. Although the recommendations emphasised on the use of potato with low concentration of reducing sugars, the study did not specify its concentrations in potatoes used by the restaurants. **Chapter 2** concluded that variable frying conditions, variation in initial concentration of reducing sugars, and food handler's variable and inadequate control of these factors in their daily practice could lead to the observed large variation and high acrylamide concentrations in French fries. In **Chapter 3**, the contribution of reducing sugars to the prediction of acrylamide formation in French fries prepared under the typical conditions in different types of FSE could not be confirmed. It was discussed that the effect of variable frying temperature and to a lesser extent frying time, possibly overshadowed the effect of reducing sugars on acrylamide concentration.

## **Effect of a technological control measure**

FSE commonly purchase frozen par-fried potato strips from different sources in local markets and they have to deal with the given concentration and variation in reducing sugars in their purchased potato strips (Grob, 2007; Sanny, Jinap, Bakker, Van Boekel, & Luning, 2012). Different studies have shown that par-fried potato strips in the markets can contain rather different concentrations of reducing sugars (Fiselier & Grob, 2005; Sanny, et al., 2012). According to Palazoğlu and Gökmen (2008), a concentration of reducing sugars of approximately 1 g/kg is considered as a normal value of reducing sugars that might be encountered in common practice, whereas a concentration of above 3 g/kg is considered as a maximum value (CIAA, 2006; FAO/WHO, 2007). It is not yet clear if the considerable variation in reducing sugars concentration contributes to the observed variation in acrylamide concentrations in real situations in-practice. The objective of this study was to obtain insight into the actual effectiveness of lowering reducing sugars concentration in par-fried potato strips on the concentration and variation of acrylamide in French fries prepared in-practice in FSE. French fries using par-fried potato strips with lower and higher concentration of reducing sugars were prepared under standardised frying conditions in FSE. They were compared with French fries made from commonly used par-fried potato strips.

### **4.2 Materials and methods**

#### *4.2.1 Characteristics of Food Service Establishments*

The chain fast-food services (CFS) and institutional caterers (IC) were selected to study the effect of potato strips with different concentration of reducing sugars. In a previous study, it was shown that these FSEs represent the common types of FSE (besides small restaurants) and they use similar types of frying equipment. The CFS and IC types use fryers that are equipped with a

100

## Chapter 4

temperature controller and a timer. The setting of frying temperature and time was identical in the CFS whereas it was variable in the IC. In the present study, frying temperature and time setting were standardised at 177°C and 2 minutes 45 seconds, respectively, to minimise the effects of these influencing factors on the formation of acrylamide and to focus on the effect of changing the concentration of reducing sugar. This setting has recently shown to correspond to the lowest mean acrylamide concentration with the least variation in French fries (Sanny, et al., 2012).

The study focused on FSEs that are located in Selangor, Malaysia. Selangor typically represents an urban area. Two different establishments with more than five workers were selected from each FSE type. The establishments consisted of local chain fast-food services for the CFS type and caterers in a college/university for the IC type.

### *4.2.2 Characteristics of par-fried potato strips*

The study comprised of three experiments, in which par-fried potato strips with three ranges of initial reducing sugars concentrations (i.e. low (LRS), normal (NRS), and high (HRS)) were used. The par-fried potato strips were purchased either from a selected supplier (for the low reducing sugars concentration) or from the market (for the normal and the high reducing sugars concentration). Reducing sugars concentrations in these par-fried potato strips were measured before frying to determine which materials to use in which experiments.

The first experiment was considered as a reference condition, in which a food handler in the FSE was asked to fry French fries using the par-fried potato strips with the normal concentration of reducing sugars (NRS). Par-fried potato strips with the low concentration of reducing sugars

## **Effect of a technological control measure**

(LRS) were used in the second experiment while the strips with the high concentration (HRS) were used in the third experiment.

### *4.2.3 Measurements and observation data.*

Frozen par-fried potato strips were thawed for a minimum of two hours at room temperature before frying. Although frying instruction on the packaging commonly recommended not to thaw the frozen par-fried potatoes to obtain crispier French fries (Lou, 2005; Products, 2009), instruction to thaw frozen par-fried potato strips was given based on literature. Tuta and co-authors (2010) showed that a shorter frying time was needed for the thawed potato strips (using microwave thawing) than for the unthawed potato strips and the formation of acrylamide was reduced by 89% with good quality attributes of French fries. The food handlers were asked to fry three servings of French fries. During frying, the actual (initial) frying temperature once the fryer reached the set temperature (thermostat light off) was measured by a digital thermometer 51 series II coupled with a temperature probe (Fluke Corporation, Everett, USA). A built-in timer measured the actual frying time once the fryer reached the set frying time (timer goes off). Palm oil was used in all experiments. Characteristics of fryers were recorded and summarised in Table 4.1.

## Chapter 4

Table 4.1. Characteristics of the fryers.

	Chain fast-food service		Institutional caterer	
	A <sup>1</sup>	B	D	E
Type of fryer	EF1 <sup>2</sup>	EF2 <sup>3</sup>	EF3 <sup>4</sup>	GF1 <sup>5</sup>
Volume of oil, L	20 <sup>6</sup>	20 <sup>6</sup>	15	20 <sup>6</sup>

<sup>1</sup>Data from an establishment of each FSE type.

<sup>2</sup>EF1 = Electric fryer, floor model FM II (Frymaster L.L.C, Shreveport, L.A., USA). This fryer has a temperature range of 60°C to 195°C and heating capability of no more than  $\pm 2^\circ\text{C}$ .

<sup>3</sup>EF2 = Electric fryer, floor model (data on the manufacturer of fryer is not available).

<sup>4</sup>EF3 = Electric fryer, floor model HEF 77 (Hobart, London, United Kingdom).

<sup>5</sup>GF1 = Gas fryer, floor model FSGDF 23M 2B (Berjaya, Malaysia).

<sup>6</sup>This fryer has a bowl of 20L oil capacity. It was equipped with two static baskets in which only one was used in the study and a regulating thermostat.

### 4.2.4 Sampling

#### 4.2.4.1 French fries samples

The study used straight-cut French fries samples that were 8 mm x 8 mm cross section and 60 - 70 mm long. Samples were collected over three days of French fries production. Each day, samples were taken from three different frying batches. In each frying batch, a total of three servings of the French fries was collected. Of these servings, two were used to determine acrylamide concentration whereas the last serving was served to a customer. The consumer accepted French fries that were prepared in all three experiments as good and comparable sensory quality.

The determination of acrylamide concentration was done in duplicate, one measurement of acrylamide concentration for each serving. Each serving weighed approximately 67 gram, the recommended serving size (Shahar et al., 2002) and coded and stored in polyethylene bags at -18°C prior to analysis.

## Effect of a technological control measure

### 4.2.4.2 *Frozen par-fried potato strips*

Frozen par-fried potato strips samples were collected over three days of French fries preparation for analysis of reducing sugars. Samples were collected before they were used for frying. Each day, samples were taken from three different frying batches. In each frying batch, a total of two serving portions of the frozen par-fried potato strips were collected. These servings' portions were used to determine reducing sugars concentration in duplicate, one measurement of reducing sugars for each serving portion. Similarly, to French fries samples, each serving portion weighed approximately 67 gram. Each serving portion was coded and stored in polyethylene bags at -18°C prior to analysis.

### 4.2.5 *Analysis methods.*

#### 4.2.5.1 *Chemicals and stock solutions.*

Acrylamide (99%) was purchased from Fluka Chemie AG (Buchs, Switzerland).  $^{13}\text{C}_3$ -labelled acrylamide (99% isotopic purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ultra pure water was used throughout the experiments (Purelab Classic UV, Elga Labwater, Lane End, UK). Solid phase extraction cartridges (Oasis Hydrophilic-Lipophilic Balance (HLB), 3cc and Oasis Mixed-mode Cation-eXchange (MCX), 3cc) were supplied by Waters Corp (Milford, Massachusetts, USA).

Stock solutions of acrylamide (0.2 mg/mL) and  $^{13}\text{C}_3$ -labelled acrylamide (4  $\mu\text{g/mL}$ ) were prepared by dissolving the compound in distilled water. Working standards were prepared by diluting the stock solution of acrylamide to concentrations of 0.5, 5, and 10  $\mu\text{g/mL}$  with water.



## Chapter 4

All stock solution and working standards were stored in a refrigerator at 4°C for maximum of 3 months.

### 4.2.5.2 *Analysis of reducing sugars.*

Reducing sugars concentrations were determined by high-performance liquid chromatography (HPLC) as described earlier (Sanny, et al., 2012). The sample was grinded in a blender (Braun multiquik ZK3, Frankfurt, Germany). A sub-sample (2 gram) was weighed in a 50 mL centrifuge tube and 10 mL of acetonitrile/water (80:20 v/v) were added. The suspension was then centrifuged at 1643RCF (g) for 10 min and the supernatant was passed through a 0.45 µm nylon syringe filter (Sartorius AG, Goettingen, Germany). Aliquots (50 µL) of the filtrate were injected into a Waters high-performance liquid chromatography (HPLC) instrument equipped with a refractive index (R.I.) detector and an µBondapack NH<sub>2</sub>, 3.9 × 300 mm column (Waters Corp, Milford, Massachusetts, USA). An isocratic mode of elution was used with the mobile phase consisting of acetonitrile/water (80:20, v/v) at a flow rate of 0.8 mL/min. Maltose was used as an internal standard. Calibration curves were linear ( $r^2 > 0.999$ ). The detection limit for fructose and glucose was 5 mg/kg. The recoveries for fructose ranged between 91.3-100.3% and for glucose ranged between 92.9-96.7%. The intra-day and inter-day precisions, expressed as the relative standard deviation, were 4.6 and 6.7% (fructose) and 11.7 and 13.2% (glucose), respectively.

### 4.2.5.3 *Analysis of acrylamide.*

#### 4.2.5.3.1 *Sample preparation procedure.*

The scheme described by McHale and co-authors (2003) was generally followed. The sample was grinded in a blender. A sub-sample (2 gram) was weighed in a 50 mL centrifuge tube and 10 mL of water containing 500 ng<sup>13</sup>C<sub>3</sub>-labelled acrylamide as the internal standard (final

## Effect of a technological control measure

concentration = 50 ng/mL) were added. The mixture was shaken at medium speed level (ca. 256 pulses / minute) on a vertical shaker (RS-1, Jeio Tech Co., Gyeonggi-do, Korea) for 10 minutes and centrifuged in a refrigerated centrifuge (3-18K, Sigma, Gillingham Dorset, United Kingdom) at 10956 RCF (g) for 30 minutes. Approximately 2ml aliquot beneath the oil layer were taken using a syringe, filtered through a 0.22 $\mu$ m nylon syringe filter (Sartorius AG, Goettingen, Germany) and the filtrate was collected. Both Oasis HLB and Oasis MCX were conditioned with 2 mL of methanol and equilibrated with 2 mL of water. The filtrate (1.0 ml) was loaded onto Oasis HLB cartridge and was allowed to pass through and discarded. The Oasis HLB cartridge was then washed with 1.0ml of water and this eluate (eluate 1) was collected and allowed to pass through onto Oasis MCX cartridge. The eluate (eluate 2) was collected and transferred to an amber vial for HPLC-MS/MS analysis.

### 4.2.5.3.2 HPLC-MS/MS analysis.

Sample extracts and calibration standards were injected into a Thermo Scientific Surveyor HPLC system, equipped with a Thermo Scientific Hypercarb column (2.1 mm x 50 mm, 5 $\mu$ m) (ThermoFisher Scientific, San Jose, USA). The detection of acrylamide was performed on a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer, operating in positive Atmospheric Pressure Chemical Ionization (APCI+). Standards contained acrylamide at concentrations of 1, 5, 10, 25, 50, 100, 250, 500, 1000 ng/mL and isotopically labelled acrylamide at 50 ng/mL. The injection volume was 10  $\mu$ L.

Separation of acrylamide was achieved under isocratic conditions using 100% water as the mobile phase at a flow rate of 0.15 mL/min. The ion transfer capillary temperature was maintained at 250°C, vaporizer temperature at 375°C and discharge current at 5  $\mu$ A. The argon

## Chapter 4

collision gas pressure was adjusted to 1.5 mTorr for MS/MS. The collision energy was kept at 9 eV for each monitored transition in selective reaction monitoring mode (SRM). The MS/MS transitions monitored were  $m/z$  72 > 55 for acrylamide and 75 > 58 for  $^{13}\text{C}_3$ -acrylamide. The scan time for each monitored transition was kept at 0.3 s, the scan width at 0.010  $m/z$  and the Q1, Q3 resolution at 0.7  $m/z$  full width half mass (FWHM).

### 4.2.5.3.3 *Quantification.*

The transitions  $m/z$  72 > 55 for acrylamide and 75 > 58 for  $^{13}\text{C}_3$ -acrylamide were used for quantification, and  $m/z$  72 > 55, 72 > 54 and 72 > 44 were used for confirmation of the peak identity. A calibration graph was constructed by plotting the peak area of acrylamide relative to the internal standard against the corresponding ratios of analyte amounts. Acrylamide concentrations in sample extracts were calculated from the calibration slope and intercept value. Calibration curves were linear ( $r^2 > 0.999$ ). The limit of detection was at 1  $\mu\text{g}/\text{kg}$  and the recoveries were in the range between 100-108%. The intra-day and inter-day precisions, expressed as the relative standard deviation, were 4.4 and 3.9%, respectively.

### 4.2.6 *Statistical analysis.*

The original data of acrylamide concentration in each serving of French fries samples were  $\log_e$  transformed to construct histograms. The  $\log_e$  transformation was done to approximate a normal distribution of the data while retaining information about the degree of variation in the distribution (Dallal, 2009; Garner, 2010; Hopkins, 2003).

An analysis of variance (ANOVA) was used to explore the mean differences in the  $\log_e$  transformed acrylamide concentration among the reducing sugars ranges as well as between the

## Effect of a technological control measure

FSE types while also investigating the variation due to different establishments, sampling days and frying batches. A mixed model was used with reducing sugars ranges and FSE types as fixed factors, and establishment, sampling day and frying batch as random factors. The mixed model was selected because reducing sugars ranges and FSE type were used as a control factor in the present study whereas the establishments, sampling days and frying batches were not fixed in advance and their effects were therefore considered as random. The reducing sugars range was represented by the low concentration or the normal concentration or the high concentration of reducing sugars whereas the FSE type was represented by the CFS or the IC. All random factors are nested within the pre-mentioned sampling level. The significances of the differences among the three ranges of reducing sugars as well as between the two FSE types were determined using Tukey's HSD test. The obtained means of  $\log_e$  transformed acrylamide concentration were back-transformed to an original scale of measurement and these are the geometric means (Bland & Altman, 1996; Petrie & Sabin, 2009). The coefficient of variation (coded as CV) was calculated using the formula of  $100(e^{SD} - 1)$ , where SD is the standard deviation of the  $\log_e$  transformed acrylamide concentration (Dallal, 2009; Hopkins, 2003). The CV compares the degree of variation from each FSE type to another.

Bivariate correlations analysis was used to investigate the strength and direction of the relationship between reducing sugars and acrylamide concentration, which was expressed as Pearson correlation coefficient. A p-value less than or equal to 0.05 was considered significant. Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL).

## Chapter 4

### 4.3 Results

In this study, frying temperature and time were standardised for both the chain fast-food services (CFS) and the institutional caterers (IC) and were set at 177°C and 2 minutes 45 seconds (165 seconds), respectively. The actual (initial) frying temperature and frying time were recorded in all experiments to verify the accuracy of these settings (data not shown). The actual (initial) frying temperature in the two FSE types ranged between 175-177°C and the coefficient of variation (CV) in actual (initial) frying temperature was 0.2%. The actual frying time (165 seconds) in all establishments corresponded to the set frying time.

