ACRYLAMIDE FORMATION AND MITIGATION IN PROCESSED POTATO PRODUCTS

by

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B.S., University of Idaho, 2008

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Approved by:
Major Professor
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Animal Sci. & Ind.

Abstract

Acrylamide is a naturally occurring compound that is formed during the Maillard reaction. The International Agency for Research on Cancer has classified acrylamide as a "probable human carcinogen" as a result of tumor development in laboratory animals when acrylamide is ingested in high concentrations. The amino acid asparagine is particularly important in the formation of acrylamide due to its structural significance; its structure is analogous to that of acrylamide. Potato tubers contain high amounts of asparagine, thus food products such as French fries and potato chips (crisps) have been flagged for their high acrylamide levels and widespread consumption. Acrylamide mitigation in potato products can take place either during raw variety selection (or breeding) or during processing. Heating potatoes at a lower temperature or for a shorter time has shown to significantly decrease acrylamide levels. Numerous studies have shown that use of acidulants, preservatives, and low pH conditions dramatically reduce acrylamide formation by protonation of the asparagine molecule. Hydrolysis and epimerization of sugars during storage, precursor concentrations, and plant physiology are agronomic factors that can be manipulated to decrease acrylamide concentrations. Asparagine has shown to be the rate limiting factor in acrylamide formation, so processing potato cultivars with low asparagine concentrations will result in lower acrylamide levels in the finished product. Continued research areas are focusing on cultivar studies and process optimization to provide a product with lower acrylamide levels but the same sensorial attributes as current products.

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Acknowledgements

I would like to acknowledge and thank Dr. J. Scott Smith for all of his support and guidance over the past two. His encouragement and patience played an integral role in my success during my time at Kansas State University. I would also like to thank Gordon Smith for all of the direction and assistance he provided; without his help this paper would not have been a possibility.

Dedication

This paper is dedicated to the people in my life who have pushed me to become the person I am today. Mom and Dad, you have always supported me 110% in all of my endeavors and challenged me to push my limits. You have taught me that I really can do anything if I set my mind to it and to always take pride in my work. I am so blessed to have you as my parents.

Michelle, you have always been an incredible role model. I have looked up to you for 25 years and you never cease to amaze me. I would have never worked as hard as I did in school, sports, or anything else if my big sister had not set the bar so high. Your laundry list of achievements is astounding and I am so proud of you. I love you, Sister.

Mike Leitner, words cannot convey how much I appreciate you. You have provided so much guidance, support, and wisdom over the 3+ years that we have worked together. You are an inspiration to me and I want you to know that the impact you have made on my life will never be forgotten.

Kirsten, you were and are my lifeblood. I am so lucky to have you as a friend; I love you!

Many other names could be mentioned; I am so fortunate to have family and friends who believe in me. Thank you all for your continued words and encouragement and support.

-Sara Marie

Chapter 1: Background Information

Acrylamide (2-propenamide, CAS No. 79-06-1) is an odorless, colorless crystalline solid. It is formed from the hydration of acrylonitrile and is soluble in water, ethanol, and acetone. It is a small compound and highly mobile in groundwater and soil (Smith et al., 1997). The chemical structure of acrylamide is displayed below in Figure 1.1.

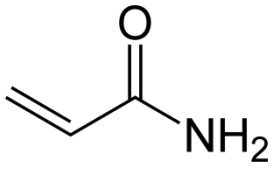

Figure 1.1 Acrylamide Chemical Structure

Acrylamide is a familiar chemical and has been used in a variety of industries for years (Friedman, 2003). This chemical is used in grout, cement, sewage, wastewater treatment, cosmetics, pesticide formulations, and soil erosion prevention as a binding, thickening, or flocculating agent. Acrylamide polymers are used in molecular biology laboratory gels for separation of proteins and chromatography, ore processing, food packaging, and plastic products. Cigarette smoke is another form of exposure (EU, 2002; IARC, 1994).

Acrylamide has shown to be a neurotoxicant in occupational settings and animal studies (Spencer & Schaumburg, 1974b). Recent studies have shown that a number of baked and fried foods naturally contain acrylamide (Tareke et al., 2000, 2002). Various proceedings built up to this finding. In southwest Sweden near the Bjare peninsula, railroad tunnel workers were developing signs of impaired nerve function. This development was traced back to exposure to acrylamide in Rhoca-Gel, a sealant that was used to waterproof cracks in the tunnel walls.

Succeeding investigations on the tunnel workers measured hemoglobin (Hb) adducts, which function as biomarkers for acrylamide exposure, in the workers' blood (Hagmar et al., 2001). Controls from this group had high levels of Hb adducts, which initiated an investigation for the source of acrylamide exposure in the control subjects.

It was known that acrylamide was formed when heating biological materials, such as tobacco, which led to the speculation of food being the source of exposure. This ultimately led to a study in which rats fed fried food contained Hb adducts which were characteristic of acrylamide exposure. These results stimulated a broad study of acrylamide in different food products, which was subsequently published by the Swedish government in 2002 (Tareke et al., 2002). The results of the study showed that starch-based foods which were baked or fried at high temperatures contained acrylamide residues. Additional studies performed by other countries (United States, Canada, Norway, United Kingdom, Australia) confirmed the Swedish results. Because acrylamide occurred naturally in foods and had shown to be a neurotoxicant in animals and humans as well as a reproductive toxicant and carcinogen in animals, the International Agency for Research on Cancer classified acrylamide as a "probable human carcinogen (IARC, 1994). Additional studies performed by the National Toxicology Program suggest that acrylamide is "reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals" (NTP, 2011).

The discovery of acrylamide in cooked food, particularly starch-based foods (Tareke et al., 2002) raised an alarm over the safety of such foods. The concern was founded on three

issues: the foods what contain acrylamide are extensively consumed; acrylamide is a probable human carcinogen, and the levels of acrylamide found in food are higher than a large number of other known foodborne carcinogens (Friedman, 2003). The possibility of acrylamide being a human carcinogen is a hot topic of debate, but research around the subject has been focused on understanding the mechanism(s) under which acrylamide is formed along with reduction/elimination techniques to minimize the possible human health risk. The United States Food and Drug Administration (FDA) has analyzed a variety of U.S. food products for acrylamide. Surveys began in 2002 and were updated every year until 2006. Table 1 lists examples of foods that have been surveyed, what year they were analyzed, the category to which they belong, and the amount of acrylamide present in the food (FDA, 2011).

Table 1.1 Acrylamide Concentration in Various Food Products (FDA, 2011)

Year Analyzed	Category	Product	Acrylamide (ppb)
2002	Potato Chips	Kettle Chips Lightly Salted Natural Gourmet Potato Chips	1265
2002	Protein Foods	Tyson Crispy Chicken Strips (baked)	35
2002	Breads	Pepperidge Farm Original White Bread	36
2002	French Fries	McDonald's French Fries	326
2002	French Fries	OreIda Golden Fries (baked)	
2002	Cereals	General Mills Cheerios	266
2002	Snack Foods	Herr's Extra Thin Pretzels	309
2002	Gravies & Seasonings	Colgin Natural Pecan Liquid Smoke	151
2002	Nuts and Nut Butters	Jif Creamy Peanut Butter	64
2002	Crackers	Keebler Town House Crackers Reduced Fat	130
2002	Chocolate Products	Hershey's Cocoa	909
2002	Canned Fruits & Vegetables	Mott's Apple Sauce	<10
2002	Cookies	Nabisco Chips Ahoy! Chewy Chocolate Chip	97
2002	Coffee	Maxwell House Slow Roast (ground, not brewed)	209
2002	Frozen Vegetables	Hanover Premium Petite Asparagus Spears	<10
2002	Dried Foods	Lipton Recipe Secrets Onion Soup & Dip Mix	1184
2002	Dairy	Carnation Instant Nonfat Dry Milk	11
2002	Miscellaneous	General Mills Lucky Charms Marshmallows	nondetect
2003	Breads & Bakery Products	Whole Foods Pumpkin Pie	29
2003	Cereals	Quaker 100% Natural Granola Oats, Honey & Raisins	84
2003	Cookies	Stauffer's Animal Crackers	432
2003	Potato Chips	Pringles Ridges Original Potato Crisps	1286
2003	Snack foods (other than potato chips)	Sun Chips Original Flavor	199
2003	Fruits and Vegetables	Giant Foods Large Pitted Ripe Olives	375
2004	Breads and Bakery Products	Krispy Kreme Original Glazed Doughnuts	22
2004	Cereal	General Mills Trix	171
2004	Fruit and Vegetable Products	French's Original French Fried Onions	125

Chapter 2: Toxicology of Acrylamide

Regulatory Levels in Drinking Water, Occupational Venues, and Body Care Products

The World Health Organization (WHO) established allowable levels of acrylamide in drinking water at 1μg/L, the United States Environmental Protection Agency (US EPA) at 0.5μg/L, and the European Union at 1μg/L. Exposure levels for ambient air in occupational venues were set by the Occupational Safety and Health Administration (OSHA) at 0.3mg/m^3 for 8 hour or 10 hour time-weighted averages. In body care products, levels are set at <0.1ppm and <0.5 ppm in other cosmetic products (SCCNFP, 1999). No current guidelines govern the presence of acrylamide in foods. Acrylamide exposure is estimated to be much greater at occupational levels than exposure levels in the diet (Marsh et al., 1999).