The histograms of reducing sugars of the three types of potato strips; LRS, NRS, and HRS were constructed to illustrate its observed variation. Figure 4.1 shows that the mean of reducing sugars was lower for the LRS (0.43 g/kg) than for the NRS (1.21 g/kg), and for the HRS (4.76 g/kg). The standard deviation of the mean reducing sugars concentration was 0.10 for the LRS, 0.34 for the NRS, and 1.71 for the HRS. Table 4.2 shows that the reducing sugars concentrations ranged from 0.27 to 0.63g/kg (a 2.3-fold difference) for the LRS, from 0.70 to 2.99 g/kg (a 4.3-fold difference) for the NRS, and from 2.63 to 9.81 g/kg (a 3.7-fold difference) for the HRS. The CV in reducing sugars concentration was lower for the par-fried potato strips with low concentration of reducing sugars (LRS) (22.1%) than for the strips with normal concentration (NRS) (28.2%), and for the strips with high concentration (HRS) (36.0%).

## Effect of a technological control measure

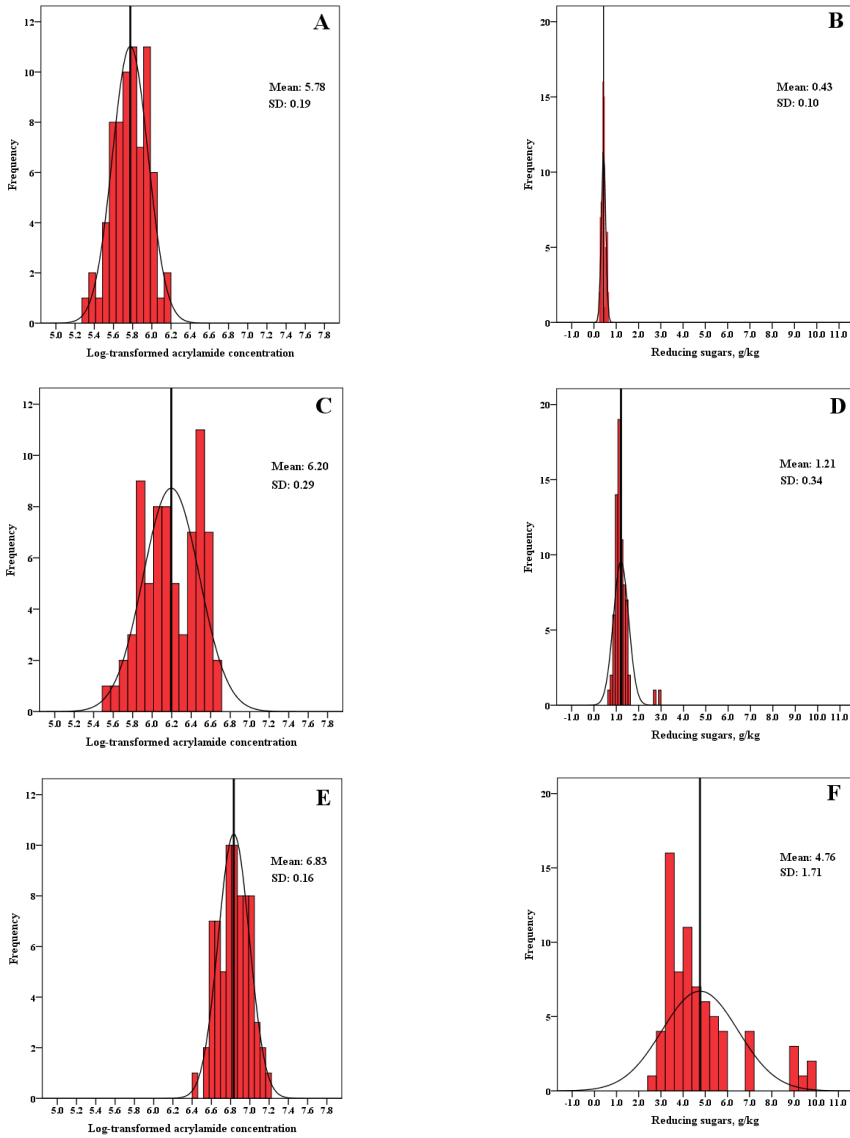


Figure 4.1. Histograms of  $\log_e$  transformed acrylamide concentrations of French fries and the corresponding histograms of reducing sugars concentration: (A) French fries using potato strips with low concentration of reducing sugars, (B) Par-fried potato strips with low concentration of reducing sugars, (C) French fries using potato strips with typical concentration of reducing sugars, (D) Par-fried potato strips with typical concentration of reducing sugars, (E) French fries using potato strips with high concentration of reducing sugars, (F) Par-fried potato strips with high concentration of reducing sugars

## Chapter 4

Table 4.2. Reducing sugars concentration of par-fried potato strips used in the CFS and the IC.

FSE types	Reducing sugars concentration, g/kg <sup>1</sup>			Mean (by FSE types)
	1 <sup>st</sup> experiment	2 <sup>nd</sup> and 3 <sup>rd</sup> experiments		
	Par-fried potato strips with normal concentration of reducing sugars (NRS)	Par-fried potato strips with low concentration of reducing sugars (LRS)	Par-fried potato strips with high concentration of reducing sugars (HRS)	
<b>Chain fast-food service</b>				
Establishment A <sup>2</sup>				
Mean <sup>3</sup> (SD <sup>4</sup> )	1.15(0.11)	0.32(0.03)	4.33(1.30)	2.05(1.94)
CV <sup>5</sup> , %	9.6	9.4	30.0	93.2
Minimum	1.00	0.27	2.63	0.27
Maximum	1.37	0.36	7.05	7.05
Range	0.37	0.09	4.42	6.78
Establishment B				
Mean (SD)	1.20(0.13)	0.41(0.03)	4.89(0.50)	
CV, %	10.8	7.3	10.2	
Minimum	0.95	0.35	4.11	
Maximum	1.44	0.46	5.79	
Range	0.49	0.11	1.68	
<b>Institutional caterer</b>				
Establishment C				
Mean (SD)	1.15(0.2)	0.43(0.02)	3.89(0.70)	2.22(2.33)
CV, %	17.4	4.7	18.0	105.0
Minimum	0.86	0.40	3.16	0.40
Maximum	1.51	0.46	5.80	9.81
Range	0.65	0.06	2.64	9.41
Establishment D				
Mean (SD)	1.33(0.63)	0.57(0.04)	5.94(2.71)	
CV, %	47.4	7.0	45.6	
Minimum	0.70	0.50	3.01	
Maximum	2.99	0.63	9.81	
Range	2.29	0.13	6.80	
Mean (by reducing sugars types)				
Mean (SD)	1.21(0.34)	0.43(0.10)	4.76(1.71)	
CV, %	28.1	23.3	36.0	
Minimum	0.70	0.27	2.63	
Maximum	2.99	0.63	9.81	
Range	2.29	0.36	7.18	

<sup>1</sup>Referring to fresh weight.

<sup>2</sup>Data from an establishment of each FSE type.

<sup>3</sup>Mean value based on N = 18.

<sup>4</sup>Standard Deviation.

<sup>5</sup>The CV was defined as the standard deviation divided by the mean, the result of which is reported as a percentage.

### Effect of a technological control measure

A  $\log_e$  transformation was done because initial data analysis indicated that the frequency distribution of acrylamide concentration was slightly skewed to the right (data not shown). Figure 4.1 illustrates that the mean of the  $\log_e$  transformed acrylamide concentration of French fries was lower for the LRS (5.78) than for the NRS (6.20) and for the HRS (6.83). The standard deviation of the (mean  $\log_e$  transformed) acrylamide concentration was 0.19 for the LRS, 0.29 for the NRS, and 0.16 for the HRS. Table 4.3 shows that there was a significant difference in acrylamide concentration among the three reducing sugars ranges ( $p < 0.05$ ). The mean acrylamide concentration of French fries was significantly lower for the LRS (322  $\mu\text{g}/\text{kg}$ ) than for the NRS (491  $\mu\text{g}/\text{kg}$ ), and for the HRS (928  $\mu\text{g}/\text{kg}$ ). The CV in acrylamide concentration was 20.9% for the LRS, 33.6% for the NRS, and 17.4% for the HRS. The acrylamide concentrations ranged from 209 to 477  $\mu\text{g}/\text{kg}$  (a 2.3-fold difference) for the LRS, from 243 to 799  $\mu\text{g}/\text{kg}$  (a 3.3-fold difference) for the NRS, and from 632 to 1357  $\mu\text{g}/\text{kg}$  (a 2.1-fold difference) for the HRS. There was no significant difference in acrylamide concentration between the two FSE types ( $p = 0.989$ ). There was also no significant difference in the interaction effect between reducing sugars and FSE types ( $p = 0.621$ ).



## Chapter 4

Table 4.3. Acrylamide concentration of French fries prepared in the CFS and the IC from the three reducing sugars types.

FSE types	Acrylamide concentration, $\mu\text{g}/\text{kg}$ <sup>1</sup>			Mean (by FSE types)
	1 <sup>st</sup> experiment	2 <sup>nd</sup> and 3 <sup>rd</sup> experiments		
	Par-fried potato strips with normal concentration of reducing sugars (NRS)	Par-fried potato strips with low concentration of reducing sugars (LRS)	Par-fried potato strips with high concentration of reducing sugars (HRS)	
<b>Chain fast-food service</b>				
Establishment A <sup>2</sup>				
Mean <sup>3,4</sup>	649(0.09)	286(0.15)	1027(0.12)	527(0.50)
(SD) <sup>5</sup>	9.9	16.7	13.1	64.7
CV <sup>6</sup> , %	655	292	985	536
Median <sup>7</sup>	508	209	865	209
Minimum	756	264	1357	1357
Maximum	248	55	492	1148
Range				
Establishment B				
Mean (SD)	413(0.15)	325(0.15)	835(0.16)	
CV, %	16.6	16.7	17.8	
Median	422	319	794	
Minimum	330	269	632	
Maximum	567	461	1096	
Range	37	192	464	
<b>Institutional caterer</b>				
Establishment C				
Mean (SD)	514(0.26)	381(0.11)	986(0.14)	528(0.48)
CV, %	30.0	11.1	15.3	61.2
Median	479	383	1009	457
Minimum	354	317	733	215
Maximum	799	477	1276	1276
Range	445	160	543	1061
Establishment D				
Mean (SD)	420(0.32)	305(0.19)	877(0.13)	
CV, %	38.0	21.5	13.9	
Median	415	300	868	
Minimum	243	215	716	
Maximum	701	421	1129	
Range	458	206	413	
Mean (by reducing sugars types)				
Mean (SD)	491(0.29) <sup>b</sup>	322(0.19) <sup>a</sup>	928(0.16) <sup>c</sup>	
CV, %	33.6	20.9	17.4	
Median	474	322	926	
Minimum	243	209	632	
Maximum	799	477	1357	
Range	556	268	725	

<sup>1</sup>Referring to fresh weight.

<sup>2</sup>Data from an establishment of each FSE type.

<sup>3</sup>The mean of  $\log_e$  transformed acrylamide concentration was back transformed to an original scale using an anti-logarithmic and expressed as geometric mean.

<sup>4</sup>Mean value based on  $N = 18$ .

<sup>5</sup>Standard Deviation (SD) of the  $\log_e$  transformed acrylamide concentration.

<sup>6</sup>The exact coefficient of variation (CV) was calculated using a formula of  $100(e^{\text{SD}} - 1)$ , where SD is the standard deviation of the  $\log_e$  transformed acrylamide concentration.

<sup>7</sup>The median of  $\log_e$  transformed acrylamide concentration in each establishment was back transformed to an original scale using an anti-logarithmic and expressed as geometric median.

Mean concentrations with different superscript within row are significant difference ( $p < 0.05$ ).

### 4.4 Discussion

The present study shows that lowering reducing sugars concentration in the par-fried potato strips is an effective control measure to reduce acrylamide concentrations in French fries prepared under standardised frying conditions in the chain fast-food services (CFS) and the institutional caterers (IC). The lowest mean acrylamide concentration in French fries was found for the par-fried potato strips with low concentration of reducing sugars (LRS) (322 µg/kg) and was significantly different ( $p < 0.05$ ) from the strips with normal (NRS) (491 µg/kg) and high concentration of reducing sugars (HRS) (928 µg/kg). There was no difference in acrylamide concentration between the two FSE types (527 µg/kg for the CFS and 528 µg/kg for the IC, Table 4.3). Our findings are consistent with studies that were conducted in controlled laboratory conditions (Fiselier & Grob, 2005; Grob, et al., 2003). For example, Fiselier and Grob (2005) reported that the average acrylamide concentration was lower (24 µg/kg) in French fries prepared from par-fried potato strips containing around 0.5 g/kg reducing sugars than strips with 1.47 g/kg sugars (118 µg/kg).

Bivariate correlations analysis was performed to investigate the strength and direction of the relationship between reducing sugars and acrylamide concentration. A strong correlation between reducing sugars concentration and acrylamide concentration ( $r = 0.758$ ,  $n = 216$ ,  $p < 0.05$ ) was obtained for the whole dataset, whereas no significant correlation between the two parameters was obtained in an earlier study in FSE (Sanny, et al., 2012). Moreover, a larger range of reducing sugars concentration (from 0.27 to 9.81 g/kg, a 36.3-fold difference, Table 4.2) was used in the present study than in the earlier study (from 0.25 to 2.22 g/kg, a 8.88-fold difference). This result implies that the differences in the concentrations of reducing sugars in real life situations must be sufficiently large to observe a strong correlation. However, the

114

## Chapter 4

Pearson correlation coefficient ( $r=0.758$ ) was much lower than the coefficient results reported in studies that were done under strict laboratory conditions. For example, Williams (2005) reported a Pearson correlation coefficient of 0.985 and Amrein and co-authors (2003) reported a coefficient of 0.993.

Unlike the obvious effect of lowering reducing sugars concentration on the mean concentration of acrylamide, no effect was observed on the variation in acrylamide concentration. Figure 4.1 shows the histograms of reducing sugars and acrylamide concentrations for the three types of potato stripes used for frying. Our data showed that the difference in the variation in reducing sugars concentration in the three types of par-fried potato strips was not reflected in the variation in acrylamide concentration in French fries. To illustrate, we observed the least variation in reducing sugars concentration in the LRS (0.27 to 0.63 g/kg, a 2.3-fold difference) and the largest variation in reducing sugars concentration in the HRS (2.63 to 9.81 g/kg, a 3.7-fold difference, Table 4.2). Nevertheless, a similar variation in acrylamide concentrations was found for the LRS (209 to 477  $\mu\text{g}/\text{kg}$ , a 2.3-fold difference) and the HRS (632 to 1357  $\mu\text{g}/\text{kg}$ , a 2.1-fold difference, Table 4.2). Apparently, other factors contribute more to the observed variation. In a previous study (Sanny, et al., 2012), we indeed found a strong correlation between frying temperature and acrylamide ( $r=0.596$ ,  $p<0.05$ ) and a small but significant correlation between frying time and acrylamide ( $r=0.104$ ,  $p<0.05$ ). It was suggested that these factors overshadowed the contribution of variation in reducing sugars to the variation in acrylamide. Possible reason for a more dominant effect of frying temperature-time than reducing sugars could be that acrylamide is sensitive to temperature changes (at low moisture contents) and its concentration increases exponentially with increasing frying temperature (De Vleeschouwer, Plancken, Van Loey, & Hendrickx, 2008; Eichner, Laible, & Wolf, 1985; Gökmen & Palazoğlu, 2008).