Neurotoxicity

The neurotoxicity of acrylamide has been extensively studied because the neurotoxic effects are the only adverse effects that have resulted in humans from occupational exposure and in laboratory animal studies. Over 35 years of research has been conducted to understand the mechanisms of action of acrylamide-induced neuropathies; the knowledge is quite extensive and a number of detailed reviews of the neurotoxic effects have been published. Spencer and Schaumburg (1974a) documented the neurotoxic effects of acrylamide in humans in occupational settings. LoPachin and Lehnig (1994) discuss the mechanism of acrylamide neurotoxicity and relate it to distal axon degeneration due to diminished Na/K-ATPase activity. LoPachin (2004) researched the direct actions of acrylamide at nerve terminal sites on the

sulfhydryl groups on presynaptic proteins and interrupt membrane fusion at nerve terminals.

The neurotoxic effects of acrylamide have been studied in many species of laboratory animals: cats, rats, mice, rabbits, guinea pigs, and monkeys. The studies showed that recurring exposure of 0.5-50 mg acrylamide/kg/day cause a myriad of effects including ataxia, hind-limb foot play, and skeletal muscle weakness, which was gauged by decreased fore- and hind-limb grip strength (Miller and Spencer, 1985). In humans, the neurotoxic effects of acrylamide from short-term occupational exposure include peripheral neuropathy, which manifested as numbness and tingling in the hands and feet, loss of toe reflexes, and weak legs (Hagmar et al., 2001). All of the symptoms were reversible. Long-term exposures caused cerebellar dysfunction, ataxia, some central neuropathy, and excessive tiredness, most of which were reversible (Hagmar et al., 2001).

The proposed mechanism for neurotoxicity via acrylamide exposure involves the interference of acrylamide with kinesin-related motor proteins in neurofilements that take part in fast anterograde transport of nerve signals between axons (Sickles et al., 1996). Slowing down the motor proteins and transaxonal transport nerve growth factors consequently impairs molecular transport from the cell body to the distal axon, which can cause a dying back of the nerve body. Current studies suggest that the acrylamide neurotoxicity mechanism involves an interference with the membrane fusion processes, which results in decreased neurotransmitter release and ultimately nerve terminal disintegration. Sulfhydryl groups on cysteines of proteins are involved in membrane fusion, and acrylamide binds to the sulfhydryl groups thereby

inhibiting proper fusion. These mechanisms also play a role in the genotoxic and negative reproductive effects of acrylamide in animals. Kinesin motor proteins are necessary for cell division and healthy sperm activity (Tyl and Friedman, 2003).

In general, the no-observable-adverse-effect level (NOAEL) for acrylamide neurotoxicity exposure is 0.2-0.5 mg/kg body weight (bw)/day and the lowest-observable-adverse-effect level (LOAEL) is 2 mg/kg bw/day (Spencer and Schaumburg, 1974a, 1974b). The WHO estimates that the average dietary exposure of acrylamide is 0.001 mg/kg bw/day (WHO, 2002). This level is used in risk assessment models and offers a 200-500-fold margin of safety. Although the average dietary exposure levels are low and will not result in neurotoxicity, a number of neurotoxicologists worry about possible cumulative neurotoxicity effects of acrylamide. Studies have shown that both low and high doses of acrylamide cause the same neurotoxic effects, low doses merely need longer exposure times for the effects to take place (LoPachin, 2004).

Genotoxicity

One major issue when evaluating potential carcinogens is the ability of the compound to cause genetic damage. Direct action on a DNA molecule is considered to be a form of the most critical damage, and that damage can be measured by specific gene locus or point mutation assays. Various tests involving both prokaryote and eukaryote systems have been accepted by regulatory agencies and validated to expose the mutagenic effects of carcinogens. The Ames forward and reverse bacterial mutations test is a common test using prokaryote systems, mostly *Salmonella* strains. The thymine kinase (TK) test or hypoxanthine-guanine phosphoribosyl

transferase (HGPRT) forward mutations of mouse lymphoma are common tests utilizing eukaryotic systems (Dearfield et al., 1988, 1995).

Unlike the neurotoxicity of acrylamide, the genotoxic effects of acrylamide have only been studied for twenty years and the resulting studies have found mixed results. A study conducted by Tsuda et al. (1993) did not show any mutagenic effects of acrylamide in the HGPRT assay at high concentrations, whereas a study by Knaap et al. (1988) reported acrylamide activity in the HGPRT assay at high concentrations. An increase in mutation frequency in the TK assay after acrylamide exposure was reported by Moore et al. (1987), but was attributed to clastogenesis and not to point mutations based on the distinct colonies that had formed. Johansson et al. (2005) reported that glycidamide induced clastogenic effects in Chinese hamster ovary cells in vitro.

Acrylamide mutagenicity is very likely attributed to the conversion of acrylamide to glycidamide. Glycidamide is a reactive epoxide of acrylamide and is formed after biotransformation by the P-450 mono-oxygenase CYP2E1. Studies on the metabolite glycidamide reveal damage in human blood cells in vitro, alterations in the HGPRT locus in V-79 cells (Baum et al., 2005), formation of adducts with guanine and adenine bases in various tissues after in vitro exposure (Doerge et al., 2005; Maniere et al., 2005), form adducts with DNA and proteins (Dearfield et al., 1995), and break DNA strands at high concentrations (Puppel et al., 2005).

Although reasonable questions regarding the mechanisms of acrylamide action exist,

evidence has not shown that acrylamide affects DNA integrity by either genotoxic or epigenetic mechanisms. Epigenetic, or gene altering, actions tend to be more dose-related and reversible. They have thresholds of exposure below the level at which their effects are negligible. This is important, because the epigenetic factors have repercussions in the application of risk assessment models where a genotoxic mechanism of action is suggested (Besaratinia and Pfeifer, 2007).

Carcinogenicity

Acrylamide is currently classified as "reasonably anticipated to be a human carcinogen" (NTP, 2011) based on sufficient evidence of carcinogenicity from studies on laboratory animals. Insufficient evidence exists for acrylamide being the cause of any carcinogenic effects in humans from occupational exposure or epidemiologic studies. Animals that are exposed to high concentrations of acrylamide in drinking water for long periods of time develop multiple tumors in many sites in both genders. In vitro and in vivo animal models have shown genotoxic effects of acrylamide in cell cultures (NTP, 2011). The chemical structure of acrylamide is analogous to other carcinogens such as vinyl carbamate and acrylonitirile.

Many long-term and highly irregular dose studies on laboratory rats were taken into consideration when acrylamide was classified as a probable human carcinogen (IARC, 1994). Male and female Fischer 344 rats were given drinking water samples with 0.01, 0.1, 0.5, and 2 mg acrylamide/kg/day for 2 years (Johnson et al., 1986). A significant change in tumor incidence was not seen in either gender when exposed to the three lower doses compared to the control, but increased tumor incidence was seen in the high dose. Female rats developed more

tumors in the mammary gland, thyroid gland, oral cavity, uterus, clitoral gland, and central nervous system. Male rats developed more tumors of the thyroid gland and scrotal mesothelium. Both genders exhibited peripheral neuropathy at the high dose.

Friedman et al. (1995) attempted to reproduce the results of the above-mentioned study due to some perceived inconsistencies with the first study. For 106 weeks, male Fisher 344 rats were exposed to levels of 0.1, 0.5, or 2 mg acrylamide/kg/day and females were exposed to 1 or 3 mg acrylamide/kg/day. Although some of the results of the later study aligned with the study by Johnson et al. (1986), significant differences did exist. The initial study indicated that female rats were more sensitive to acrylamide exposure whereas the second study showed higher mortality in male rats. Various tumors reported in the first study were unseen in the second study, including tumors of the oral cavity, clitoral gland, uterus, and central nervous system. The only malignant tumors were of the scrotal mesothelium, and it is practically unknown in humans and unusual in the rat.

Bull et al. (1984a, 1984b) conducted risk assessments that investigated the effects of acrylamide in classical tumor initiation/promotion assays in mice. Female SENCAR mice were dosed with 12.5, 25, or 50 mg/kg acrylamide by oral, intraperitoneal, or dermal application 6 times a week for 2 weeks. 12-O-tetradecanoyl phorbol 13-acetate (TPA) was then administered to the mice as a promoter for 52 weeks. The mice that were given TPA and acrylamide developed excessive skin tumors with decreased dormancy in a dose-responsive way by all methods of exposure that were tested. Tumors were not developed in the absence of TPA

treatment, signifying that acrylamide was acting as an initiator but was not able to induce cancer when solely administered.

In the same study, A/J strain male and female mice were orally given doses of 6.25, 12.5, 25, or given an intraperitoneal injection with 1, 3, 10, 30, or 60 mg/kg/bw acrylamide 3 times a week for 3 weeks. Lung adenoma frequency increased in a dose-responsive manner in both genders of mice by both routes of exposure. When given a 60 mg/kg intraperitoneal injection of acrylamide, the mice developed frank peripheral neuropathy after numerous injections and were subsequently eliminated from the study.

Epidemiology Studies

Clear evidence of the carcinogenicity of acrylamide exists when applied to laboratory rodents at relatively high doses (NTP, 2011). However, the same effect has yet to be seen in humans when acrylamide is included in the diet. Numerous epidemiologic studies have failed to show any connection between ingesting acrylamide in the diet and an increased incidence of any type of cancer. Conversely, the epidemiological studies may lack statistical power needed to assess a lower cancer incidence induced by a compound in the diet; initial studies were very limited in range. A study performed by Sobel et al. (1986) examined mortality in 371 workers in manufacturing facilities making acrylamide monomers and polymers, emphasizing cancers at sites seen in animal studies. A relationship between acrylamide exposure and cancer was not detected, but the small population size, a one-time exposure to other potential carcinogenic organic dyes, insufficient follow-up studies, and short-time exposure to some of the participants

rendered the study inadequate for drawing any conclusions. Collins et al. (1989) performed a larger study involving 8500 workers in 3 plants making water-soluble polymers of acrylamide. The 60-year cohort study did not show a relationship between acrylamide exposure and increased risk of any kind of cancer. Marsh et al. (1999) performed a follow-up study and looked at cancer deaths for the following 11 years after the larger study by Collins et al. had ended. No evidence was found to link acrylamide exposure in the diet with cancer-related deaths.

Several epidemiologic studies have been conducted to determine if a link between acrylamide intake and increased cancer risk exists. In 2003, Mucci et al. have conducted a population-based case control study that examined the incidence of large bowel, kidney, and bladder cancer linked to acrylamide exposure in 14 different foods. The potential cancer sites were thought to be appropriate due to intestinal exposure to acrylamide in food and its excretion in the urine. The acrylamide concentrations in the foods were moderate (100-299 μg/kg) to high (300-1200 μg/kg), but no connection between acrylamide exposure and increased risk of cancer was detected. However, the group witnessed a reduction in bowel cancer incidence, which could be linked to the high amount of fiber in the foods that were eaten. A similar, more specific study conducted by Pelucchi et al. (2003) also saw a decrease in bowel cancer when examining the incidence of cancer in relation to acrylamide intake.

In 2004, Mucci et al. re-analyzed the data from the 2003 study for incidence of renal cell cancer. Limited statistical power to detect an association with higher risk of the studied cancers

with acrylamide intake was a concern of the 2003 study, thus the data was re-visited. No positive association between acrylamide intake and increased risk of renal cell cancer existed (Mucci et al., 2004).

Increased incidence of mammary gland tumors in rats exposed to acrylamide prompted Mucci et al. (2005) to examine the relationship between acrylamide intake and increased risk of breast cancer in women. The study involved 43,404 Swedish women that were categorized based on acrylamide intake. The women were asked to fill out a semi-quantitative food questionnaire that asked about their intake of foods with higher acrylamide levels (coffee, fried potatoes, crackers, etc.). No evidence of association between acrylamide ingestion and increased risk of breast cancer existed.

The NTP (2011) reported various case-control and prospective cohort population-based studies have been conducted since 2010. The prospective cohort studies used case-cohort or nested case-control analyses along with food-frequency questionnaires to evaluate dietary exposure to acrylamide and the risks of cancer at specific tissue sites. Some of the prospective cohort studies include the Swedish Women's Lifestyle and Health Cohort, the Netherlands Study on Diet and Cancer, the U.S. Nurses' Health Study, and the Danish Diet, Cancer, and Health Study (NTP, 2011). The case-control studies also used food-frequency questionnaires and assessed cancer and dietary exposure of acrylamide among Swedish, French, and United States populations. Breast cancer was the main focus of the studies, however, the studies found no association between dietary exposure of acrylamide to increased risk of breast cancer (NTP,

2011).

Risk Assessment

Due to the lack of epidemiological evidence that dietary acrylamide increases the risk of cancer in humans, a number of regulatory agencies in the Sweden, Norway, Belgium, the Netherlands, and Europe are using risk assessment models to calculate theoretical risk. There is no standardized risk assessment model that is used by all agencies, but most regulatory agencies conducted risk assessment studies that used an average exposure level of 1 μg acrylamide/kg bodyweight/day in a 65-70 kg person. The study by the Norwegian group approximated an increased cancer incidence of 6 out of 10,000 individuals on average, with the increase for children a bit higher based on eating habits (Dybing and Sanner, 2003). Other estimates using this level of exposure estimated increased incidence of cancer in groups of 10,000 to range from 7 TO 45 (WHO, 1996).

The estimated average daily intake of acrylamide in μg/kg/bw/d from several studies was 0.46-0.49 (Dybing and Sanner, 2003), 0.46 (Konings et al., 2003), 0.5 (Svensson et al., 2003), and 0.3-0.8 (Mucci et al., 2003). The standard dose of 1 μg/kgbw/day used in the risk assessment studies is high compared to the estimated average daily intake. A few fundamental problems exist when using models to approximate dietary exposure levels: not all foods have been tested for acrylamide levels; acrylamide concentrations vary greatly in foods that have been tested, variation exists within the same batch of food (Friedman, 2003); and significant differences in exposures exist based on cultural eating habits in different countries

(Dybing et al., 2005). Foods with low levels of acrylamide could offer significant exposure based on the consumption volume (e.g., coffee) whereas foods with high levels of acrylamide may contribute very little exposure.

Chapter 3: Acrylamide Formation in Food Products

The Maillard Reaction

The Maillard reaction plays an integral part in improving the appearance and taste of assorted foods. The first proposal for the general pathways of the Maillard reaction was demonstrated by Hodge (1953). In an early stage a reducing sugar condenses with a compound containing a free amino group to give a condensation product, *N*-substituted glycoamine. The free amino group can be an amino acid in proteins (mainly the ε-amino group of lysine), or the α-amino groups of terminal amino acids. The condensation product rearranges to form the Amadori rearrangement product (ARP).

The succeeding degradation of the Amadori product is reliant on the pH of the system. Below pH 7, it undergoes 1,2-enolization mainly with the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At a pH higher than 7 the degradation of the Amadori compound involves mostly 2,3-enolization, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF), and an assortment of fission products, including acetol, pyruvaldehyde, and diacetyl are formed (see Figure 2.1). These compounds are exceedingly reactive and join in additional reactions.

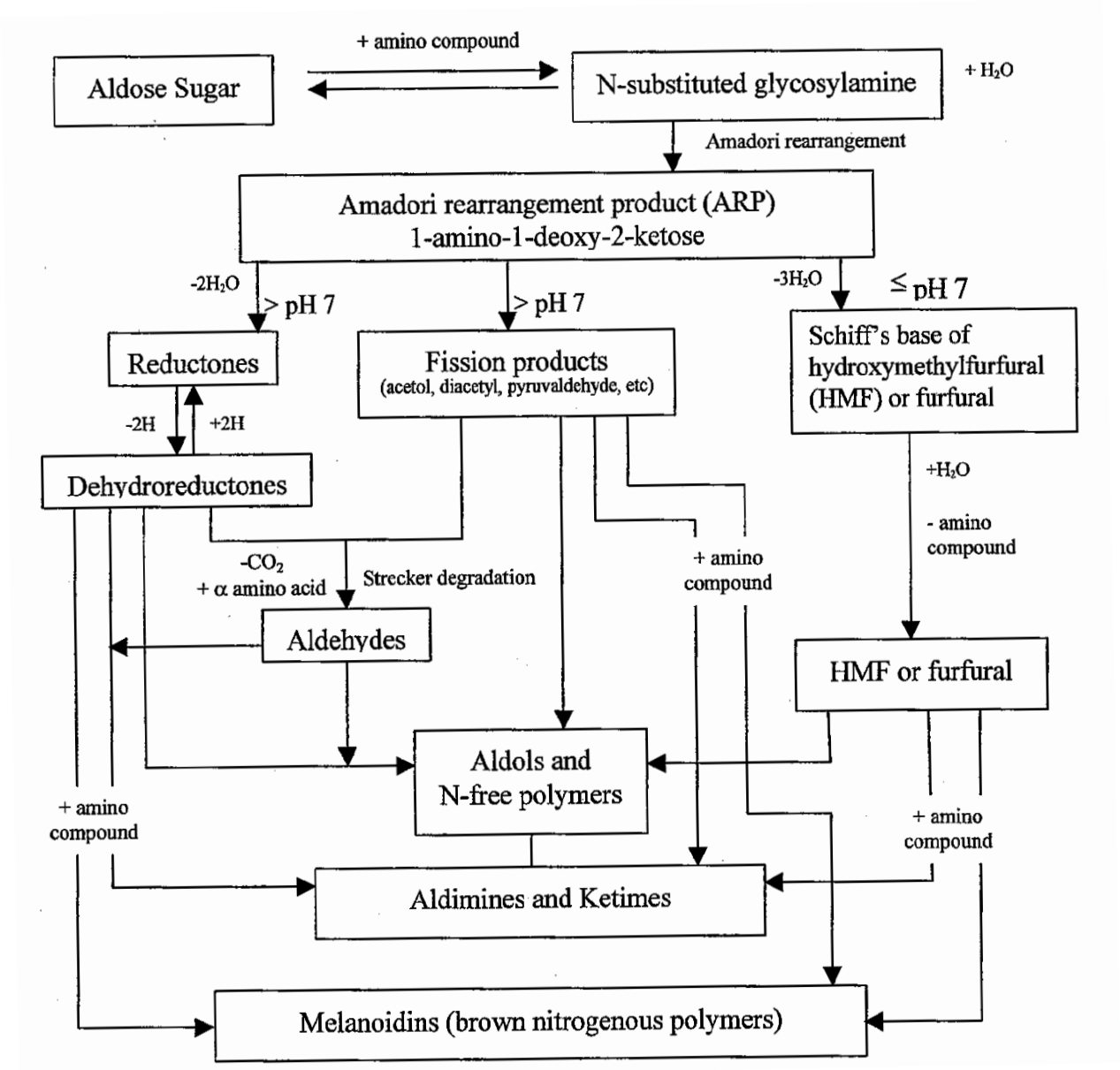

Figure 2.1: General Pathway of the Maillard Reaction (Hodge 1953)

Carbonyl groups condense with free amino groups, resulting in the inclusion of nitrogen into the reaction products. Dicarbonyl compounds react with amino acids to form aldehydes and α-aminoketones. This reaction is recognized as the Strecker degradation. Later, in an advanced stage, a series of reactions take place involving cyclizations, retroaldolizations, enolizations, rearrangements, further condensations, and isomerizations to the form melanoidins, or brown

nitrogenous polymers (Martins et al., 2001).