## Effect of a technological control measure

Fiselier and co-authors (2006) emphasised that the profile of actual frying temperature during frying is more important than that regulated by the thermostat. We did not monitor the actual temperature profile of frying oil during frying but standardisation of the frying conditions, which resulted in very small differences in initial actual frying temperature (175 to 177°C, CV=0.2%) and actual frying time (165 seconds for all FSE). However, the FSE used different frying equipment purchased from different equipment suppliers (Table 4.1), which could have resulted in differences in energy input to the potato strips. Romani and co-authors (2009) showed that diverse types of fryers give different temperature profiles, which affected the surface temperature of potato strips in a different way. Various authors discussed that the temperature-time profile of frying oil and the resulting energy input to the potato strips during frying can influence the formation of acrylamide (Gökmen, Palazoğlu, & Şenyuva, 2006; Palazoğlu & Gökmen, 2008; Romani, et al., 2009). For example, Palazoğlu & Gökmen (2008) showed that acrylamide concentration in French fries can be reduced by 58% when a temperature program was used during frying. The temperature program controlled the temperature-time profile of frying oil, i.e. setting a lower frying temperature of 150 °C for 3 min at the final stage of frying process than the starting frying temperature of 170°C for 1 min. Gökmen and co-authors (2006) discussed that a greater energy input to potato strips causes a faster drying of potato strips, so that the temperatures attained in the surface were much higher than in the core, and the formation of acrylamide mainly takes places at the surface regions. Also other factors such as portion size and oil volume of fryer (product/oil ratio) and thawing practice influence the temperature-time profile of frying oil (Fiselier, et al., 2006; Grob, et al., 2003; O'Connor, Fisk, Smith, & Melton, 2001; Taiwo, Baik, & Farinu, 2007; Tuta, et al., 2010). Apparently, these factors need to be also

## **Chapter 4**

controlled to further reduce the variation in acrylamide in French fried prepared in real food service establishment conditions.

### **4.5 Conclusion**

The present study demonstrated that lowering the concentration of reducing sugars in par-fried potato strips can be an effective control measure to reduce acrylamide concentrations in French fries prepared under standardised frying conditions in chain fast-food services and institutional caterers. However, other factors that affect the temperature-time profile of frying oil need to be strictly controlled to further reduce the variation in acrylamide in French fries prepared in real food service establishment conditions.

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## Effect of a technological control measure

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# Chapter 5

## **Effect of frying instructions to food handlers on acrylamide concentration in French fries: An explorative study**

This chapter is submitted as Sanny, M., Luning, P.A., Jinap, S., Bakker, E.J., and van Boekel, M.A.J.S. Effect of frying instructions to food handlers on acrylamide concentration in French fries: An explorative study



## Chapter 5

### Abstract

The objective of this study was to obtain insight into the effect of frying instructions on food handlers' control decisions in the restaurant type of FSE and to investigate the impact of control decisions on the concentration and variation of acrylamide in French fries. Acrylamide and reducing sugars concentrations were analysed, frying temperature and time were measured, and thawing practices were observed. The obtained results before and after instructions were compared for restaurants as a group as well as for each restaurant. The study found that frying instructions supported food handlers' decisions to start frying when the oil temperature reached 175°C, although an inconsistent effect of the instructions on the food handlers' decisions on frying time was observed. Providing instructions did not result in significant difference in the mean concentration of acrylamide for the restaurants as a group. However, data analysis for each restaurant showed that when food handlers properly followed the instructions, the mean concentration of acrylamide was significantly lower (169 µg/kg) compared to before instructions (1517 µg/kg). When food handlers did not comply with the frying instructions, this resulted even in higher mean concentrations of acrylamide after instructions. Although all started frying at the right temperature (175°C), most of them increased the frying time beyond 240 seconds to achieve crispier French fries with a final colour that fitted with their own preference. Two different strategies were discussed to overcome the non-compliance behaviour of food handlers, namely setting minimal requirements on characteristics of a commercial fryer and strict monitoring on compliance to instructions.

### 5.1 Introduction

Potato-based products such as French fries can contain high and variable concentrations of acrylamide, a probable carcinogenic compound for humans (DiNovi, 2006; Friedman, 2003; IARC, 1994). Acrylamide concentration in French fries as high as 3500 µg/kg was reported in the European Union acrylamide monitoring database (Mills, Mottram, & Wedzicha, 2009). French fries are a predominant source of dietary exposure of acrylamide because they are widely consumed (Fiselier & Grob, 2005). The high acrylamide concentrations combined with high consumption could increase the dietary exposure for an individual by a factor of 5, from the average exposure of 2.8 µg/day (Grob, 2007). Control measures to reduce acrylamide exposure should therefore be focused on food products that are widely consumed and with high acrylamide concentrations such as French fries (Grob, 2007).

Different studies done under controlled laboratory conditions established the effect of lowering the concentration of reducing sugars on reduction of acrylamide formation (Fiselier & Grob, 2005; Grob et al., 2003). A study to validate the effectiveness of lowering sugars concentrations as a control measure in real food service establishments (FSE) showed that a lower mean concentration of acrylamide was obtained using par-fried potato strips with low concentration of reducing sugars compared to commonly use potato strips (**Chapter 4**). Besides the concentration of reducing sugars, frying condition (time-temperature) is identified as a major technological factor that influences the variation in acrylamide concentration while food handler's variable and inadequate decisions to control this factor could also contribute to the large variation (**Chapter 2**). **Chapter 3** showed the differences in frying equipment (digitally controlled fryers versus simple frying pans) and frying instructions (oral instructions versus no

## Chapter 5

instructions at all) resulted in considerable differences in frying practices among food handlers in FSE and it lead to large variation and high acrylamide concentration in French fries. Various authors found indeed that the actual frying practices among FSE types can differ considerably (Al-Kahtani, 1991; Gere, 1985; Morley-John, Swinburn, Metcalf, Raza, & Wright, 2002). For example, setting of frying temperature in fast food establishments in New Zealand ranged from 175°C to 190°C whereas actual frying temperature ranged from 136°C to 233°C (Morley-John, et al., 2002). Another study showed that frying temperature in nearly 70 % of restaurants and public catering facilities in Budapest was not controlled at all (Gere, 1985). It was argued that food handlers' variable and inadequate decisions could have a considerable impact on food quality, but its effects have been scarcely studied (Luning & Marcelis, 2006, 2007, 2009).

Food handlers make decisions on out-of-tolerances situation of product properties (e.g., the golden yellow colour of French fries) and processing conditions (e.g., actual frying temperature and time, portion size, oil volume etc.) (CIAA, 2006; Luning & Marcelis, 2007, 2009). They decide to do these actions (or not) to control product properties and processing conditions; so-called 'control decisions'. Likewise, managers can control food handler's behaviour by taking decisions on their non-compliance actions (out-of-control) and by taking decisions on administrative conditions (like providing procedures, instructions, decision-support tools, training, etc.). Administrative conditions aim to direct and harmonise peoples' decision-making behaviour (Luning & Marcelis, 2007). Food handlers in restaurants, in many cases, use frying pans to fry French fries (Al-Kahtani, 1991; Sanny, Jinap, Bakker, Van Boekel, & Luning, 2012). With a frying pan, they can only decide on starting of the frying (by visually inspecting the oil to estimate its temperature) and on ending the frying (by visually checking the colour of the French

## **Effect of a managerial control measure**

fries). To our knowledge, no data exists in literature on the quantitative contribution of food handlers' decisions during frying on the concentration and variation of acrylamide. The objective of this study was to obtain insight into the effect of frying instructions on food handlers' control decisions in the restaurant type of FSE and to investigate the impact of control decisions on the concentration and variation of acrylamide in French fries. Frying instructions that specified the acceptable frying practices were provided to influence the food handlers' daily decisions to control frying conditions in this explorative study and compliance to the frying instructions was observed.

### **5.2 Materials and methods**

#### *5.2.1 Characteristics of restaurants*

The study focused on restaurants that are located within a 25 km radius of Serdang, Selangor, Malaysia. This city represents typically an urban area. Ten different restaurants with more than five workers was selected. The restaurants consisted of family style, vegetarian, and café restaurants.

#### *5.2.2 Development and implementation of instructions*

The frying instructions were developed using data from literature. Thawing for a minimum of two hours at room temperature was selected to follow normal practice in fast food establishment (Glenn et al., 1993). In addition, thawing potato strips (using microwave thawing) required a shorter frying time than unthawed potato strips and the formation of acrylamide was reduced by 89% with good quality attributes of French fries (Tuta, Palazoğlu, & Gökmen, 2010). A frying temperature of 175°C was selected because this temperature is recommended for restaurants



## Chapter 5

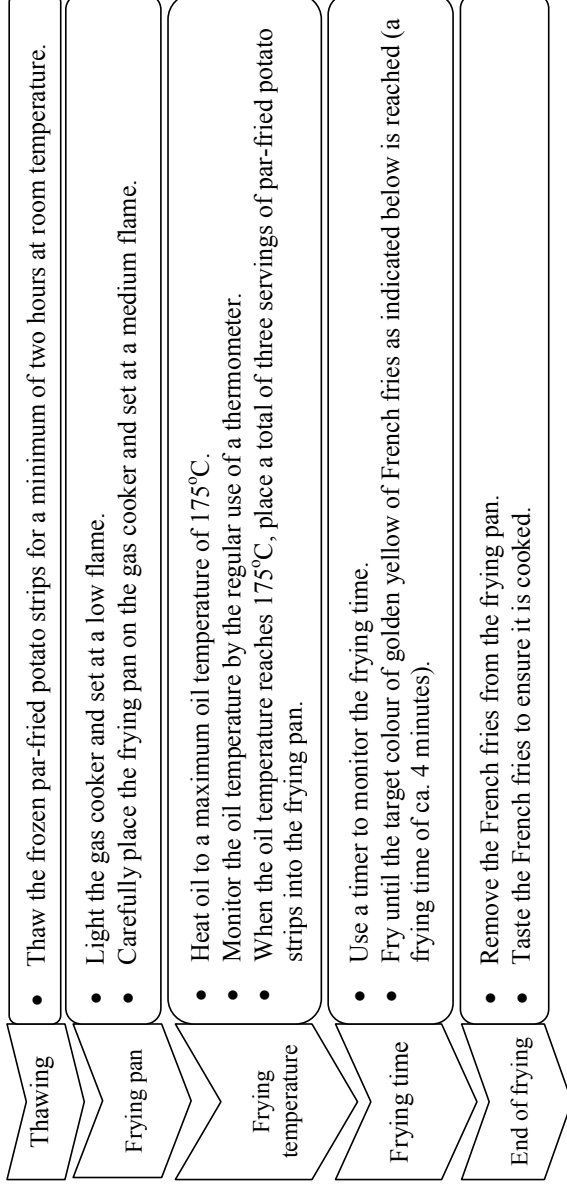
(CIAA, 2006; Fiselier, Bazzocco, Gama-Baumgartner, & Grob, 2006; Gertz & Klostermann, 2002; Williams, 2005). A frying time of 240 seconds (4 minutes) was selected because acrylamide concentration started to increase linearly with time after 4 minutes of frying (at 180°C) (Romani, Bacchiocca, Rocculi, & Rosa, 2008). Grob and co-authors (2003) optimised the preparation of French fries (of the size of 8 x 8 mm) in collaboration with cooking experts and found that a frying time of 255 seconds (4.25 minutes at 175°C) was required to achieve optimum culinary quality combined with a minimum acrylamide concentration (65 µg/kg). A colour card was included in the frying instructions to illustrate the target colour, based on the advice of the European Food and Drink Federation (CIAA) Acrylamide “Toolbox” (2006) to aim for golden yellow colour of French fries before ending the frying. The 10% rule of product/oil ratio as recommended by Grob and co-authors (2003) was not included in the frying instructions because it was found in a recent study that FSE in daily practice did not implement this rule (Sanny, et al., 2012). Therefore, the food handlers were asked to fry three servings of French fries in each frying batch before and after instructions to indicate that the obtained results were a result of the frying instructions. The food handlers had to prepare a portion that should be approximately the amount sufficient for three servings of French fries to customers. The food handlers were allowed to use the serving size and oil volume that they normally do. The actual serving size and oil volume were measured.

We verified the frying instructions in a preliminary experiment in the laboratory and the specified frying conditions indeed resulted in the target colour of golden yellow of French fries after frying time of 4 minutes at the initial temperature of 175°C. In the preliminary experiment,

### **Effect of a managerial control measure**

using a frying pan, three servings of French fries were added into 2 L of oil. Each serving weighed approximately 67 gram, i.e., the recommended serving size (Shahar et al., 2002).

The study comprised of two stages of experiments; the experiment before and the experiment after instructions. The first experiment was considered a reference, in which a food handler was asked to fry three servings of French fries as he normally does. In the second experiment, the written frying instructions (Figure 5.1) were explained to the food handlers in a training session. The frying instructions specified the following requirements: to thaw the frozen par-fried potato strips for a minimum of two hours at room temperature; to heat oil to a maximum frying temperature of 175°C; to place a total of three servings of potato strips when the oil temperature reached 175°C; to fry until the target colour of golden yellow of French fries was reached as indicated in the colour card (corresponding with a frying time of ca. 4 minutes). Besides the frying instructions, the food handler received a digital thermometer and a digital stopwatch to measure the frying temperature and time. The digital stopwatch produced a beep to alert the food handler that the frying time of 4 minutes was reached. To create awareness, the food handlers also received an explanation about the formation of acrylamide and the need to reduce exposure to acrylamide.



Yellow (undercooked)



Golden yellow



Brown (overcooked)

Figure 5.1. Scheme of frying instructions

## **Effect of a managerial control measure**

The training session started with a frying demonstration (1<sup>st</sup> frying) on how to fry the French fries following the frying instructions. Next, the food handler had to practice frying as demonstrated (2<sup>nd</sup> frying) and to reproduce it in the third frying. In the training session, the food handler was instructed to start the frying at 175°C (by regularly checking the temperature reading of a digital thermometer). The food handler was also instructed to end the frying after the target colour of golden yellow of French fries, as illustrated in the colour card embedded into the frying instructions, was achieved. In addition, the food handler was asked if he understood the frying instructions to verify that the correct frying procedure had been learned. In the next three days of French fries preparation, the food handler was instructed to reproduce the frying following the frying instructions. Ten different restaurants participated in the experiment before instructions. However, only seven out of ten restaurants participated in the experiment after instructions because three restaurants ceased their business in the course of the present study.

### *5.2.3 Measurements and observations*

In all experiments, frying temperature and time were measured and thawing practices were observed and recorded. The actual (initial) frying temperature was measured using a digital thermometer 51 series II coupled with a temperature probe (Fluke Corporation, Everett, USA), just before par-fried potato strips were inserted into the oil. The temperature probe was placed into the oil bath at the same location between different measurements and away from the frying pan's wall. The actual frying time was measured using a digital stopwatch SW01 (ALBA, Japan) just after the removal of French fries from the oil. Palm oil was used in all experiments.

## Chapter 5

### 5.2.4 *Sampling*

#### 5.2.4.1 *French fries samples*

The present study used straight-cut French fries samples that were 8 mm x 8 mm in cross section and 60-70 mm long. Frozen par-fried potato strips were used as raw materials. In all experiments, samples were collected over three days of production. Each day, samples were taken from three different frying batches. In each frying batch, a total of three servings of the French fries was collected. Of these servings, two were used to determine acrylamide concentration whereas the last serving was served to a customer. The determination of acrylamide concentration was done in duplicate, one measurement of acrylamide concentration for each serving. Each serving was coded and stored in polyethylene bags at -18°C prior to analysis.