Findings from Mottram et al. (2002) demonstrated that the Maillard reaction involving asparagine could create acrylamide and might explain the higher concentrations of acrylamide in particular plant-derived foods after cooking. They also deduced projected pathways for the formation of acrylamide after Strecker degradation of the amino acids methionine and asparagine in the company of dicarbonyl products from the Maillard reaction. Research conducted by Stadler et al. (2002) showed that *N*-glycoside formation could be supported in food processing matrices that involved high temperature with water loss. When condensation occurs between reducing sugars and amino acids, a direct pathway is opened up to the prospective source of acrylamide.

Acrylamide Formation Mechanics

After the announcement of the discovery of acrylamide in heat-treated foodstuffs, several research groups in industry, academic schools, and laboratories instigated studies into the potential sources and subsequent mechanisms. Numerous hypotheses on formation pathways were discussed at the initial stages of research, focusing firstly on lipids and vegetable oils, since the predicament largely included carbohydrate-rich foods that were deep fat fried or baked. On the whole, some significant and direct precursors causing to the formation of acrylamide were 3-aminopropionamide, acrylic acid, and acrolein (Yasuhara et al., 2003), decarboxylated Schiff base (Zyzak et al., 2003), and decarboxylated Amadori product (Yaylayan et al., 2003).

In the beginning, parameters affecting the formation of acrylamide, including heating

time, heating temperature, pH, the ratio of amino acid and reducing sugar, etc. were the researchers' main focus. Heating equimolar amounts of asparagine and glucose at 180°C for 30 minutes resulted in the formation of 368 μmol of acrylamide per mol of asparagine (Stadler et al., 2002). The addition of water to the reaction mixture produced an increase of acrylamide up to 960 μmol/mol. A temperature-dependent study proposed that increased acrylamide formation occurs when processing temperatures range from 120 to 170°C.

Methionine formed about one-sixth the amount of acrylamide under the same conditions. Comparable temperature dependence was observed by Tareke et al. (2002) when lean beef, cod, lean pork, chicken, soy flour, potatoes, beetroots, and spinach were heated in a frypan, microwave, and boiling. Moderate amounts of acrylamide (5-50 μg/kg) were detected in cooked protein-rich foods and elevated levels (150-400 μg/kg) in carbohydrate-rich foods such as potatoes. However, Ezeji et al. (2003) showed that acrylamide is formed when starch is boiled or autoclaved. Other amino acids such as alanine, arginine, aspartic acid, cysteine, glutamine, methionine, threonine, and valine have been found to produce low amounts of acrylamide (Friedman, 2003).

The main method of acrylamide formation in foods is related to the Maillard reaction; especially when the amino acid asparagine is present (Mottram et al., 2002). Asparagine provides the backbone chain of the acrylamide molecule, and the connection of acrylamide to asparagine was determined by isotope labeling experiments (Zyzak et al., 2003). Mass spectral studies showed that the three carbon atoms and the nitrogen atom of acrylamide are all derived

from asparagine. The mechanism of acrylamide formation from a decarboxylated Amadori product of asparagine is shown in Figure 2.2. In contrast, lipid oxidation has been proposed as a minor pathway, with acrylic acid as a direct precursor formed by way of acrolein by oxidative degradation of lipids (Gertz and Klostermann, 2002).

The first step is the amino-carbonyl reaction between asparagine and a carbonyl substance (Figure 2.2). The reaction results in the corresponding *N*-glycosyl conjugation and formation of the Schiff base as a crucial intermediate after dehydration. Both the *N*-glycosyl conjugation and the Schiff base are relatively stable under low moisture conditions (Robert et al., 2004). However, in aqueous systems the Schiff base may hydrolyze to the precursors or rearrange to the Amadori compound, which is not an effective precursor in acrylamide formation (Yaylayan et al., 2003).

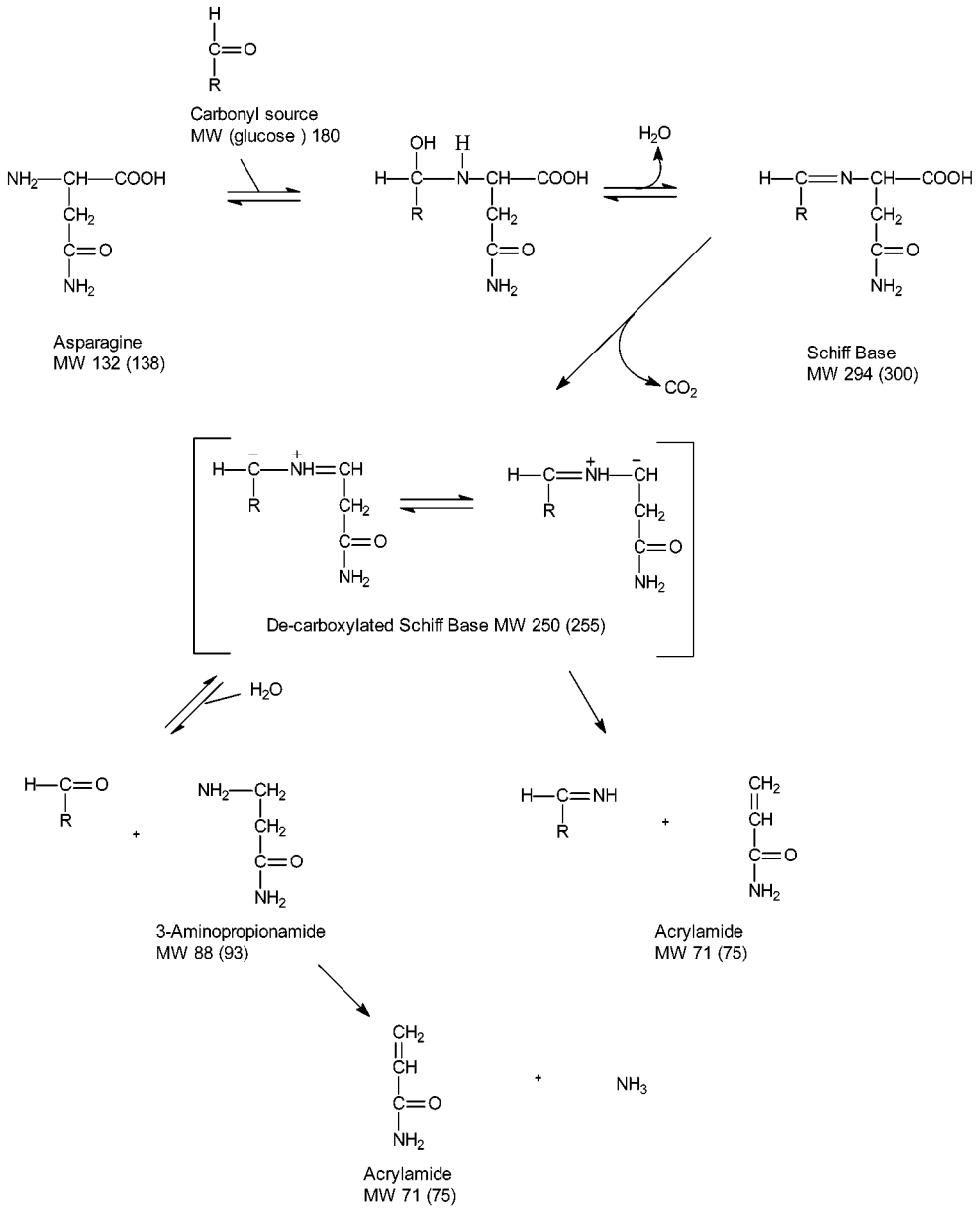

Figure 2.2: The Mechanism of Acrylamide Formation from a Decarboxylated Amadori Product of Asparagine (Mottram et al., 2002)

Even under low moisture conditions, this reaction is presumably the major pathway initiating the early Maillard reaction stage that leads to 1- and 3-deoxyosones, which decompose further, producing color and flavor. This is in accordance with the relatively low conversion yield of asparagine to acrylamide (Blank, 2005). The Schiff base also undergoes

decarboxylation either directly by means of the Schiff betaine or through the intermediary oxazolidine-5-one to generate the azomethine ylide I, which provides the decarboxylated Amadori product after tautomerization (Yaylayan et al., 2003).

In summary, acrylamide may be released via the following pathways: directly from azomethine ylide I (Zyzak et al., 2003); β-elimination reaction from the Maillard intermediate, i.e. decarboxylated Amadori product (Yaylayan et al., 2003); and loss of ammonia from 3-aminopropionamide deriving from the azomethine ylide II. Such reactions have shown to proceed favorably under aqueous conditions in the absence of sugars (Granvogl et al., 2004).

Besides the main precursors (amino acids and reducing sugars) and key intermediates, it is proposed that fat or oils are capable of playing an integral role in the acrylamide formation pathway. Oils can serve as the carbonyl source for the Maillard reaction. Yasuhara et al. (2003) investigated the effect of using triolein as a carbonyl reactant and heated it with asparagine to 180°C. Ammonia and glycerol were formed during the reaction and acrylamide was formed at the level of 88.6 μg/g of asparagine. Yasuhara et al. hypothesize that that when asparagine reacted with glycerol from the triolein. Figure 2.3 depicts the hypothesized pathway of acrylamide formation from lipids and amino acids.

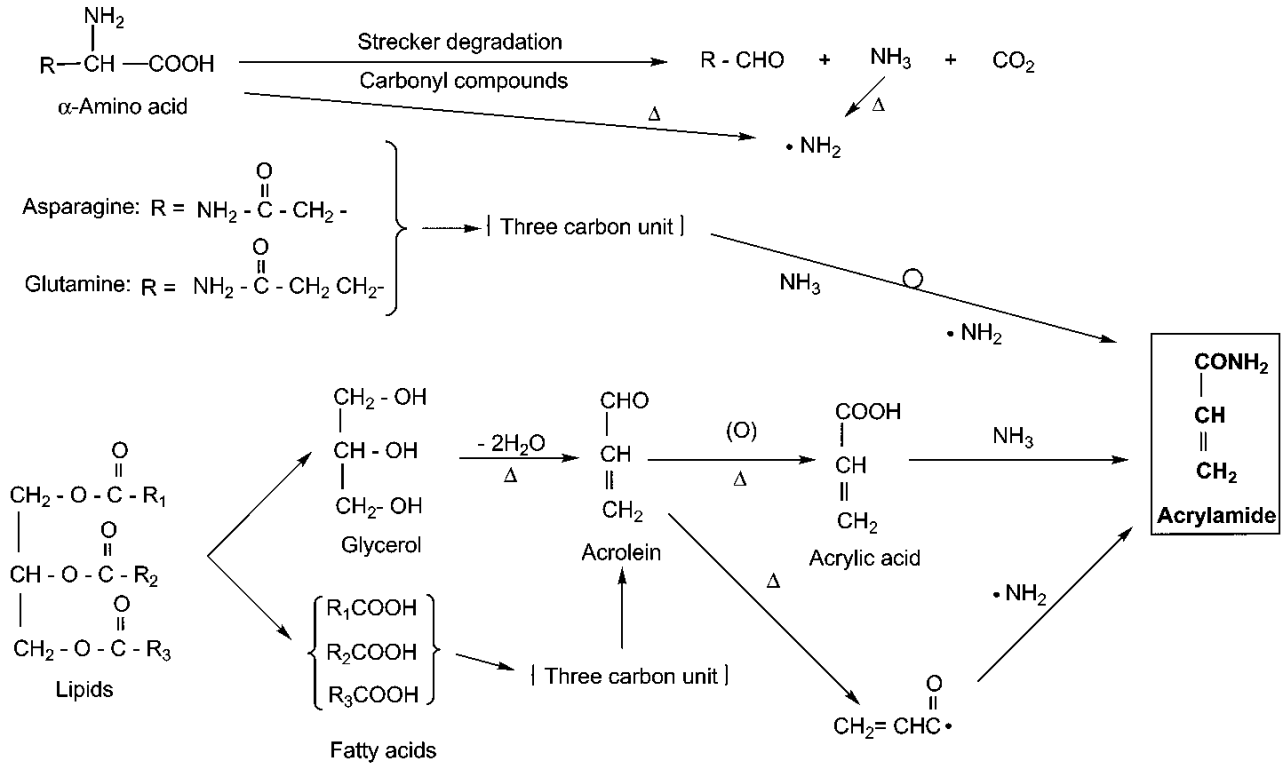

Figure 2.3: Proposed Formation Mechanisms of Acrylamide from a Lipid and an Amino Acid (Yasuhara et al., 2003)

Significance of Asparagine

In 2002, independent research groups presented the first tangible evidence of the role of asparagine in acrylamide formation (Stadler and Scholz, 2004). Experiments using ^{15}N-labeled asparagine and mass spectral studies showed that the three-carbon backbone of acrylamide and the amide nitrogen originated from analogous arrangements in the asparagine molecule (Becalski et al., 2003). Theoretically, structural significance determines that asparagine could be changed thermally into acrylamide through decarboxylation and deamination reactions. The major product of the thermal decomposition of asparagine is maleimide, mostly due to the rapid intromolecular cyclization reaction that inhibits the formation of acrylamide. However, in the presence of reducing sugars, asparagine can generate acrylamide along with maleimide

(Yaylayan et al., 2003). Exposing asparagine by itself to high temperatures does not cause the formation of considerable amounts of acrylamide (less than 1 ppb) (Yasuhara et al., 2003) and the early studies previously cited explained the requirement of reducing sugars for the reaction to advance, characteristically at temperatures above 100°C (Stadler and Scholz, 2004).

Controlling the biosynthesis of free asparagine in foodstuffs has the potential to be a useful method of mitigating acrylamide since asparagine has shown to be the major precursor. Researchers have investigated the asparagine level in a variety of plants. Martin and Ames (2001) found the level of free asparagine in potatoes to be about 939 mg/kg; the highest of any investigated plant. Variances in asparagine content have shown to be a good indicator of the change in nitrogen metabolism of plants (Friedman, 2003). A study performed by De Wilde et al. (2006) analyzed three different cultivars of potatoes that were raised and given three different nitrogen regimes. The results showed that free asparagine strongly correlates with the nitrogen availability in the soil. The results of suppressing genes that control the formation of enzymes required for asparagine biosynthesis remain unknown (Friedman, 2003).

Significance of Sugars

Reactions in asparagine model systems involving fructose, glucose, sucrose, sorbitol, glyceraldehydes, glycolaldehyde, or 2,3-pentanedione have been tested (Yaylayan et al., 2003). Acrylamide was formed at 250°C in each system, and the concentration of acrylamide increased with higher temperatures. At 350°C, the asparagine/sucrose system was shown to be more efficient than the asparagine/glucose system. The asparagine/2,3-pentanedione system was the

least effective at forming acrylamide; creating only trace amounts at both 250°C and 350°C (Yaylayan et al., 2003).

In a related study, Biedermann et al. (2002b) reported that fructose is more efficient than glucose when forming acrylamide in a potato model. Stadler et al. (2002) investigated various sugars and the different efficiencies in acrylamide formation. Lactose, galactose, fructose, and sucrose form acrylamide with similar yields. Although many researchers have found that acrylamide is formed when a specific amino acid reacts with a reducing sugar in the presence of heat, the reaction of sucrose and an amino acid in the presence of heat resulted in acrylamide formation comparable to the levels formed by fructose and glucose (Stadler et al., 2002). This could be due to the hydrolysis of sucrose under high temperatures into glucose and fructose, both of which are reducing sugars. In theory, one sucrose molecule could give rise to two reducing hexoses resulting in a molar ration of 2:1 sugar to amino acid (Taeymans et al., 2004). If sufficient reactants are present to react with the α-NH_2 group of asparagine by way of the Maillard pathway acrylamide can be formed.

Kinetic Studies

The kinetic study of acrylamide includes both the elimination and formation processes when the kinetics of the Maillard reaction are examined (Claeys et al., 2005b). The formation of acrylamide can be represented by a second-order reaction when the model system reaction involves asparagine and a reducing sugar because the two reactants are present at equimolar levels. The concentration of asparagine (C_{Asn}) and reducing sugar (C_{Sugar}) can be conveyed as

the concentration of reactant (C_R). Conversely, the elimination of acrylamide is observed as a first-order kinetic process. When observing the elimination of acrylamide by D_3-acrylamide, Biedermann et al. (2002) described the process in terms of first-order kinetics. However, although asparagine and sugar are both involved in the Maillard reaction, the sugar is used in caramelization reactions as well. In the Maillard reaction, reducing sugar loss occurs at a faster rate than amino acid loss. Thus, the kinetics of acrylamide formation relies on the concentration of sugar (C_{Sugar}). If the above statements hold true, the acrylamide yield (C_{AA}) can be described by a first-order formation/first-order elimination kinetic model and a resultant kinetic equation can be written as follows, with k_F, k_E, and t the formation rate constant, elimination rate constant, and the treat time.

$$\frac{d\,C_{AA}}{dt} = k_F C_R - k_E C_{AA} \qquad (1)$$

Equation (1) is especially useful in reflecting the kinetics of acrylamide under isothermal conditions and where K values are presumed to be constant. However, the cooking process of samples does not involve isothermal conditions; the temperature does not remain constant. Thus, an integrated effect of temperature on the reaction rate constant must be taken into consideration (Claeys et al., 2005). The Arrhenius equation demonstrates the effect of temperature on the reaction rate constant k, wherein the temperature reliance of k is quantified by the activation energy E_a (J/mol).

$$k = k_0 \exp\left[(E_a/R)((1/T_0) - (1/T)\right) \quad (2)$$

In the equation (2), R is the universal gas constant, T is the absolute temperature, k_0 is the

reaction rate constant, and T_0 is the reference temperature (Claeys et al, 2005). The kinetic parameters describing the formation and elimination of acrylamide can be predicted by nonlinear regression consistent with Gauss-Newton algorithm in the modeling phase.