#### 5.2.4.2 *Frozen par-fried potato strips*

In all experiments, frozen par-fried potato strips samples were collected over three days of French fries production before they were used for frying. Each day, samples were taken from three different frying batches. In each frying batch, a total of two serving portions of the frozen par-fried potato strips were collected. These servings' portions were used to determine reducing sugars concentration in duplicate, one measurement of reducing sugars for each serving portion. Each serving portion had a weight of approximately 67 gram. Each serving portion was coded and stored in polyethylene bags at -18°C prior to analysis.

### 5.2.5 *Analysis methods*

#### 5.2.5.1 *Analysis of reducing sugars*

## Effect of a managerial control measure

Reducing sugars concentrations were determined by high-performance liquid chromatography (HPLC) as described earlier (Sanny, et al., 2012). Briefly, after extraction with mobile phase consisting of acetonitrile/water (80:20, v/v) and addition of maltose as an internal standard, the supernatant was filtered and injected into a Waters HPLC instrument equipped with a refractive index (R.I.) detector and a  $\mu$ Bondapak NH<sub>2</sub>, 3.9 × 300 mm column (Waters Corp, Milford, Massachusetts, USA).

### 5.2.5.2 Analysis of acrylamide

Acrylamide was determined by LC-MS/MS as described earlier (Sanny, Jinap, Bakker, van Boekel, & Luning, Submitted for publication). Briefly, after aqueous extraction using <sup>13</sup>C<sub>3</sub>-labelled acrylamide as internal standard, the acrylamide extract was further cleaned by solid phase extraction. The extract was analysed using LC-MS/MS with positive Atmospheric Pressure Chemical Ionization (APCI+) (ThermoFisher Scientific, San Jose, USA).

### 5.2.6 Statistical analysis

The original data of acrylamide concentration in each serving of French fries samples were log<sub>e</sub> transformed to construct histograms. The log<sub>e</sub> transformation was done to approximate a more normal distribution of the data while retaining information about the degree of variation in the distribution (Dallal, 2009; Garner, 2010; Hopkins, 2003).

Three out of ten restaurants did not participate in the experiment after instructions because they ceased their business in the course of the study and were therefore excluded from data analysis. For each restaurant, the mean of log<sub>e</sub> transformed acrylamide concentration for the experiments

## Chapter 5

before and after instructions was calculated. Using the pair of these means, a paired-sample t-test was performed to analyse differences in mean acrylamide concentration in a group of restaurants before and after instructions. The obtained means of  $\log_e$  transformed acrylamide concentration were back-transformed to an original scale of measurement and these are the geometric means (Bland & Altman, 1996; Petrie & Sabin, 2009). The coefficient of variation (coded as CV) was calculated using the formula of  $100(e^{SD} - 1)$ , where SD is the standard deviation of the  $\log_e$  transformed acrylamide concentration (Dallal, 2009; Hopkins, 2003). The CV compares the degree of variation of a group of restaurants before and after instructions. The paired-sample t-test was also used to analyse differences in frying temperature, frying time and reducing sugars in a group of restaurants before and after instructions. The CV was calculated as the standard deviation divided by the mean, the result of which was reported as a percentage.

An analysis of variance (ANOVA) with a mixed model was used to analyse the pair of the means of acrylamide concentration of each restaurant before and after instructions. Experiment type (before or after instructions) was included as a fixed factor but establishment, sampling day and frying batch as random factors. The mixed model was selected because the establishments, sampling days and frying batches were not fixed in advance and their effects were therefore considered as random. All random factors were nested within the pre-mentioned sampling level. A mixed ANOVA model with the same factors was used to analyse the pair of the means of frying temperature and frying time of each restaurant. In tests, a p-value of 0.05 or less was considered significant. Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL).

### 5.3 Results

The food handlers indicated that the frying instructions were clear to them. When they did not comply with frying instructions (i.e. they extended the frying time beyond 240 seconds), the reasons for their behaviour were asked after they finished frying. The typical answers were to obtain crispier French fries and to achieve a more appropriate colour according to their own preference.

The concentrations of reducing sugars in the par-fried potato strips were measured to verify if the concentrations were within the range commonly found in practice. Table 5.1 shows that the mean concentrations of reducing sugars in potato strips was lower in the experiment with (0.97 g/kg) than without instructions (1.27 g/kg); but the difference was not significant ( $p=0.327$ ). However, the coefficient of variation (coded as CV) in reducing sugars concentration was higher in the experiment with (79.4%) than without instructions (51.2%).

Table 5.2 shows that the mean of actual frying temperature for restaurants as a group was lower after (176°C) than before instructions (185°C), but the difference was not significant ( $p=0.113$ ). The CV in actual frying temperature was also lower after (0.68%) than before instructions (7.5%). The actual frying temperature for restaurants as a group ranged from 175 to 185°C after instructions and from 145 to 235°C before instructions. When analysing data for each restaurant, the mean of actual frying temperature for restaurant A, C, D, E and F was significantly lower ( $p<0.05$ ) after than before instructions whereas in restaurant G, it was significantly higher. There was no significant difference in the mean of actual frying temperature for restaurant B.



Table 5.1. Reducing sugars concentrations of par-fried potato strips used by different restaurants before and after instructions.

	Restaurant										Total <sup>2</sup>
	A <sup>1</sup>	B	C	D	E	F	G	H	I	J	
<b>Experiment before instructions</b>											
Reducing sugars, g/kg <sup>3</sup>	1.47(0.21)	1.28(0.16)	2.46(0.30)	1.00(0.17)	1.61(0.15)	0.68(0.06)	0.53(0.15)	1.00(0.08)	1.31(0.14)	1.49(0.19)	1.27(0.65)
CV <sup>5</sup> , %	14.3	12.5	12.2	17.0	9.3	8.8	28.3	8.0	10.7	12.8	51.2
Minimum	1.21	0.81	1.69	0.76	1.28	0.58	0.33	0.91	1.08	1.21	0.33
Maximum	1.94	1.38	2.80	1.27	1.84	0.77	0.80	1.19	1.54	1.78	2.80
Range	0.73	0.57	1.11	0.51	0.56	0.19	0.47	0.28	0.46	0.57	2.47
<b>Experiment after instructions</b>											
Reducing sugars, g/kg	0.12(0.01)	0.48(0.06)	1.56(0.05)	0.78(0.11)	2.40(0.12)	0.09(0.03)	0.56(0.10)	ND <sup>6</sup>	ND	ND	0.97(0.77)
CV, %	8.3	12.5	3.2	14.1	5.0	33.3	17.9				79.4
Minimum	0.10	0.41	1.48	0.58	2.23	0.06	0.37				0.06
Maximum	0.15	0.58	1.66	0.99	2.64	0.16	0.68				2.64
Range	0.05	0.17	0.18	0.41	0.41	0.10	0.31				2.58

<sup>1</sup>Data from a different restaurant.

<sup>2</sup>Mean of data from seven restaurants.

<sup>3</sup>Referring to fresh weight.

<sup>4</sup>Mean value based on N = 18.

<sup>5</sup>The coefficient of variation (CV) was defined as the standard deviation (SD) divided by the mean, the result of which is reported as a percentage. It compares the degree of variation from each restaurant to another.

<sup>6</sup>ND = no data.

Table 5.2. Actual frying temperature of different restaurants before and after instructions.

	Restaurant										Total <sup>2</sup>
	A <sup>1</sup>	B	C	D	E	F	G	H	I	J	
<b>Experiment before instructions</b>											
Actual frying temperature, °C	185(9)	179(22)	185(7)	189(24)	208(18)	188(8)	161(7)	186(10)	192(13)	187(14)	185(13.9)
Mean <sup>3</sup> (SD)	4.9	12.3	3.8	12.7	8.7	4.3	4.3	5.4	6.8	7.5	7.5
CV <sup>4</sup> , %	170	148	174	145	175	178	148	178	166	175	145
Minimum	200	210	195	218	235	200	170	210	210	225	235
Maximum	30	62	21	73	60	22	22	32	44	50	90
Range											
<b>Experiment after instructions</b>											
Actual frying temperature, °C	176(1.9)*	175(0.4)	176(1.0)*	176(0.7)*	179(2.6)*	175(0.3)*	175(0.5)*	ND <sup>5</sup>	ND	ND	176(1.2)
Mean (SD)	1.1	0.2	0.6	0.4	1.5	0.2	0.3				0.68
CV, %	175	175	175	175	176	175	175				175
Minimum	180	176	178	177	185	176	176				185
Maximum	5	1	3	2	9	1	1				10
Range											

<sup>1</sup>Data from a different restaurant.

<sup>2</sup>Mean of data from seven restaurants.

<sup>3</sup>Mean value based on N = 9.

<sup>4</sup>The coefficient of variation (CV) was defined as the standard deviation (SD) divided by the mean, the result of which is reported as a percentage. It compares the degree of variation from each restaurant to another.

<sup>5</sup>ND = no data.

\*The mean difference within column is significant at the 0.05 level.

## Chapter 5

Table 5.3 shows that the mean of actual frying time for restaurants as a group was longer after (386 seconds) than before instructions (365 seconds), but the difference was not significant ( $p=0.654$ ). The CV in actual frying time was higher after (27.2%) than before instructions (19.9%). The actual frying time for restaurants as a group ranged from 240 to 644 seconds after instructions and from 221 to 694 seconds before instructions. When analysing data for each restaurant, the mean of actual frying time for restaurant E and F was significantly lower ( $p<0.05$ ) after instructions than before instructions whereas in restaurant A, D, and G, it was significantly higher. There was no significant difference in the mean of actual frying time for restaurants B and C. Four out of seven restaurants did thaw the frozen par-fried potato stripes before instructions and after instructions; all restaurants thawed the frozen par-fried potato stripes for a minimum of two hours at room temperature.

The actual serving size and oil volume were measured to obtain insight into these frying practices in restaurants, and the actual portion size and product/oil ratio were calculated. Table 5.4 shows that the mean portion size (which was used for the three servings) was 435 gram after instructions and 423 gram before instructions, whereas the serving size ranged from 91 to 233 gram after instructions and from 70 to 213 gram before instructions. The oil volume ranged from 1.5 L to 15 L for both the experiment after and before instructions. The mean product/oil ratio was 9.5 % after instructions and 9.2 % before instructions.

Table 5.3. Actual frying time and thawing practice of different restaurants before and after instructions.

	Restaurant										Total <sup>2</sup>
	A <sup>1</sup>	B	C	D	E	F	G	H	I	J	
<b>Experiment before instructions</b>											
Actual frying time, second	477(112)	477(112)	403(28)	361(42)	368(84)	397(72)	264(10)	244(27)	276(30)	221(32)	365(72.6)
Mean <sup>3</sup> (SD)	285(64)	285(64)	403(28)	361(42)	368(84)	397(72)	264(10)	244(27)	276(30)	221(32)	365(72.6)
CV <sup>4</sup> , %	22.5	23.5	7.0	11.6	22.8	18.1	3.8	11.1	10.9	14.5	19.9
Minimum	222	322	361	305	221	262	244	200	224	170	221
Maximum	435	694	456	421	510	480	276	271	306	270	694
Range	213	372	95	116	289	218	32	71	82	100	524
Thawing	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No	Four out of seven restaurants
<b>Experiment after instructions</b>											
Actual frying time, second	478(47)	478(47)	384(38)	519(58)*	271(17)*	262(25)*	319(46)*	ND <sup>5</sup>	ND	ND	386(105)
Mean (SD)	473(89)*	473(89)*	384(38)	519(58)*	271(17)*	262(25)*	319(46)*	ND <sup>5</sup>	ND	ND	386(105)
CV, %	18.8	9.8	9.9	11.2	6.3	9.5	14.4				27.2
Minimum	372	423	327	425	255	240	240				240
Maximum	644	569	451	630	312	320	370				644
Range	272	146	124	205	57	80	130				404
Thawing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	ND <sup>5</sup>	ND	ND	All restaurants practice thawing

<sup>1</sup>Data from a different restaurant.

<sup>2</sup>Mean of data from seven restaurants.

<sup>3</sup>Mean value based on N = 9.

<sup>4</sup>The coefficient of variation (CV) was defined as the standard deviation (SD) divided by the mean, the result of which is reported as a percentage. It compares the degree of variation from each restaurant to another.

<sup>5</sup>ND = no data.

\*The mean difference within column is significant at the 0.05 level.

Table 5.4. Actual serving size, portion size, oil volume, product/oil ratio of different restaurants before and after instructions.

	Restaurant										Total <sup>2</sup>	
	A <sup>1</sup>	B	C	D	E	F	G	H	I	J		
<b>Experiment before instructions</b>												
Serving size, gram												
Mean <sup>3</sup> (SD)	138(23.4)	187(25.1)	158(12.7)	85(10.5)	153(16.5)	141(27.4)	129(11.1)	133(15.0)	90(12.3)	101(4.6)	139(32.8)	
CV <sup>4</sup> , %	17.0	13.4	8.0	12.4	10.8	19.4	8.6	11.3	13.7	14.5	23.6	
Minimum	92	152	131	70	128	85	109	111	67	70	70	
Maximum	178	262	184	109	184	193	153	170	109	127	213	
Range	86	110	53	39	56	108	45	59	42	57	143	
Portion size (for three servings), gram												
Mean <sup>5</sup> (SD)	415(62)	561(68)	473(36)	254(27)	459(43)	415(64)	387(28)	400(38)	271(31)	303(32)	423(99.6)	
CV <sup>4</sup> , %	15.0	12.1	7.6	10.2	9.4	15.4	7.2	9.5	11.4	10.6	23.5	
Minimum	345	483	425	221	405	298	357	359	219	248	221	
Maximum	526	713	538	301	527	490	446	480	315	348	713	
Range	181	230	113	80	122	192	89	121	96	100	492	
Oil volume <sup>6</sup> , L	2	5	2.5	1.5	3	3	15	8	2	4	4.6	
Product/oil ratio, %	20.8	11.2	18.9	16.9	15.3	13.8	2.6	5.0	13.6	7.6	9.2	
<b>Experiment after instructions</b>												
Serving size, gram												
Mean (SD)	147(7.3)	166(20.6)	123(12.1)	127(16.5)	156(17.9)	174(26.1)	121(14.2)	ND <sup>7</sup>	ND	ND	145(26.3)	
CV, %	5.0	12.4	9.8	13.0	11.5	15.0	11.7				18.1	
Minimum	131	137	92	98	123	135	91				91	
Maximum	156	219	138	150	189	233	147				233	
Range	25	81	46	52	66	98	57				142	
Portion size (for three servings), gram												
Mean (SD)	442(20)	499(58)	368(22)	381(37)	468(45)	523(73)	364(34)	ND	ND	ND	435(74)	
CV, %	4.5	11.6	6.0	9.7	9.6	14.0	9.3				17.0	
Minimum	401	429	336	318	405	454	314				314	
Maximum	460	625	395	443	541	677	415				677	
Range	59	196	59	125	136	223	101				363	
Product/oil ratio, %	22.1	10.0	14.7	25.4	15.6	17.4	2.4	ND	ND	ND	9.5	

<sup>1</sup>Data from a different restaurant.

<sup>2</sup>Mean of data from seven restaurants.

<sup>3</sup>Mean value based on N = 27.