Initially, a kinetic study exhibited acrylamide formation and elimination in the Maillard reaction (Claeys et al, 2005). However, precursor deterioration, the balance between formation and elimination of key intermediates, and the development of other important compounds like melanoidins are not able to be observed based on a kinetic study. The aim of a more in-depth kinetic study is to fill the aforementioned gaps. A simplified reaction system is shown in Figure 3.1 (Stadler et al., 2004). The kinetic rate constants, k_1, k_2, k_3, k_4, k_5, and k_6 are conveyed as loss of asparagine and glucose, formation of fructose, loss of fructose and asparagine, formation of acrylamide, formation of melanoidins, and elimination of acrylamide, respectively. At each reaction step, a differential equation was arranged by the use of the law of mass action, and the acquired differential equations were answered by numerical integration. The temperature reliance, which plays a crucial role in the Maillard reaction, was also considered by incorporating an Arrhenius relationship (Equation 2) among the rate constants (k) of the different reactions (Knol et al., 2005).

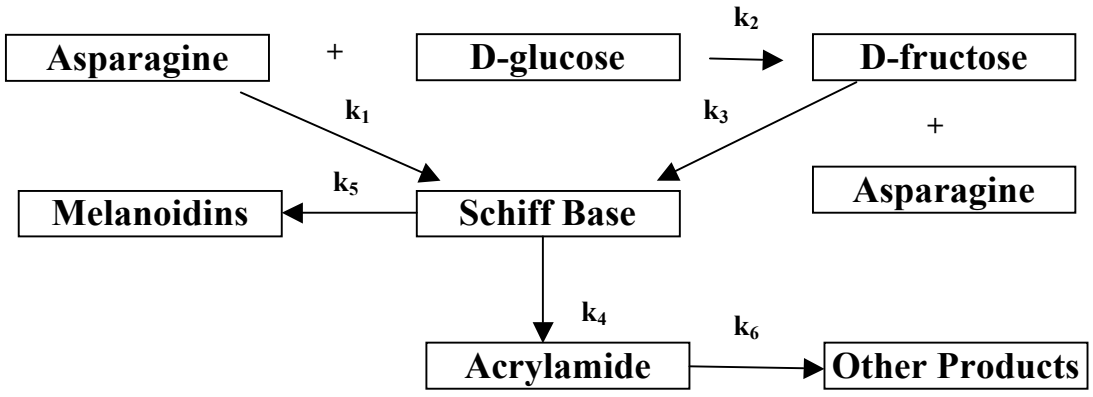

Figure 3.1: Simplified Acrylamide Reaction System (Stadler et al., 2002)

Model Studies in Potatoes

Potato tubers contain substantial amounts of free asparagine, glucose, and fructose, which helps explain the high levels of acrylamide in various potato products (Amrein et al., 2003). The Erntestolz cultivar was studied to observe the variance in reducing sugar concentration when the potatoes were stored at 4°C for 15 days. The reducing sugar content increased from 80 to 2250 mg/kg (fresh weight). Today, French fries serve as the primary source of acrylamide, and it is important to try to reduce acrylamide formation as much as possible. Laboratory trials and small scale tests have produced French fries with acrylamide levels below 100 μg/kg (Grob et al., 2003) by modifying the reducing sugar content of the raw product. Fiselier and Grob (2005) discovered that an average concentration of 50 μg/kg acrylamide in French fries could be achieved by controlling the reducing sugars in the potatoes to 0.7 g/kg and the frying temperature to 170°C.

Chapter 4: Agronomic Factors Affecting Acrylamide Formation

Acrylamide Precursors in Commercial Potatoes

Breeding or suppressing genes that program enzymes directing the biosynthesis of free asparagine in potato tubers could provide a raw product with lower asparagine content. Obviously, selecting a variety that inherently contains low levels of asparagine for dietary use provides another method to reduce acrylamide content.

Studies conducted by Elmore et al. (2007) and by Muttucumaru et al. (2006) highlighted three important factors for asparagine content in raw tubers. Soil with low sulfur content resulted in a reduction of acrylamide formation and augmented free amino acid and sugar levels. The raised reducing sugar levels resulting from the low-sulfur soils did not show a relationship with high acrylamide levels; however, free asparagine levels as a percentage of the total free amino acid composition did correlate with acrylamide levels. This information suggests that free asparagine is the rate limiting factor of acrylamide formation. When sugar content is low, competition between amino acids and asparagine as a reactant in the Maillard reaction is a key determinant of the quantity of acrylamide developed during processing.

Biedermann-Brem et al. (2003) studied the reducing sugar content of potatoes and how it relates to acrylamide formation during frying and roasting. They found that potatoes with less than 0.2 g/kg of fresh weight fructose and glucose have been found to be unsuitable for roasting because of insufficient flavor and browning. Roasted potato products containing more than 1 g/kg reducing sugars contain over 500 μg/kg acrylamide. Thus, potatoes used for roasting and

frying should contain less than 1 g/kg reducing sugars to reduce acrylamide levels. A lower level of reducing sugars can be achieved by avoiding cold storage of the raw potatoes (4°C or lower). Finally, the study showed that a 250 gram serving of hash browns or roasted potatoes could potentially have up to 1000 μg of acrylamide.

A related study conducted by Grob (2005) for Switzerland-grown potatoes reported that fructose was two times as effective as glucose and ten times more effective than lactose in creating acrylamide in potato flour. Roasted potatoes have the potential to contain more than 10,000 μg/kg acrylamide which greatly exceeds that acrylamide content of potato chips (about 500 μg/kg). Acrylamide levels were lessened when the products were fried at 170-175°C.

Fiselier and Grob (2005) found that limiting reducing sugars in raw potatoes for French fries is a straightforward and efficient measure to reduce the exposure to acrylamide from the predominant source for a majority of consumers. Meanwhile, Grob et al. (2003) showed that the acrylamide level in potato chips produced from potatoes stored at low temperature (<10°C) was much higher than chips produced from potatoes stored at high temperature (>10°C).

Another critical factor which affects acrylamide levels is the potato cultivar (Williams, 2005). The concentrations of fructose, glucose, sucrose, and asparagine in nine different potato varieties sold in Italy and twenty-two varieties sold in the United States were measured and compared (Vivanti et al., 2006). Fructose content varied from 1.73 mmol/kg of fresh weight for the Fingerling Ozette to 33.6 mmol/kg of fresh weight for the red variety, a 19.3-fold range. Glucose levels had a 31.3-fold variation; the levels ranged from 1.11 mmol/kg (Jelli) to 34.73

(Yukon Gold). Sucrose values varied greatly, ranging from 1.16 (Fingerling Ozette) to 40.6 (Marabel). Asparagine levels ranged from 1.17 (Agata) to 57.7 (Russet variety), a 49.3-fold range (Vivanti et al., 2006). Another study analyzing reducing sugar content in Irish potatoes showed an 80-fold variation in reducing sugar concentrations (Brunton et al., 2007). The wide ranges of precursor concentrations in various potatoes sold worldwide implies that varieties with low levels of precursors can be selected and utilized for commercial potato products.

Acrylamide Precursors in Other Potato Cultivars

Amrein et al. (2003) found that acrylamide formation in 74 different potato cultivars was directly related to the fructose and glucose levels of the potatoes. Asparagine content was not as influential on acrylamide formation. A variety of studies have been conducted on acrylamide precursors in various potato cultivars, and most of them have come to the same conclusions: acrylamide levels in fried potato products correlates with glucose and fructose levels (Chuda et al., 2003 and Williams, 2005); acrylamide levels can be reduced by using potato varieties that have low concentrations of reducing sugars (Becalski et al., 2004); and cooling of American potatoes to temperatures below 10°C brings about an increase in reducing sugar concentration and an increase in acrylamide levels in fried potatoes (Rydberg et al., 2003).

It should be mentioned that when measuring acrylamide precursors, especially reducing sugars, in raw potato varieties at a specific time may not be the proper indication of precursor levels. If the potatoes have been stored in cold temperatures they should be reconditioned after they are brought out of storage before processing (Friedman and Levin, 2008). It has been

suggested that potatoes should be labeled for their reducing sugar content to reduce the use of potatoes with high reducing sugar content for high heat processing/cooking (Grob, 2005).

Lowering the acrylamide precursors asparagine and/or reducing sugars glucose and or fructose is expected to result in reduced acrylamide formation. The following studies show that the balance of the precursors may be just as important a factor, if not more so. Use of these methods to reduce acrylamide must take into account consumer acceptance and expectations of a certain amount of browning for desirable flavor, color, and texture.

Potato Storage: Hydrolysis and Epimerization of Sugars

Fructose, glucose, sucrose, and starch are inherent in potato tubers. The proportions fluctuate based on postharvest treatment. These components take part in what is called cold-sweetening induced hydrolysis and epimerization, or inversion, reactions illustrated below in Figure 4.1 (Friedman and Levin, 2008).

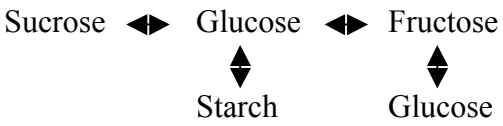

Figure 4.1: Cold Sweetening Reactions (Friedman and Levin, 2008)

The above pathways are reversible, cultivar-dependent, and are able to advance in any direction. The sugars and starch ratio stays balanced above 10°C; free sugars either become starch or are utilized in other reactions. Below 10°C, reducing sugars accrue in which they are available to participate in the Maillard reaction which ultimately results in acrylamide formation in the processed product. Because the pathways are reversible, the level of reducing sugars can be diminished via reconditioning of the potatoes at room temperature after cold storage,

usually at 4°C (Friedman and Levin, 2008).

Silva and Simon (2005) studied the glucose, fructose, sucrose, and asparagine contents of American potatoes and reported that when American potatoes are stored at 2°C glucose, fructose, sucrose, and asparagine levels are elevated. Fructose and glucose content correlate with resulting acrylamide formation in fried potato products but sucrose and asparagine contents do not. Preconditioning of the potato tubers before storing them at 2°C by decreasing the temperature by 0.2°C per day produced an outcome of significantly lower acrylamide levels in French fries. Silva and Simon (2005) noted that when high levels of nitrogen fertilizer were used on potatoes the potatoes contained higher levels of free amino acids.

A study on the storage of Japanese potatoes for 18 weeks at 2, 6, 8, 10, and 18°C conducted by Matsuura-Endo et al. (2006) resulted in the discovery that reducing sugar and acrylamide levels dramatically increased when potatoes were stored below 8°C. The study also showed that the levels of the reducing sugars correlated with acrylamide levels for potatoes with a fructose/asparagine ratio of less than 2, and for potatoes with ratios greater than 2 the asparagine, not the reducing sugar content, was the limiting factor for the formation of acrylamide. A similar study was conducted on Canadian and Swedish potatoes and showed that acrylamide formation during frying can be reduced by using potatoes with low sugar concentrations (Becalski et al., 2004), and asparagine levels in Swedish potatoes increased during storage while asparagine levels of Japanese potatoes remained stagnant (Olsson et al., 2004). The above studies imply that potato varieties all over the world are different in their

vulnerabilities to structural change during storage.

Effect of Surface-to-Volume Ratios of Potato Tubers

Acrylamide levels in deep fat fried potato products depend on the storage temperature, surface to volume ratio, reducing sugar level, and temperature and time of processing. An associated study on the effect of processing parameters on acrylamide formation during frying of potatoes indicated that acrylamide content constantly increased with increasing temperature from 120-230°C for potatoes with low surface-to-volume ratios. Acrylamide levels also increased in these potatoes with increased frying time, reaching maximum levels of 2500 µg/kg (Taubert et al., 2004). Low-fat potato snacks have been found to contain approximately 489 µg/kg acrylamide which is one-third of the reported value for potato chips (Majcher and Jelen, 2007).

On the contrary, potatoes with medium to high surface-to-volume ratios, maximum acrylamide levels of 18,000 µg/kg for high surface-to-volume ratios grated potatoes were reached at 160-180°C. Higher temperatures and longer processing times resulted in lowered acrylamide levels in potatoes with higher surface-to-volume ratios. This could indicate that the acrylamide formation reaction is nearly complete and supplementary treatment leads to other reactions or degradation.

Chapter 5: Modification of Processing Parameters for Acrylamide Mitigation

The effects of temperature and time, the influence of water activity, the formation of final products, and recognizing the rate-limiting steps in food matrix systems are all important aspects in acrylamide mitigation techniques (Blank, 2005). Controlling specific processing parameters such as heating temperature, heating time, and oil type could be looked upon as the most direct way to reduce acrylamide. Acrylamide formation from asparagine is temperature dependent and is favored above 100°C; very high temperatures are not necessary for acrylamide formation (Mottram et al., 2002). A simple linear increase between heating time and the acrylamide level does not exist, but it has been noted that acrylamide concentrations increase with heating time. Additionally, foods fried or cooked using palm olein or oils containing silicone were found to contain much higher levels of acrylamide than foods which were not cooked in those mediums (Gertz and Klostermann, 2002). Low temperature heating, like low temperature vacuum frying, short time heating, and not using palm olien during processing could result in lower acrylamide levels in food.

Mechanisms of Acrylamide Reduction

Acrylamide reduction can be attained by preventing the Schiff base of *N*-glycosylasparagine from stabilizing itself through intramolecular cyclization instigated by the carboxylate anion and formation of oxazolidin-5-one (Manini et al., 2001). Two significant stabilization forms of the Schiff base of *N*-glycosylasparagine exist, Amadori rearrangement and intramolecular cyclization (Yaylayan et al., 2003). The Amadori product removes the carboxylic

acid via intramolecular cyclization at increased temperatures and produces an Amadori product with *N*-substituted succinimide. The main residue generated from the amino acid moiety identified in the pyrolysates of asparagine model systems was succinimide. This pathway, similar to that occurring in asparagine alone, stops the formation of acrylamide. According to the means of competitive inhibition, such formation pathway should be encouraged because it allows the Amadori product to decarboxylate and inhibits the intramolecular cyclization of the Schiff base (Yaylayan et al., 2003).

Moisture Content

Water plays a multifarious part in acrylamide formation and elimination in potato products. When moisture levels in the potato are low, the activation energy for acrylamide formation is larger (calls for more heat) than the equivalent activation energy for browning to occur (Amrein et al., 2006). During high-heat processing, acrylamide formation in products with higher water activities is elevated compared to products with very high moisture levels, in which the rate of browning and acrylamide formation are slower. The rate constant for acrylamide formation in a glucose/asparagine model system fluctuated slightly with initial water activity, but the acrylamide elimination rate was at a minimum at the water activity level of 0.82. This is the same water activity level at which the Maillard reaction rate constant was highest (De Vleeschouwer et al., 2006). This suggests that the formation and elimination of acrylamide and Maillard reaction products can be influenced in different ways by the water activity of the product. It could be possible to disengage the concurrent reactions by controlling moisture;

desirable browning could be accomplished without a corresponding increase in the level of acrylamide.

Frying Conditions

Heating parameters have a direct influence on acrylamide formation in fried potatoes. Commercial White-Rose potatoes fried at 165°C for 4 min at atmospheric conditions contained 5021 μg/kg acrylamide; Atlantic potatoes, 646 μg/kg; and Shepody potatoes, 466 μg/kg (Granda et al., 2004). This study also illustrated that vacuum frying at 118°C caused a 94% reduction in acrylamide levels of potato chips compared to normal frying conditions at 165°C. Acrylamide levels ranged from 50 to 1800 μg/kg in 66 potato samples which were fried at 180°C for 3.5 minutes (Becalski et al., 2004). These results imply that considerable reductions in acrylamide concentrations can be accomplished by using varieties that produce low levels of acrylamide during high heat processing, i.e. have low levels of reducing sugars.

A comprehensive study on processing conditions that mitigate acrylamide formation in deep-fried potato products was conducted by May et al. (2006). They found that blanching raw potato chips in hot water for 1-3 minutes at 95°C exponentially decreased the reducing sugar concentration in the chips by 51-62% of the original levels. Unblanched shallow-fried chips cooked at 180°C contained 667% more acrylamide than blanched deep-fried chips, whereas blanched shallow-fried chips cooked at 180°C contained 406% more acrylamide than blanched deep-fried chips. The study also showed that the acrylamide formation rate as a function of temperature in the range of 140-180°C increased linearly with an inflection point at 165°C.

May et al. (2006) found that the frying temperature was more influential on acrylamide formation at temperatures around 160°C and the frying time was more influential at temperatures around 170°C. It was also discovered that shallow frying causes sizeable increases in acrylamide levels compared to deep-frying. Also, simply lowering the temperature of the frying oil from 190°C to 150°C has shown to significantly diminish acrylamide formation (Pedreschi et al., 2007).

Studies on the effects of various cooking oils on acrylamide formation in potato chips and French fries have indicated that the cooking oil type (canola, cotton seed, olive, peanut, safflower, shortening, soybean, and sunflower) is not a significant variable for acrylamide formation, with the exception of olive oil. Use of olive oil produced a 300% increase in acrylamide content compared to control potato chips cooked in corn oil (Becalski et al., 2003). Olive oil has distinct characteristics that distinguish it from other commercial oils. It is less refined and not normally used for frying. It was shown to significantly increase acrylamide formation in potato products (Becalski et al., 2003). The FDA has indicated that olives, both black and green, have relatively high levels of acrylamide (FDA, 2011). Experiments involving adding olive oil to potatoes before cooking at a lower temperature (150°C) for 30 minutes did not result in an increased acrylamide level compared to the control (Biedermann et al., 2002). This shows that acrylamide formation is more dependent on the cooking temperature rather than the type of oil used in cooking.