<sup>4</sup>The coefficient of variation (CV) was defined as the standard deviation (SD) divided by the mean, the result of which is reported as a percentage.

<sup>5</sup>Mean value based on N = 9.

<sup>6</sup>Data for both experiments.

<sup>7</sup>ND = no data.

## Effect of a managerial control measure

Figure 5.2 illustrates the variation profiles of acrylamide concentrations for restaurants as a group after and before instructions. The  $\log_e$  transformation was performed because initial data analysis indicated that the frequency distribution of acrylamide concentration was slightly skewed to the right (data not shown). The mean of  $\log_e$  transformed acrylamide concentration of French fries was 6.42 after and 6.39 before instructions and the standard deviation was lower after instructions (i.e. 0.85 after and 0.91 before).

Table 5.5 shows that there was no significant difference in the mean concentration of acrylamide for restaurants as a group ( $p=0.93$ ). The mean concentration of acrylamide was higher (614  $\mu\text{g}/\text{kg}$ ) after than before (596  $\mu\text{g}/\text{kg}$ ) instructions, but the CV in acrylamide concentration was lower after (134%) than before instructions (148%). The acrylamide concentration ranged from 85 to 3369  $\mu\text{g}/\text{kg}$  after instructions and from 77 to 4283  $\mu\text{g}/\text{kg}$  before instructions. When analysing data for each restaurant, it appeared that the mean concentration of acrylamide for restaurant E was significantly lower ( $p<0.05$ ) after (169  $\mu\text{g}/\text{kg}$ ) than before instructions (1517  $\mu\text{g}/\text{kg}$ ). The mean concentrations of acrylamide after instructions for restaurant B, C and F were significantly higher (715  $\mu\text{g}/\text{kg}$ , 961  $\mu\text{g}/\text{kg}$ , and 239  $\mu\text{g}/\text{kg}$ ) than before instructions (271  $\mu\text{g}/\text{kg}$ , 506  $\mu\text{g}/\text{kg}$ , and 134  $\mu\text{g}/\text{kg}$ , respectively). For restaurants A, D, and G, no significant difference in acrylamide concentration between the experiments were found.

## Chapter 5

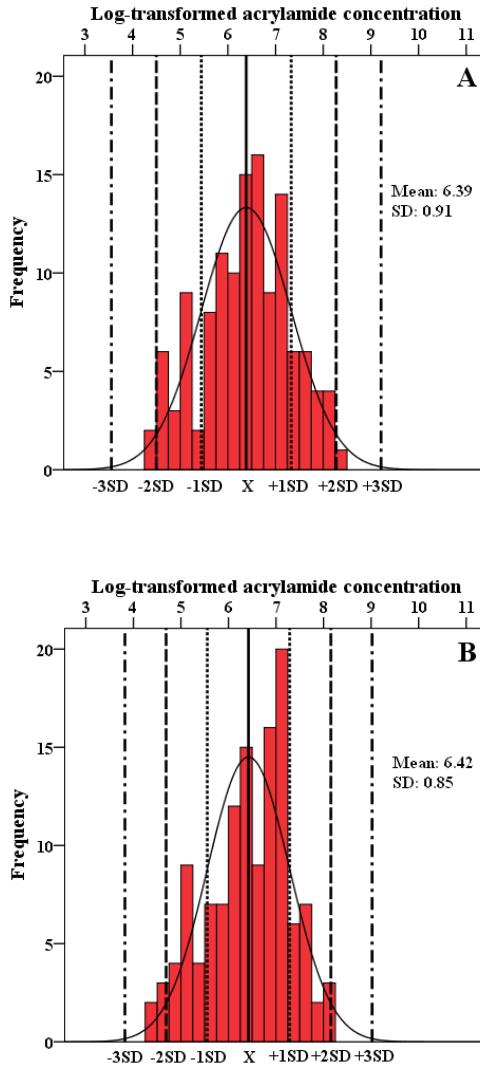


Figure 5.2. Histograms of  $\log_e$  transformed acrylamide concentration (A) Experiment before instructions (B) Experiment after instructions.

Effect of a managerial control measure

Table 5.5. Acrylamide concentrations of French fries prepared by different restaurants before and after instructions.

	Restaurant										Total <sup>2</sup>	
	A <sup>1</sup>	B	C	D	E	F	G	H	I	J		
<b>Experiment before instructions</b>												
Acrylamide, µg/kg <sup>3</sup>												
Mean <sup>4,5</sup> (SD) <sup>6</sup>	541(0.28)	271(0.38)	506(0.25)	1353(0.69)	1517(0.51)	134(0.42)	1271(0.20)	286(0.14)	439(0.37)	181(0.34)	596(0.91)	
CV <sup>7</sup> , % <sup>8</sup>	32.3	46.2	28.4	99.4	66.5	52.2	22.1	15.0	44.8	40.5	148	
Median	558	272	513	1285	1522	132	1226	280	426	187	580	
Minimum	325	152	290	506	796	77	824	220	249	90	77	
Maximum	787	562	749	4283	3816	329	1814	345	723	311	4283	
Range	462	410	459	3777	3020	252	990	125	483	221	4206	
<b>Experiment after instructions</b>												
Acrylamide, µg/kg												
Mean (SD)	565(0.22)	715(0.40) <sup>*</sup>	961(0.26) <sup>*</sup>	1633(0.53)	169(0.49) <sup>*</sup>	239(0.36) <sup>*</sup>	1296(0.21)	ND <sup>9</sup>	ND	ND	614(0.85)	
CV, %	24.6	49.2	29.7	69.9	63.2	43.3	23.4				134	
Median	545	636	989	1,914	160	242	1288				657	
Minimum	406	364	593	537	85	125	890				85	
Maximum	954	1346	1478	3369	372	414	1964				3369	
Range	548	982	885	2832	287	289	1074				3284	

<sup>1</sup>Data from a different restaurant.

<sup>2</sup>Mean of data from seven restaurants.

<sup>3</sup>Referring to fresh weight.

<sup>4</sup>The mean of log<sub>e</sub> transformed acrylamide concentration in a restaurant was back transformed to an original scale using an anti-logarithmic and expressed as geometric mean.

<sup>5</sup>Mean value based on N = 18.

<sup>6</sup>Standard Deviation (SD) of the log<sub>e</sub> transformed acrylamide concentration.

<sup>7</sup>The exact coefficient of variation (CV) was calculated using a formula of 100(e<sup>SD</sup> - 1), where SD is the standard deviation of the log<sub>e</sub> transformed acrylamide concentration. It compares the degree of variation from each restaurant to another.

<sup>8</sup>The median of log<sub>e</sub> transformed acrylamide concentration in each establishment was back transformed to an original scale using an anti-logarithmic and expressed as geometric median.

<sup>9</sup>ND = no data.

<sup>\*</sup>The mean difference within column is significant at the 0.05 level.



## Chapter 5

### 5.4 Discussion

To our knowledge, this is one of the first studies that quantified the effect of frying instructions on food handlers' control decisions on food quality. After instructions, a lower mean frying temperature (176°C) with smaller variation (0.68%) was found compared to before instructions (Mean: 185°C; CV: 7.5%), although the differences in the means of frying temperature and time for the restaurants as a group were not significant. On the contrary, a longer mean frying time (386 seconds) with larger variation (27.2%) was found after instructions compared to before instructions (Mean: 365 seconds; CV: 19.9%). When analysing data for each restaurant, we found the mean frying temperature in all restaurants (except in restaurant G) to be significantly lower after instructions than before instructions (Table 5.2). Apparently, the instructions and measuring equipment supported food handlers' decisions to start frying when oil reached 175°C, but an inconsistent effect of the instructions on the food handlers' decisions to end frying was observed. For two restaurants (restaurant E and F) the mean frying time was significantly lower after instructions than before instructions, but for the other three restaurants (restaurant A, D and G) it was significantly higher; no significant differences were found for restaurant B and C (Table 5.3).

Data showed that the maximum concentration was 3369 µg/kg after providing instructions as compared to 4283 µg/kg before instructions. The concentrations reported in our study are consistent with the acrylamide monitoring database of Institute for Reference Materials and Measurements (2006), which found acrylamide concentration as high as 4653 µg/kg from approximately 1377 samples of French fries. We found no significant difference in the mean concentrations of acrylamide for the restaurants as a group before and after instructions.

### **Effect of a managerial control measure**

Nevertheless, when analysing data for each restaurant, we found significant differences in the means for four restaurants; in one (restaurant E) the mean concentration after instructions was lower whereas in the others (restaurant B, C and F) it was higher. The food handler in restaurant E indeed controlled the frying conditions as instructed and attained a significantly lower mean frying temperature (179°C) and frying time (271 seconds) compared to the reference situation (208°C, 368 seconds). As expected, the mean concentration of acrylamide was significantly lower after instructions (169 µg/kg) than before instructions (1517 µg/kg). However, food handlers in the other restaurants (restaurant A, D and G) increased the frying time much longer than the instructions and they fried French fries until the colour of their own preference to obtain crispier French fries. For example, the mean frying time in restaurant D was 519 seconds and ranged from 425 to 630 seconds, which indicates that they applied longer frying times than the prescribed 240 seconds. The mean concentration of acrylamide in restaurant D was indeed higher after instructions (1633 µg/kg) than before instructions (1353 µg/kg), although the difference was not significant ( $p=0.711$ ). Romani and co-authors (2008) reported that acrylamide concentration increased exponentially with increasing of frying time after circa 240 seconds of frying (at 180°C). Although the actual frying temperature was controlled at 175°C in the restaurants, the longer frying times than prescribed in the instructions resulted in higher acrylamide concentrations (but lower variation in the dataset). This is possibly because of the exponential character of the relationship between formation of acrylamide and frying time. Apparently after the critical time of 240 seconds, a small increase in frying time can lead to a large increase in the concentration of acrylamide.

## Chapter 5

The inconsistent compliance to instructions remains a challenge, although training was given to instruct the food handlers on how to fry the French fries, and they were made aware of the need to comply with the instructions. It seems difficult to change the frying behaviour because the food handlers tended to have a different preference of target colour, even though a colour card was included in the frying instructions to illustrate the target colour of golden yellow of French fries. Food handlers seem to maintain their normal habit and they use their own target colour. Our observations are consistent with different authors who suggested that food handlers in restaurants tend to develop individual preparation techniques to meet their preferences of target colour of French fries (Haase, 2006; Vinci et al., 2011). Another reason might be that food handlers were not yet familiar with frying instructions, although they practiced frying in the training session to create familiarity. Different authors emphasised the need to create familiarity because when new information becomes available, it must first trigger attention, then achieve comprehension, and only then it can influence decision-making (Breakwell, 2000; Wilcock, Pun, Khanona, & Aung, 2004).

Two different strategies, i.e., one emphasising a managerial and one a technological approach may overcome the problem of non-compliance behaviour of the food handlers in the FSE. Seaman and Eves (2010) discussed that feedback from supervisors encourages safe food handling practices among food handlers in real work situations. Other authors also emphasised that feedback through on-going supervision is necessary to correct any non-compliance actions, besides demonstration and practice in a training program (Palmen, Didden, & Korzilius, 2010; Rennie, 1994; Seaman & Eves, 2008). Seaman and Eves (2008) showed that food handlers' perception of social normative pressures (due to the on-going supervision) significantly

## **Effect of a managerial control measure**

influenced their intention to perform food safety behaviour. Supports from the manager/supervisor, in terms of motivation of staff and commitment to safe working practices, are also required in the training program in order to have a greater effect on intention and actual behaviour of the food handler (Seaman & Eves, 2006).

A technological approach is to set minimal requirements on characteristics of commercial fryers, for example that it is equipped with a temperature controller and a timer, and automatic lifting of the basket from the oil once the fryer reaches set frying time. These technological features support food handlers in taking control decisions away from them to facilitate behaviour change. Inadequate equipment (frying facility) is certainly one of the barriers that would prevent food handlers from complying with instructions (Clayton, Griffith, Price, & Peters, 2002; Quaglia, Comendador, & Finotti, 1998; Sanny, Luning, Marcelis, Jinap, & van Boekel, 2010; Seaman & Eves, 2006). It is perhaps the responsibility of health authorities to set rules for dedicated fryers to be used in restaurants instead of frying pans to fry French fries and to conduct regular inspection to ensure that restaurants comply with this requirement.

## **5.5 Conclusion**

The present study showed that frying instructions supported food handlers' decisions to start frying when oil reached 175°C, but an inconsistent effect of the instructions on the food handlers' decisions to end frying was observed. The impact of frying instructions on the mean and extent of variation in acrylamide concentration was not consistent because of the inconsistent compliance to the frying instructions for which food handlers were trained. The majority of the food handlers exceeded the prescribed frying time because they fried the French

## **Chapter 5**

fries until the colour and crispiness were in line with their own preference. When instructions were followed strictly, the acrylamide concentration was significantly lowered. However, in case of non-compliance, even higher means acrylamide concentrations were found compared to before instructions.

### **5.6 Acknowledgments**

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## **Effect of a managerial control measure**

**Chapter**

**6**

**General Discussion**



## Chapter 6

### 6.1 Background of the research

Control measures to reduce the concentration of acrylamide in French fries are of importance because acrylamide is known as a probable human carcinogen and French fries are a predominant source of dietary intake of acrylamide (Fiselier & Grob, 2005; IARC, 1994). Especially the final preparation of French fries in food service establishment (FSE) needs to be controlled because acrylamide is formed towards the end of the frying process (Amrein, Andres, Escher, & Amadó, 2007; Fiselier, Bazzocco, Gama-Baumgartner, & Grob, 2006). The Joint FAO/WHO Expert Committee of Food Additives (2005) advised to put appropriate efforts to reduce acrylamide concentration in food to protect human health. However, they identified the large variation in acrylamide concentrations as one of the constraints in developing control measures (FAO/WHO, 2007). Capuano & Fogliano (2011) also stated that the large variation complicates the estimation of actual dietary exposure to acrylamide. Possible factors contributing to the significant variation need to be researched (FAO/WHO, 2007) and first understood before effective control measures can be developed.

A number of control measures was proposed and tested in controlled laboratory conditions (Fiselier & Grob, 2005; Ishihara, Matsunaga, Nakamura, Sakuma, & Koga, 2006; Palazoğlu & Gökmen, 2008). Different authors proposed that the results of these studies could differ from field studies (De Graaf et al., 2005; Snyder & Matthews, 1983) and they must be validated under actual food processing conditions (CIAA, 2006; Stadler, 2005). Although French fries are widely prepared in FSE, data on the effects of control measures in a real situation of French fries preparation are limited (Stadler, 2005; Taeymans et al., 2004) compared to the data available for industrial production (Vinci, Mestdagh, de Muer, van Peteghem, & de Meulenaer, 2010; Vinci et

al., 2011). In the present thesis, a techno-managerial research approach was used to investigate both technological and managerial causes of variation. Also, the effects of technological as well as managerial control measures on the concentration and variation of acrylamide in the real preparation of French fries in-practice in FSE were examined. This chapter discusses the main findings of the research described in this thesis and positions them in a broader perspective. The findings from the qualitative study (**Chapter 2**) that provided insight into technological and managerial causes of variation were used to develop a conceptual model. The results of the observational study (**Chapter 3**) and intervention studies (**Chapter 4 – 5**) were evaluated to establish the role of technological and managerial factors, to show that these factors are interacting and to test their relationships that were assumed to contribute to the variation in acrylamide concentration. The advantages and challenges of the techno-managerial research approach and the introduced control measures are discussed and future research efforts are recommended.

## 6.2 Main findings

### 6.2.1 Factors contributing to variation in acrylamide concentration

**Chapter 2** describes a qualitative study in which literature was analysed to identify the major product properties and processing conditions (i.e., the technological factors), and their possible contribution to variation in acrylamide concentrations. A ‘food quality decision model’ (Figure 1.1), which was introduced in Chapter 1, was used to analyse systematically the decisions of managers and food handlers (i.e., the managerial factors) at critical points of control in the preparation of French fries. The findings of the qualitative study were incorporated into the food quality decision model that was used as a basis to develop a conceptual model (Figure 6.1). The

## Chapter 6

conceptual model contains the typical processes in the preparation of French fries in FSE that result in the outcome factor, i.e., variation in acrylamide concentration in French fries. It also contains the technological and managerial factors that influence the processes as well as the relationship between all elements that are assumed to contribute to the outcome.

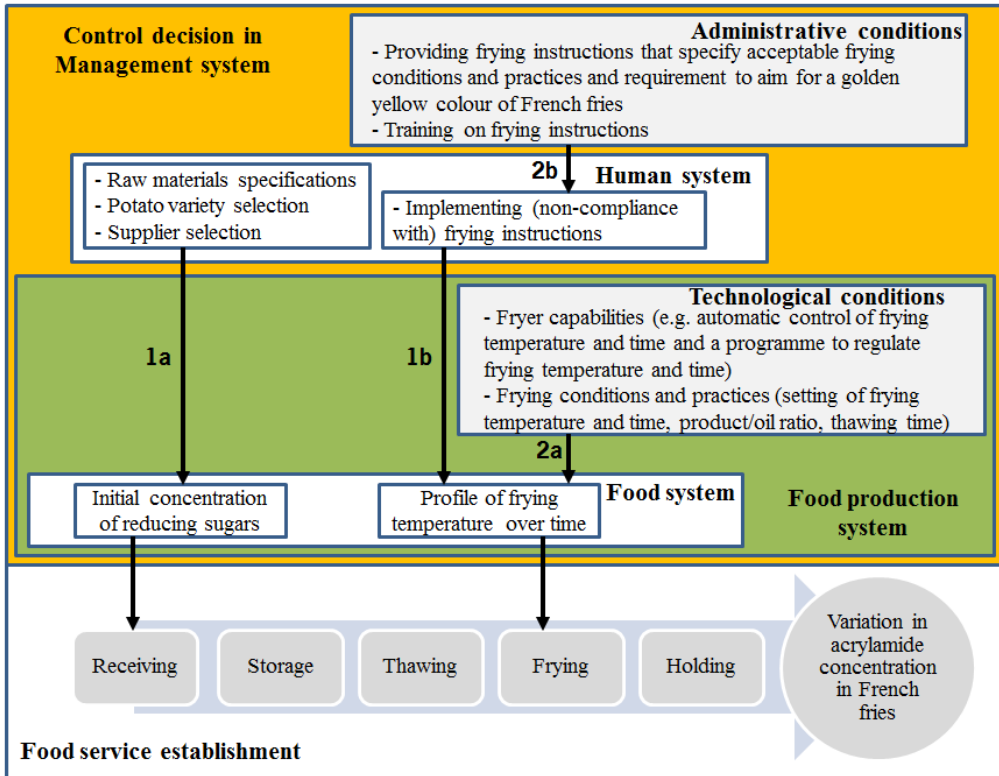


Figure 6.1. A conceptual model of the technological and managerial causes of variation in acrylamide concentration in French fries prepared in FSE. (1a) control decisions on food dynamics, (1b) control decisions on human dynamics, (2a) control decisions on technological conditions affecting food dynamics and (2b) control decisions on administration conditions affecting human dynamics.

Figure 6.1 shows the technological and managerial causes of variation and how they interact and contribute to variation in acrylamide concentration in French fries prepared in FSE. Technological causes of variation include those that relate to the variation in product properties (product factor) and the variation in technological conditions, such as equipment and process conditions (process factor). Managerial causes of variation refer to variation in (daily) control decisions that aim at keeping the product and the process status within acceptable tolerances by measuring, comparing, and taking corrective actions if necessary. Similarly, it aims at keeping peoples' actual behaviour (e.g. executing certain tasks) within certain desired tolerances (Luning & Marcelis, 2007, 2009). Managers are usually the owner of a FSE, and food handlers are the ones who do the actual frying in a FSE, they daily take control decisions. Managers typically take decisions on resources (also called resource control), such as recruiting people with adequate competences, purchasing appropriate equipment, selecting specific suppliers, providing procedures and instructions, etc. Food handlers typically take decisions on out-of-tolerance situations of product properties and process conditions (also called product and process controls), such as reducing frying time when frying a smaller portion of par-fried potato strips (CIAA, 2006; Luning & Marcelis, 2007, 2009). Figure 6.1 shows the control decisions of managers and food handlers in daily operating conditions in FSE (managerial factors), and how they affect the product properties and process conditions (technological factors) that contribute to variation. More specifically, manager's decisions to select a certain potato variety, or a specific supplier, or define strict raw materials specifications (indicated as '1a' in Figure 6.1). These decisions determine the actual concentration and variation of reducing sugars in the incoming raw materials. Similarly, manager's decisions to select certain frying equipment, to specify frying

160



## Chapter 6

conditions ('2a') and to provide specific frying instructions and training ('2b') influence the profile of frying temperature over time. Likewise, food handler's decisions to control frying conditions as instructed (or not) ('1b') affect the profile of frying temperature over time as well. It was concluded from the study (**Chapter 2**) that the variation in initial concentration of reducing sugars and the variable frying conditions (time-temperature) are the major technological factors that can influence the variation in acrylamide concentration. Consequently, the receipt of raw materials and the frying practices were identified as crucial points of control to lower acrylamide concentration and to reduce its variation. These findings are consistent with Cummins and co-authors (2009) who concluded, based on Monte Carlo simulations, that low reducing sugar concentrations as well as strict control of frying conditions in preparation of French fries are most crucial.

The current situation in FSE was examined (**Chapter 2**) and showed that manager's decision to control the appropriate supplies and suppliers (indicated as '1a') is restricted because potatoes with low concentration of reducing sugars are not widely available (Fiselier & Grob, 2005). Manager's decision to select appropriate fryers ('2a') is also restraint because fryers normally used and available for FSE have no features for regulating a temperature profile that is needed to reduce acrylamide formation (Fiselier, et al., 2006). However, managers can decide to select fryers equipped with automatic control of frying temperature and time because this type of fryer is nowadays widely available and used in chain fast-food services (Al-Kahtani, 1991; Sanny, Jinap, Bakker, Van Boekel, & Luning, 2012). Managers can also decide to provide procedures and instructions to support food handler's daily decisions. Procedures on standardised control of frying conditions, for example, require food handlers to avoid frying above 175°C and to take

corrective actions such as reducing frying time when frying smaller portion of frozen par-fried potato strips (CIAA, 2006). The actual compliance to food safety practices is however affected by various factors such as training, motivation, adequate resources and an appropriate management culture can limit the actual compliance to food safety practices (Clayton, Griffith, Price, & Peters, 2002; Griffith, 2006). These factors are expected to affect food handler's daily decisions on setting of frying conditions and controlling them between acceptable tolerances (indicated as '1b'). Food handler's variable and inadequate decisions in controlling the frying conditions are major managerial factors that could affect the variation in acrylamide concentrations. Researchers commonly dedicate causes of variation in acrylamide concentration to technological factors such as frying time-temperature regimes and concentrations of reducing sugars and amino acids (Birlouez-Aragon, Morales, Fogliano, & Pain, 2010; Boon et al., 2005). We have indicated in Figure 6.1 that managerial factors also contribute to significant variation. The finding directed us to investigate how these factors and practices contribute to the actual variation in acrylamide concentration during a preparation of French fries in real FSE-conditions in an observational study in **Chapter 3**.

**Chapter 3** describes an observational study to obtain insight into the actual variation in acrylamide concentrations in French Fries and to investigate the contribution of technological and managerial factors to the actual variation in acrylamide concentrations. The actual practices at receiving, thawing and frying (processes in the preparation of French fries described in Figure 6.1) were observed to get an insight into the typical frying practices in three FSE types, i.e., chain fast-food services (CFS), institutional caterers (IC), and restaurants (R). The study found that the least variation in acrylamide concentration was found in French fries prepared in the

## Chapter 6

CFS compared to the IC and the R, although the mean concentration of acrylamide among the three FSE types was not significantly different. The mean concentration of acrylamide was 231 µg/kg for the CFS, 254 µg/kg for the IC, and 354 µg/kg for the R. In addition, the highest concentration of acrylamide (1023 µg/kg) was found in the R type of FSE (Table 3.2). The high concentration of acrylamide reported in this observational study is consistent with Mills and co-authors (2009) who found an acrylamide concentration as high as 3500 µg/kg as reported in the European Union acrylamide monitoring database.

Comparing the frying practices among the three FSE types revealed that the setting of frying temperature of 177°C for 2 minutes and 45 seconds of digitally controlled fryers in the CFS apparently resulted in a small range of actual frying temperature (170-177°C) and time (150-165 seconds). Correspondingly, the lowest variation in acrylamide concentration (i.e. 150 to 392 µg/kg, a 2.6-fold difference) was found in the CFS type. In the R type, frying pans were used in which the temperature and time cannot be regulated. The food handlers visually inspected the oil to estimate its temperature to start frying and checked the colour of French fries to end frying, which apparently resulted in a large range of actual frying temperature (148-215°C) and time (199-694 seconds). Correspondingly, the largest variation in acrylamide concentrations (i.e. 152 to 1023 µg/kg, a 6.7-fold difference) was found in the R type. The study concluded that lack of standardised control of temperature and time (due to inadequate frying equipment) and variable frying practices of food handlers seem to contribute to large variation and high acrylamide concentration in French fries prepared in the R type compared to the other FSE types. These findings, which were obtained under actual practices in FSE, confirm experimentally the two sections of the conceptual model that are indicated as '2a' and '1b' in Figure 6.1, as assessed

## General Discussion

based on literature. It provided a first indication that frying equipment ('2a') and variable frying practices of food handlers ('1b') are the main technological and managerial causes of variation, respectively. It also shows that the technological (inadequate frying equipment) and managerial (food handler's decisions to start and to end frying) factors are interacting and result in the large range of frying temperature and time, which contributed to the largest variation in acrylamide. The finding is in line with earlier suggestions that fryer type influenced the concentrations of acrylamide (Gertz, Klostermann, & Kochhar, 2003; Romani et al., 2006).

Multiple regression analysis showed that frying temperature contributed the most ( $\beta=0.513$ ) to the prediction of acrylamide formation, followed by thawing practice ( $\beta=-0.359$ ), and frying time ( $\beta=0.143$ ) (Table 3.3). The finding suggested that the variation in actual frying temperature contributed the most to the variation in acrylamide concentrations, followed by the variation in actual frying time. These results provided another indication that frying condition (time-temperature) (indicated as '2a') is a major technological cause of variation. Various authors discussed that acrylamide concentration increases exponentially with increasing frying temperature (Amrein, Limacher, Conde-Petit, Amadó, & Escher, 2006; Gökmen, Palazoğlu, & Şenyuva, 2006; Matthäus, Haase, & Vosmann, 2004). This might explain why the large range of frying temperature and time as recorded in restaurants corresponded with the largest variation in acrylamide. Interestingly, no significant correlation between reducing sugars and acrylamide concentration was found ( $r=0.102$ ,  $n=360$ ,  $p=0.053$ ). Moreover, reducing sugars also did not significantly contribute to the prediction of acrylamide formation ( $\beta=-0.083$ ,  $p=0.078$ , Table 3.3). Therefore, an intervention study (**Chapter 4**) was conducted to establish the role of

## Chapter 6

reducing sugars on the concentration and variation in acrylamide in French fries prepared in real FSE.

### 6.2.2 Effect of control measures in food service establishments

In developing the conceptual model, the roles and interactions of technological and managerial factors as well as their relationships were assumed to contribute to the variation in acrylamide concentration. The results in the observational study (**Chapter 3**) provided insight into the roles and interactions of some of the technological and managerial factors (indicated as '2a' and '1b', respectively). The roles and interactions of the remaining technological and managerial factors were investigated and their relationships that were assumed to contribute to the variation in acrylamide concentration were tested in intervention studies in the following chapters.

**Chapter 4** describes an intervention study to evaluate the effect of lowering reducing sugars concentration in par-fried potato strips (product factor) on the concentration and variation of acrylamide in French fries. French fries using par-fried potato strips with lower (LRS) and higher concentration of reducing sugars (HRS) were compared to the normal concentration (NRS). The food handlers in the CFS and the IC prepared French fries under standardised frying conditions, in which frying temperature and time of fryer were set at 177°C and 2 minutes 45 seconds. The study found that the use of par-fried potato strips with lower concentrations of reducing sugars than the commonly used potato strips is an effective measure to reduce acrylamide concentrations in French fries. The lowest mean acrylamide concentration in French fries was found for the par-fried potato strips with low concentration of reducing sugars (LRS) (322 µg/kg) and was significantly different ( $p < 0.05$ ) from the strips with normal (NRS) (491

## General Discussion

$\mu\text{g/kg}$ ) and high concentration of reducing sugars (HRS) (928  $\mu\text{g/kg}$ ) (Table 4.3). A strong correlation between reducing sugars concentration and acrylamide concentration ( $r=0.758$ ,  $n=216$ ,  $p<0.05$ ) was found. A larger range of reducing sugars concentration in **Chapter 4** (from 0.27 to 9.81 g/kg, a 36.3-fold difference, Table 4.2) than in **Chapter 3** (from 0.25 to 2.22 g/kg, a 8.88-fold difference, Table 3.2) seems to contribute to the strong correlation between reducing sugars and acrylamide concentration. In other words, the technological factor ‘concentration of reducing sugars’ only becomes visible if its range is large enough, otherwise its effect remains hidden and overruled by other factors.

The study found that the observed variation in reducing sugars concentration in the par-fried potato strips was not reflected in the variation in acrylamide concentration in French fries. To illustrate, the study found the least variation in reducing sugars concentration in the LRS (0.27 to 0.63 g/kg, a 2.3-fold difference) and the largest variation in reducing sugars concentration in the HRS (2.63 to 9.81 g/kg, a 3.7-fold difference, Table 4.2). The difference in variation of reducing sugars concentration did not correspond with variation in acrylamide concentrations; similar variation was found for the LRS (209 to 477  $\mu\text{g/kg}$ , a 2.3-fold difference) and the HRS (632 to 1357  $\mu\text{g/kg}$ , a 2.1-fold difference, Table 4.3). We have shown in the above paragraph that the range of reducing sugars concentration must be sufficiently large to obtain the strong correlation. The strongest correlation between frying temperature (followed by frying time) and acrylamide concentration was found in **Chapter 3**, and these factors can overshadow the contribution of variation in reducing sugars to the variation in acrylamide. Possible reason for a more dominant effect of frying temperature-time than reducing sugars could be that acrylamide is sensitive to temperature changes (at low moisture contents) and its concentration increases exponentially

## Chapter 6

with increasing frying temperature (De Vleeschouwer, Plancken, Van Loey, & Hendrickx, 2008; Eichner, Laible, & Wolf, 1985; Gökmen & Palazoğlu, 2008). Fiselier and co-authors (2006) emphasised that the profile of the frying temperature is more important than that regulated by the thermostat because acrylamide is formed towards the end of frying. Various authors discussed that additional factors such as setting a lower frying temperature at the final than at the start of frying, product/oil ratio and thawing practice affect the profile of frying temperature over time (Fiselier, et al., 2006; Grob et al., 2003; O'Connor, Fisk, Smith, & Melton, 2001; Palazoğlu & Gökmen, 2008; Tuta, Palazoğlu, & Gökmen, 2010). These technological factors are indicated as '2b' in Figure 6.1 and they also influence the actual temperature-time profile during frying and apparently need to be controlled to further reduce the variation in acrylamide. However, fryers normally used in FSE have no capabilities for regulating a temperature profile (Fiselier, et al., 2006). FSE in daily practice also did not implement the 10% rule of product/oil ratio and did not consistently practice thawing (Sanny, et al., 2012). The practical situations in FSE indicated that the possibilities to reduce further the variation in acrylamide are restricted. This situation underpinned the need to provide instructions that specify the acceptable frying conditions and practices to direct peoples' daily decisions on the process status within acceptable tolerances. The role of implementing (or non-compliance with) frying instructions (indicated as '1b' in Figure 6.1), as a managerial cause of variation in acrylamide, was investigated in **Chapter 5**.

**Chapter 5** describes an intervention study to obtain insight into the effect of frying instructions on food handlers' control decisions (process factor) in restaurant type of FSE and the impact on the concentration and variation of acrylamide in French fries. The frying instructions that specified the acceptable frying conditions and practices were provided to influence the food

## General Discussion

handlers' daily decisions to control frying conditions and compliance to the frying instructions was observed. The study found that frying instructions supported food handlers' decisions to start frying when the oil temperature reached 175°C, but an inconsistent effect of the instructions on the food handlers' decisions to end frying was observed. Although providing instructions did not result in a significant difference in the mean concentration of acrylamide for the restaurants as a group, data analysis for each restaurant showed that implementing frying instructions can reduce the concentration of acrylamide in French fries. The impact of frying instructions on the mean and extent of variation in acrylamide concentration was not consistent because of the inconsistent compliance to the frying instructions for which food handlers were trained. The finding confirms the role of implementing (or non-compliance with) frying instructions as a managerial cause of variation, and its relationship that was assumed to contribute to the variation in acrylamide concentration (indicated as '1b' in Figure 6.1). When instructions were strictly followed, the acrylamide concentration was significantly lowered. In case of non-compliance, even higher means of acrylamide concentrations were found compared to before instructions. The finding also shows that knowledge about technological factors (frying conditions indicated as '2a') are required for providing instructions and training (managerial factor indicated as '2b' in Figure 6.1) and these factors are interacting in developing and providing instructions.

Most of the food handlers were not following the frying instructions, although they were trained in it. A technological and a managerial strategy were proposed to overcome the non-compliance behaviour of food handlers in the FSE. The technological one involved setting strict requirements on equipment features and specifications. Restaurants are required to use a commercial fryer equipped with a temperature controller and a timer, in which its basket can be



## Chapter 6

automatically lifted from the oil once the fryer reached the set frying time. These features support food handlers by taking control decisions away from them to facilitate behaviour change and this technological strategy is indicated as '2a' in Figure 6.2. Despite the fact that fryers normally used in FSE have no capabilities for regulating a temperature profile (Fiselier, et al., 2006), practicing a standardised frying temperature and time seems the closest option as an effective strategy to lower acrylamide concentration and to reduce its variation in French fries. Setting of frying temperature and time of fryers in all types of FSE should be standardised at 177°C for 2 minutes 45 seconds to aim for golden yellow colour of French fries. This setting has recently shown to correspond to the lowest mean acrylamide concentration with the least variation in French fries prepared in CFS (**Chapter 3**) (Sanny, et al., 2012). Different authors showed that fryer type influences the concentrations of acrylamide (Gertz, et al., 2003; Romani, et al., 2006; Sanny, Luning, Marcelis, Jinap, & van Boekel, 2010). Future study may investigate the effect of the standardised frying temperature and time on various types of fryers that are available on the market. Such insight can be used to standardise frying time-temperature regime because manufacturers advised various types of frying time-temperature regime on the packaging. For example, some manufacturers specify frying French fries at 190°C for 5 minutes whereas others specify frying French fries at 175°C for 3-4 minutes until golden yellow (ALEC-International, 2009; FSA, 2009).

The managerial strategy included feedback through on-going supervision to correct any non-compliance actions (indicated as '2b' in Figure 6.2), besides demonstration and practice in a training program (Palmen, Didden, & Korzilius, 2010; Rennie, 1994; Seaman & Eves, 2008). Future studies could investigate the effect of strict monitoring on compliance to instructions on

acrylamide. This managerial strategy is expected to have a strong influence on food handlers' control decisions on setting of frying conditions and controlling them between acceptable tolerances to result in significant reduction in variation in acrylamide concentration.

The food quality relationship model reflects that food quality is dependent on food and human behaviour (Luning & Marcelis, 2006). In this thesis, food behaviour and human behaviour were quantified to realise food quality (concentration and variation of acrylamide in French fries). Food behaviour, i.e., the considerable variation in reducing sugars concentration in potato strips (food dynamics) as a result of applied technological conditions (standardised frying temperature and time in the preparation of French fries), were quantified in **Chapter 4**. Human behaviour, i.e., the variation in (daily) control decisions of food handler (human dynamics) as a result of applied administration conditions (instructions and training), were also quantified in **Chapter 5**. Quantifying food and human behaviour and comparing their effects serve as an essential component in this thesis because it leads to a broader insight into which factors contributes more to large variation and high concentration of acrylamide. The estimation of actual dietary exposure to acrylamide deals with ambiguity because of variable concentration of acrylamide (Besaratina & Pfeifer, 2007), but researchers often neglect its presence and they regularly reflect variability as natural variation (Röös, Sundberg, & Hansson, 2010; Smith, 2011). Varying the reducing sugars (from low to high) concentration in **Chapter 4** resulted in acrylamide concentration ranging from 209 to 1357  $\mu\text{g}/\text{kg}$  (a 6.5-fold difference) in French fries. The food handlers made variable decisions in controlling frying conditions (**Chapter 5**), which resulted in acrylamide concentration ranging from 85 to 3369  $\mu\text{g}/\text{kg}$  (a 39.6-fold difference). Comparing the highest concentrations of acrylamide between the two chapters provides the first indication

## **Chapter 6**

that lack of control over frying conditions (managerial factor) seem to contribute more to high concentration of acrylamide than the high concentration of reducing sugars (technological factor). The occurrence of high acrylamide concentrations that are reported in literature (IRMM, 2006; Mills, et al., 2009) could be attributed to the lack of control over frying conditions in the food production chain more than to the concentration of reducing sugars. Different authors discussed that a better understanding of process conditions and product properties responsible for the large variation in acrylamide concentration can be exploited as a major opportunity to reduce acrylamide concentration in food (FAO/WHO, 2007; Levine & Smith, 2005). The importance to control frying condition more than reducing sugars might provide a key direction of future research. In efforts to reduce high concentrations of acrylamide in French fries because of public health concern, genetic and agronomic approaches that aim to decrease acrylamide precursors in potatoes could be less important research areas than the final preparation in FSE and in home preparation.

### **6.3 Advantages, challenges and future research efforts**

This thesis introduced a different approach from a classical one because it considered the contribution of control decisions of manager and food handler to significant variation in acrylamide besides the effect of technological factors alone. Managerial factors that can contribute to significant variation were identified based on a food quality decisions model, besides current knowledge on causes of variation due to technological factors. Literature from management sciences (managerial discipline) was analysed to operationalise the food quality decisions model to examine control decisions of manager and food handler (human behaviour). Literature from food sciences (technological discipline) was analysed to understand the

underlying technological factors that influence the initial concentration of reducing sugars and the profile of frying temperature over time (food behaviour). The gained knowledge from these sciences was integrated to investigate how control decisions in a management system influence the technological factors that contribute to variation. Luning and Marcelis (2006, 2007, 2009) stated the techno-managerial approach involves integrative use of technological and managerial theories to explain and predict food quality from food and human behaviour. In this thesis, we have shown the basic principle of this approach that it starts with understanding behaviour of food and human systems to explain and predict food quality. Introduction of any managerial control measure makes only sense if it is combined with knowledge about technological factors that affect food quality. For example, many other types of managerial measures can be introduced, but knowledge about the acceptable frying conditions and practices (technological factors) affecting the variation in acrylamide concentration directed us to introduce frying instructions as a managerial control measures in **Chapter 5**.

The food quality decision model (Figure 1.1) proposed that human systems make decisions to influence food quality via these four factors: food and human dynamics, technological and administrative conditions. In this thesis, the four factors were translated into practice to analyse how control decisions influence the technological factors that contribute to high and variable concentration of acrylamide in the real preparation of French fries in FSE. However, researchers must have a strong background in both technological and managerial theories to work in this type of multidisciplinary research approach. This can be a problem because researchers usually receive education on one of them (Luning & Marcelis, 2009). This limitation is identified as one of the barriers for a researcher to be critical in analysing a problem situation and to perceive food

## Chapter 6

quality from a broader perspective. Especially strong knowledge in food science and technology is of importance to enable a researcher to make a useful integration on how other possible factors (such as managerial factors) can affect the underlying technological factors to realise food quality.

Acrylamide is generated in parallel with desired flavour and browning compounds because reducing sugars and free amino acids are also the precursors of flavour components and of browning formed in the Maillard reaction (Amrein, et al., 2006). Grob (2005) reported that potato products with low concentration of reducing sugars (<0.3 g/kg) become crispy but browning and flavour compounds formation remain unsatisfactory whereas when reducing sugars exceed 3 g/kg, the product gets dark and bitter before the desired crispiness is achieved. However, preliminary testing in **Chapter 4** showed that the consumer accepted French fries that were prepared in all three experiments as good and comparable sensory quality. As a matter of fact, the chain fast-food service A is serving this type of quality of French fries to their consumers because they use the par-fried potato strips with low concentration of reducing sugars in their daily practice. Furthermore, different authors reported that it is possible to prepare French fries with good culinary quality using low concentration of reducing sugars (Biedermann et al., 2010; Fiselier & Grob, 2005). Future studies should objectively measure the quality attributes of French fries prepared in FSE, using a sensory method to compare French fries prepared using the low concentration of reducing sugars with products of normal and high concentration of reducing sugars.

## General Discussion

The managerial intervention to provide frying instructions to food handlers as a control measure is a challenge because of inconsistent compliance. Future attempts to provide frying instructions should consider that FSE in actual practice are using various serving sizes and oil volume and it makes the guideline of portion size (10% potato referring to the oil) not practical to be implemented in FSE. Different authors showed that portion size affect the profile of frying temperature over time and consequently the formation of acrylamide (Fiselier, et al., 2006; Grob, et al., 2003). Providing a specific frying instruction for a specific condition could be a solution. Besides the temperature program as suggested by Palazoğlu and Gökmen (2008), standardising the capacity of fryer makes it possible to provide a specific frying instruction; specifying frying temperature and time setting, portion size and oil volume that are appropriate for intended conditions.

The technological and managerial control measures that were evaluated gave more insight into possible strategies to reduce the concentration and variation of acrylamide in French fries prepared in FSE. Practical situations in FSE were considered in proposing future research efforts to make the control measures more effective and complete to be used as tools to reduce acrylamide exposure from French fries. These control measures are intended to be generic in nature to facilitate its implementation in other settings such as home preparation.

## Chapter 6

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## Chapter 6

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## SUMMARY

Dietary studies observed significant variations in acrylamide intake, a probable human carcinogen, which complicates the estimation of actual dietary exposure to acrylamide. Possible factors contributing to significant variation in acrylamide concentrations need to be understood first before effective control measures can be developed. Various control measures have been tested in controlled laboratory conditions but they need to be validated under actual food processing and food preparation conditions. Although French fries are widely prepared in food service establishments (FSE), data on the effects of control measures of French fries preparations in real life situations are limited. Because such preparation is highly relying on food handlers, their control decisions are expected to influence the status of product properties and processing conditions that affect variation in acrylamide concentration. The topic is introduced in **Chapter 1** and the outline of the thesis is given. The aim of this thesis is first to identify the major technological and people related factors and to investigate their contribution to variation in acrylamide concentrations in French fries. The second aim is to investigate the effect of technological and managerial control measures on the concentration and variation of acrylamide in the preparation of French fries in real life FSE.

**Chapter 2** reviews both technological and managerial factors that contribute to variation in acrylamide concentration in French fries based on a comprehensive literature analysis. The major product properties and processing conditions (i.e., technological factors) that affect the formation of acrylamide were first identified, followed by an analysis of studies that provided insights into their possible contribution to variation in acrylamide concentrations. *A food quality*

*decision model* was used to systematically analyse the decisions of managers and food handlers (managerial factors) at the identified critical points of control in the preparation of French fries. The literature study indicated that glucose and fructose, and actual frying conditions (time-temperature) are major technological factors in acrylamide formation and food handler's inadequate control of these factors can result in variable and high acrylamide concentrations in French fries upon consumption. Analysis of the common control situation in FSE revealed that control decisions on resources by management (i.e. appropriate suppliers, specified raw materials, adequate frying equipment, and competent personnel) are restricted in practice. Moreover, food handlers' daily control decisions (at receipt and during frying) are also an important cause of variation even under appropriate technological (advanced equipment, low reducing sugars in raw materials) and managerial conditions (competent people, appropriate procedures, etc.).

**Chapter 3** describes how insight was obtained into the actual variation in acrylamide concentrations in French Fries prepared under typical conditions in FSE and what was the contribution of technological and managerial factors to this variation. The actual practices at receiving, thawing and frying were observed to get an insight into the typical frying practices in three FSE types, i.e., chain fast-food services (CFS), institutional caterers (IC), and restaurants. Although the mean concentration of acrylamide among the three FSE types was not significantly different, the variation was the least in French fries prepared in the CFS compared to the IC and the restaurants. The variation in actual frying temperature contributed most to the variation in acrylamide concentrations, followed by the variation in actual frying time; no obvious effect of

reducing sugars was found. The lack of standardised control of frying temperature and frying time (due to inadequate frying equipment) and the variable frying practices of food handlers seem to contribute most to the large variation and high acrylamide concentrations in French fries prepared in restaurants as compared to the two other FSE types.

In the second part of the thesis, the effects of a technological as well as a managerial control measure on the concentration and variation of acrylamide in French fries made in real FSE were investigated. **Chapter 4** evaluated the effect of lowering reducing sugars concentration in par-fried potato strips on the variation and concentration of acrylamide in French fries in real FSE practices. French fries using par-fried potato strips with lower (LRS) and higher concentration of reducing sugars (HRS) were compared to the normal concentration (NRS). The CFS and the IC types of FSE prepared French fries under standardised frying conditions; frying temperature and time of fryer were set at 177°C and 2 minutes 45 seconds. The results showed that the use of par-fried potato strips with lower concentrations of reducing sugars (i.e., than in the commonly used potato strips) is indeed an effective measure to reduce acrylamide concentrations in French fries. However, the small variation in reducing sugars concentrations in the LRS and NRS in combination with the standardised frying conditions still resulted in large variation in acrylamide concentrations in French fries. Other factors such as setting a lower frying temperature at the final than at the start of frying, product/oil ratio and thawing practice that affect the temperature-time profile of frying oil need to be controlled to further reduce the variation in acrylamide. Possibilities to control these factors however are restricted because fryers normally used in FSE have no features for regulating a temperature profile. Moreover, the FSE did not implement the

10% rule of product/oil ratio and did not consistently practice thawing. This situation underlined the need to provide instructions that specify the acceptable frying conditions and practices to direct peoples' daily decisions on the process status within acceptable tolerances.

**Chapter 5** evaluated the effect of frying instructions on food handlers' control decisions in the restaurant type of FSE and investigated the impact of control decisions on the concentration and variation of acrylamide in French fries. The frying instructions that specified the acceptable frying conditions and practices were provided to influence the food handlers' daily decisions to control frying conditions, and compliance to the frying instructions was observed. The results showed that frying instructions supported food handlers' decisions to start frying when the oil temperature reached 175°C, although an inconsistent effect of the instructions on the food handlers' decisions to end frying was observed. Providing instructions did not result in a significant difference in the mean concentration of acrylamide for the restaurants as a group. However, data analysis for each restaurant showed that when the food handler in a restaurant properly followed the instructions, the mean concentration of acrylamide was significantly lower as compared to before instructions. The food handlers in other restaurants did not comply with the frying instructions, which resulted in higher mean concentrations of acrylamide after instructions. The food handlers increased the frying time beyond 240 seconds to achieve crispier French fries with a final colour that fitted with their own preference; although they did start frying at the right temperature (175°C). The impact of frying instruction on the mean and extent of variation in acrylamide concentration was not consistent because of the variable compliance to the frying instructions. It was discussed how different strategies may overcome the non-



compliance behaviour of food handlers, namely the use of a commercial fryer (a technological approach) equipped with a temperature controller and a timer, and supported with strict monitoring on compliance to instructions (a managerial approach).

**Chapter 6** discusses the main findings of the research described in this thesis and positions them in a broader perspective. A conceptual model was developed, incorporating findings from the qualitative study (**Chapter 2**) that provided insight into technological and managerial causes of variation. The results of the observational study (**Chapter 3**) and intervention studies (**Chapter 4 – 5**) were evaluated to investigate the role of technological and managerial factors and their relationships that were assumed to contribute to the variation in acrylamide concentration. The basic principle of the techno-managerial approach was shown to start with understanding behaviour of food and human system to explain and predict food quality. Quantifying food and human behaviour and comparing their effects serve as an essential component of this thesis because it leads to a broader insight into which factor contributes more to large variation and high concentration of acrylamide. Lack of control over frying conditions (managerial factor) had a greater effect on the variation in acrylamide concentration than did the effect of the reducing sugars (technological factor). Finally, future research efforts were recommended to make the evaluated control measures more effective and complete to be used as tools to reduce acrylamide exposure from French fries.



## SAMENVATTING

Acrylamide is een component die gevormd kan worden in bepaalde voedingsmiddelen bij verhitting, waarbij de zogenaamde Maillard reactie de voornaamste bron is, een reactie tussen reducerende suikers en aminogroepen. Acrylamide is een potentieel carcinogeen, en daarom ongewenst in voedsel. Echter, omdat acrylamide tegelijkertijd gevormd wordt met gewenste Maillard reactie componenten (vanuit sensorisch oogpunt) is het niet goed mogelijk om vorming compleet te vermijden, maar men kan wel streven naar minimalisering van het gehalte aan acrylamide met behoud van de gewenste kwaliteit. Analyse van het voedsel dat mensen eten laat zien dat de variatie in gehalten aan acrylamide die men binnenkrijgt erg groot is; het is daarom erg moeilijk in te schatten wat nu eigenlijk de werkelijke belasting is die mensen aan acrylamide binnen krijgen. Dit is uiteraard ongewenst en de vraag rijst welke maatregelen genomen kunnen worden om niet alleen het gehalte aan acrylamide omlaag te krijgen maar ook de variatie daarin. Het onderzoek dat tot nu toe is verricht aangaande acrylamide vorming is vooral uitgevoerd in laboratoria. Dat levert heel veel inzicht op in de technologische factoren die invloed hebben op acrylamide vorming. De vraag is echter of dat voldoende is voor de situatie in de praktijk van voedselbereiding, en dit proefschrift nam als uitgangspunt aan dat behalve technologische ingrepen ook het gedrag van mensen in de praktijksituatie van voedselproductie van belang is om mee te nemen. De hypothese is dat zowel technologische als management factoren van belang zijn om tot een daadwerkelijke beheersing van kwaliteit te kunnen komen, de zogenaamde '*techno-managerial approach*'. Om deze hypothese te kunnen testen is gekozen voor de productie van frites in verschillende typen horeca; frites is een bekende bron van acrylamide, en dit stofje wordt vooral in de eindfase van de productieketen, namelijk het bakken, gevormd, vandaar de keuze voor de

horeca. **Hoofdstuk 1** introduceert het probleem, formuleert de doelstelling van het onderzoek en geeft de opbouw van het proefschrift weer.

**Hoofdstuk 2** beschrijft een diepgaand literatuuroverzicht aangaande de technologische als ook de management aspecten die bij kunnen dragen aan de variatie in gehalten aan acrylamide in frites. De belangrijkste product- en proceseigenschappen (de technologische factoren) die een effect hebben op acrylamide vorming in frites bleken respectievelijk het suikergehalte (de reducerende suikers glucose en fructose) en de tijd-temperatuur combinaties bij het bakken te zijn. Wat het effect van gedrag van mensen betreft is het vooral het niet goed in de hand hebben van de bakcondities en van de keuze van de grondstof dat kan leiden tot grote variatie. Het gaat dan om de keuze van betrouwbare leveranciers, keuze van de soort aardappel, de betrouwbaarheid van de bakapparatuur en de betrouwbaarheid en competenties van het personeel. Het literatuuroverzicht werd ingekaderd in een zogenaamd '*food quality decision model*' dat systematisch de beslissingen van managers en uitvoerend personeel beschrijft in het geval van de productie van frites.

**Hoofdstuk 3** beschrijft hoe inzicht werd verkregen in de variatie in acrylamide in frites geproduceerd bij verschillende horeca bedrijven. Dit gebeurde door te observeren wat mensen precies deden bij het bakken van frites: het in ontvangst nemen van de ingevroren voorgebakken frites, hoe ontdooid werd en hoe gebakken werd. Verder werden monsters genomen van de grondstof om suikergehaltes te meten en van het gebakken product voor analyse op acrylamide gehalten. Dit gebeurde bij drie typen horeca, een '*fast food*' restaurant, bedrijfskantines, en bij restaurants. Als resultaat werd gevonden dat weliswaar het gemiddelde gehalte aan acrylamide niet significant verschilde tussen de drie horeca types, maar wel dat de variatie erin erg verschilde. De resultaten toonden overduidelijk aan dat voor dit soort

situaties gemiddelden een verkeerd beeld geven van de werkelijkheid en dat de variatie expliciet in beschouwing moet worden genomen. In de *'fast food'* restaurants was de variatie aanzienlijk minder dan in bedrijfskantines en restaurants. Met name in de restaurants bleek nauwelijks enige standaardisatie te zijn op de tijd en temperatuur bij het bakken, mede door de afwezigheid van professionele bakapparatuur. De belangrijkste bron van variatie bleek een wisselende baktemperatuur te zijn, gevolgd door een wisselende baktijd. In deze studie kon geen relatie gevonden worden tussen variatie in gehalte aan reducerende suikers en acrylamide.

**Hoofdstuk 4** beschrijft onderzoek waarin het gehalte aan reducerende suikers werd gevarieerd in de grondstof (door verschillende aardappelrassen te gebruiken), waarna werd bestudeerd of dit effect heeft op de acrylamide gehalten in frites geproduceerd in bedrijfskantines en *'fast food'* restaurants. Om andere omstandigheden zoveel mogelijk constant te houden werd de baktijd en begintemperatuur gestandaardiseerd op 165 seconden en 177°C. Door deze maatregel bleek nu wel dat een lager gehalte aan reducerende suikers leidt tot een lager gehalte aan acrylamide. Er werd echter geconstateerd dat ook bij een laag gehalte aan reducerende suikers de variatie in acrylamide nog steeds erg groot was. Er spelen dus nog andere variatiebronnen mee. Mogelijke bronnen zijn de variatie in verhouding product/olie, verschillende manieren van ontdooien van de voorgebakken frites, variatie in temperatuurverloop nadat de frites in de olie is gebracht, afhankelijk van de gebruikte apparatuur. Dit gegeven onderstreept de noodzaak tot specifieke instructies om zoveel mogelijk het gedrag van mensen te sturen en te beheersen.

**Hoofdstuk 5** beschrijft onderzoek waarin getracht werd om het gedrag van mensen m.b.v. gedetailleerde instructies te beïnvloeden. Dit werd gedaan in restaurants omdat daar de meeste

variatie werd gevonden, en het effect van instructies dus het duidelijkst gezien zou kunnen worden. De instructies werden uitgelegd en geoefend, en betroffen vooral het exact instellen van de begin temperatuur van de olie en de baktijd. Geobserveerd werd of men zich aan de instructies hield. Het resultaat was dat het gemiddelde gehalte aan acrylamide voor alle restaurants tezamen niet verschilde en niet verlaagd werd. Echter, voor de restaurants die zich goed aan de instructies hielden bleek wel degelijk een significante verlaging in acrylamide op te treden. De frites met hogere acrylamide gehalten bleek geproduceerd te zijn in restaurants waar mensen zich niet hielden aan de instructies. Geconstateerd werd dat wel de begintemperatuur goed werd aangehouden, maar niet de baktijd. Sommige fritesbakkers bleken niet tevreden over de kleur en de textuur van de frites na de gespecificeerde tijd en gingen dus langer door, met als gevolg een hoger acrylamide gehalte. Een belangrijk resultaat is dus dat ondanks instructies mensen zich niet blijken te houden aan deze instructies omdat ze andere kwaliteitsaspecten belangrijker vinden. Bediscussieerd werd in hoeverre dit gedrag kan worden beïnvloed. Een mogelijkheid zou zijn om de baktijd en –temperatuur te laten overnemen door de apparatuur (een technologische ingreep) en door te zorgen voor strikte naleving van de regels (een management ingreep). Strikte naleving van regels is veel beter geregeld in ‘fast food’ restaurants hetgeen dan ook leidt tot minder variatie in acrylamide gehalten, zoals gevonden in hoofdstuk 3.

**Hoofdstuk 6** bediscussieert de resultaten van de verschillende onderzoeken en plaatst die in een breder perspectief. Dit heeft geleid tot een conceptueel model waarbij de technologische zowel als de management factoren zijn geïncorporeerd. De gevolgde *techno-managerial approach* bleek een zinvolle benadering te zijn geweest. Een belangrijk aspect daaraan is dat eerst en vooral begrepen moet worden welke factoren van belang zijn qua product- en proceseigenschappen en qua gedrag van mensen, en deze factoren moeten in observationele

en interventie studies worden ontrafeld. Verder is belangrijk gebleken dat de effecten van technologische maar ook menselijk gedrag gemeten kunnen worden, in dit geval in de gehalten aan acrylamide, en met name de variatie daarin. Het is dus mogelijk gebleken om deze effecten, ook van het gedrag van mensen, te kwantificeren. Dit onderzoek toonde aan dat gedrag van mensen als management factor een belangrijker effect heeft op de variatie in acrylamide dan een technologische factor als het gehalte aan reducerende suikers. Hoewel dit laatste als zodanig een effect heeft op het acrylamide gehalte wordt het effect compleet overruled door variatie in baktijd en –temperatuur door personeel dat naar eigen inzichten handelt. Indien men tot een daadwerkelijke vermindering in acrylamide gehalte wil komen is het dus zaak zowel aan technologische factoren als aan menselijk gedrag aandacht te besteden. Dit proefschrift heeft dit kwantitatief aangetoond. De consequenties van deze bevinding gaan verder dan alleen over acrylamide gehalten in frites; bediscussieerd is dat dit ook zal moeten gelden voor andere kwaliteitsproblemen.





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## CURRICULUM VITAE



Maimunah Sanny was born on August 5<sup>th</sup> 1971 in Johor, Malaysia. She obtained her Bachelor degree in Food Science and Technology in the Universiti Putra Malaysia in year 1994. From December 1995 to September 1999, she started her working career as a Quality System Executive in Palm Oleo Sdn. Bhd. in Selangor Malaysia. She moved on to work as a Laboratory Executive in BASF (Malaysia) Sdn. Bhd. from January 2000 to October 2001 and as a Quality Assurance Manager in the same company from November 2001 to February 2003. She developed an interest in research and development and from July 2003 to March 2006, she worked as a Head of Analytical Unit in Combinatory Technology & Catalysis Research Centres (COMBICAT) in Universiti Malaya. While working in this research centre, she enrolled as a part time student in Universiti Malaya and completed her Master degree in Analytical Chemistry and Instrumentation in year 2005. Since April 2006, she started to work as a Tutor in Universiti Putra Malaysia. She took a study leave to pursue a PhD degree in Wageningen University from September 2007 to March 2012. She will return to the Universiti Putra Malaysia to start her new career as a Senior Lecturer.

## List of publications

### *Peer reviewed*

Sanny, M., Jinap, S., Bakker, E. J., van Boekel, M. A. J. S., and Luning, P. A. (2012). Possible causes of variation in acrylamide concentration in French fries prepared in food service establishment: An observational study. *Food Chemistry*, 132(1), 134-143.

Sanny, M., Luning, P. A., Marcelis, W. J., Jinap, S., and van Boekel, M. A. J. S. (2010). Impact of control behaviour on unacceptable variation in acrylamide concentration in French fries. *Trends in Food Science and Technology*, 21(5), 256-267.

### *Submitted for publication*

Sanny, M., Luning, P.A., Jinap, S., Bakker, E.J., and van Boekel, M.A.J.S. Effect of frying instructions to food handlers on acrylamide concentration in French fries: An explorative study.

Sanny, M., Jinap, S., Bakker, E. J., van Boekel, M. A. J. S., & Luning, P. A. Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments?

### *Oral/poster presentation*

Sanny, M., Jinap, S., Bakker, E. J., van Boekel, M. A. J. S., and Luning, P. A. Effect of frying instructions to food handlers on acrylamide concentration in French fries. In Food Denmark PhD Congress, 2011

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Sanny, M., Luning, P. A., Jinap, S., Bakker, E. J. and van Boekel, M. A. J. S. Frying practices affecting variation in acrylamide concentration in French fries production in Malaysia food service establishments. In *International Conference on Food Safety and Security under Changing Climate*, Penang, Malaysia, December, 2010.

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Sanny, M., Luning, P. A., Marcelis, W. J., Jinap, S., & Van Boekel, M. A. J. S. Sources of variation affecting acrylamide concentration French fries preparation in food service establishments: A techno-managerial approach. In *Malaysian Institute of Food Technology (MIFT) 6<sup>th</sup> Food Science and Technology Annual Seminar (2009)*, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

## **Overview of completed training activities**

### **Discipline specific activities**

#### Courses and conferences

VLAG Advanced Food Analysis, Wageningen University, 2010

Risk Assessment, Wageningen University, 2011

Malaysian Institute of Food Technology (MIFT) 6<sup>th</sup> Food Science and Technology Annual Seminar, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia, 2009

Invention, Research and Innovation Exhibition, Universiti Putra Malaysia, Selangor, Malaysia, 2010

International Conference on Food Safety and Security under Changing Climate, Penang, Malaysia, 2010

5th International Conference 'Quality and Safety in Food Production Chain', Wroclaw, Poland, 2011

Food Denmark PhD Congress, 2011

### **General courses**

Information Literacy for PhD (including Endnote Introduction), Wageningen University, 2007

Techniques for Writing and Presenting Scientific Papers, Wageningen University, 2008

Scientific Writing, Wageningen University, 2011

Presentation Skills, Wageningen University, 2011

### **PhD excursions**

PhD study trip of Product Design and Quality Management Group, Australia, 2010