Another important factor related to acrylamide formation in fried products is fat oxidation

products. The physical affects that influence chemical reactions leading to acrylamide formation at high temperatures may be due to fat oxidation products. Gertz and Klostermann (2002) suggest that the presence of silicone in palm oil increased acrylamide formation due to the increased heat transfer.

Pectinmethylesterase Activation

Previous studies using potatoes as a model system have resulted in the discovery that introducing polyanionic substances into glucose and asparagine Maillard reactants significantly reduces acrylamide during frying (Lindsay and Jang, 2005). Pectic acid formation via low temperature blanching (50-75°C) activates pectinmethylesterase and firms the texture of vegetable materials (Bartolome and Hoff, 1972). When this technology is applied to the processing of fried potato products low temperature blanching was found to inhibit acrylamide formation. It is thought that pectic acid-containing polymers surround the cell matter and confine Maillard reactive ionic substances as they attempt to diffuse from the thermally injured cells (Lindsay and Jang, 2005).

Table 6.1: Use of Pectinmethylesterase Activation (70°C blanch and 30 min hold) Processing for Acrylamide Reduction in Potato Chips (Lindsay and Jang, 2005)

Sample Treatment	Acrylamide (µg/kg; ppb)	Reduction
Cut, wash, hold in deionized water at 21°C for 30 min	661	--
Cut, wash, blanch in deionized water at 70°C for 30 min, hold in deionized water at 21°C for 1 min	59	91%

Typically, French fries are blanched in water at 80-84°C for 1-4 minutes to inactivate the browning enzyme polyphenoloxidase and to leach sugars from the potato pieces. Blanching at

80-84°C for 1-4 minutes is not as effective for acrylamide reduction because at the elevated blanching temperatures pectinmethylesterase is thermally inactivated (Bartolome and Hoff, 1972).

Table 6.2: Use of 70°C Blanch/Hold Plus Sodium Chloride/Sodium Acid Pyrophosphate (NaCl/SAPP) Processing for Suppression of Acrylamide in French Fry Strips (Lindsay and Jang, 2005)

French Fry Treatment	Acrylamide (µg/kg; ppb)	Reduction
Cut and wash, finish fry immediately	489	--
Cut, wash, blanch 80°C for 4 min; soak in 1.5% NaCl + 0.5% SAPP at 21°C for 3 min; par-fry 190°C for 30 sec; freeze; finish fry 190°C for 1.75 min	141	71%
Cut, wash, blanch 70°C for 30 min; soak in 1.5% NaCl + 0.5% SAPP at 21°C for 3 min; par-fry 190°C for 30 sec; freeze; finish fry 190°C for 1.75 min	30	95%

Blanch and Soak in Acid Solution

Various studies have tested the effectiveness of soaking potato strips/chips in acidic solutions to mitigate acrylamide formation. Soaking raw potato chips in an acetic acid solution for 60 minutes at 20°C before frying caused a 90% decrease in acrylamide levels (Lindsay and Jang, 2005). The pretreatment most likely extracted free amino acids and sugars that partake in the Maillard reaction (Kita et al., 2004). Immersing potato strips in distilled water and in a citric acid solution decreases acrylamide formation after frying. Long-time blanching (50°C for 80 min or 70°C for 45 min) has shown to be one of the most effective acrylamide mitigation techniques. Blanching removes more glucose and asparagine than simply soaking in water (Pedreschi et al., 2007).

A linear relationship exists between the color of the finish fried potato product and the level of acrylamide in the product. The amount of surface browning directs the final acrylamide

concentration in French fries. The potency of surface browning, therefore, seems to be a good indicator for estimating acrylamide formation in French fries during frying (Rydberg et al., 2003).

Protective Effects of Metal Ions

Other researchers have investigated the effects of various metal ions on acrylamide formation. Kolek et al. (2006) found that adding 1% NaCl to a heated asparagine/glucose model system significantly limited acrylamide formation. Gökmen and Senyuva (2007) dipped potatoes into a calcium chloride solution and the resulting product had acrylamide levels that were 5% of the control. In a fructose/asparagine system calcium chloride completely inhibited acrylamide formation whereas sodium chloride partially mitigated acrylamide. The Ca^{2+} ions may inhibit the formation of the intermediate Schiff base that is integral in acrylamide formation.

Use of Chelators

Chitosan is polymer of D-glucosamine and N-acetyl-D-glucosamine units that could be used to block the carbonyl groups of neutral reducing sugars on the cut potato surface. Lindsay and Jang (2005) reported that chitosan treatments alone did affect acrylamide formation in fried potato products, but chitosan is more effective when used in tandem with other acrylamide reducing methods. Table 6.3 depicts the results of a study which employed low temperature blanching of potato slices then holding the slices in a 1000 ppm chitosan solution.

Table 6.3: Use of Pectinmethylesterase Activation (70°C blanch) and Chitosan Surface Submersion Processing to Block Reducing Sugar Carbonyl Groups to Reduce Acrylamide Formation (Lindsay and Jang, 2005)

Sample Treatment	Acrylamide (µg/kg; ppb)	Reduction
Cut, wash, and fry	317	--
Cut, wash, blanch in 5% NaCl deionized water at 70°C for 1 min, hold in deionized water at 21°C for 1 min, fry	159	50%
cut, wash, blanch in 5% NaCl deionized water at 70°C for 1 min, hold in deionized water at 21°C for 1 min, hold additional 3 min in 1000 ppm chitosan at 50°C, fry	37	88%

The results showed that significant acrylamide reduction was achieved in the finished chips. Using acetic acid in the solubilization of chitosan did not produce perceptible acid carryover flavors and the chips had a very crisp texture. It is possible that the amino groups on the chitosan monomer units react with reducing sugars to bring the mobilization of sugar molecules to a standstill (Lindsay and Jang, 2005).

In 2002, the FDA analyzed the acrylamide content of grain-based foods such as bagels, biscuits, cereals, cookies, breads, tortillas, wheat-based snacks, etc (FDA, 2011). The study showed that wheat and corn-based products contain relatively low acrylamide levels compared to products made from potatoes (OEHHA, 2011). Phytate is a naturally occurring chelator and is present in relatively high levels in corn and whole wheat products. Phytate has been known to bind multivalent cations and inhibit the absorption of the cations in the intestine. The acrylamide formation reaction involves an interaction between asparagine and carbonyl compounds and the existence of a phytate ion may hinder the reactions necessary for acrylamide formation. Calcium ions may enhance the effects of phytate by forming phytate and calcium ion complexes.

Park et al. (2005) tested the effects of phytate and calcium treatments in a French fry

model and employed the treatments in blanching and/or soaking. The most effective treatment involved phytate plus calcium chloride blanching followed by a calcium chloride soaking treatment and resulted in approximately a 60% reduction of acrylamide compared to the control. Simply blanching in acidified water (pH 3) did not significantly lower acrylamide levels; phytate and calcium were needed for a significant reduction. Soaking the strips in calcium chloride or calcium chloride plus phytate provided significantly reduced acrylamide levels, but soaking in phytate alone did not produce significant results. Further testing needs to be done before a definitive statement that acrylamide reduction in a potato model involving phytate and calcium is due to chelation rather than ion complexation between intermediates can be made.

Effects of Acidulants, Preservatives, and Acid pH

Park et al. (2005) investigated the effects of other acidulants and preservatives on acrylamide formation in potato chip and French fry models. The pH levels of the various solutions are displayed below in Tables 6.4 and 6.5. The solutions for the French fries contained 1.5% salt and 0.5% sodium acid phyrophosphate (SAPP), thus the pH levels were slightly different than those for the chips.

Table 6.4: The Effects of Various Acidulants on Acrylamide Formation (Park et al., 2005)

Acidulant	Chips Acrylamide (% of control)	Chips Solution pH	Fries Acrylamide (% of control)	Fries Solution pH
Adipic acid (1%)	$79^a \pm 11$	2.54	$78^a \pm 16$	2.88
Fumaric acid (sat. about 0.6%)	$24^a \pm 4$	2.16	$28^a \pm 8$	2.25
Gluconic acid (1%)	111 ± 7	2.58	$71^a \pm 9$	2.72
Malic acid (1%)	$39^a \pm 5$	2.22	$54^a \pm 21$	2.33
Phosphoric acid (1%)	$40^a \pm 9$	1.72	$25^a \pm 9$	1.68
Succinic acid (1%)	$55^a \pm 1$	2.43	$50^a \pm 13$	2.68
Tartaric acid (1%)	$37^a \pm 6$	2.09	$29^a \pm 14$	2.12

[a] Potato slices for the chips were soaked in solutions for 20 minutes, dried, and fried at 177°C for 90 sec. Fries were cut 10 mm thick and 76 mm long without the skin, blanched at 80°C for 10 minutes, soaked in solutions for 10 minutes, dried, par-fried at 191°C for 1 minute, frozen overnight and re-fried at 171°C for 2:45. Corn oil was used as the frying medium for both chips and fries. Numbers are mean ± standard deviation errors of two independent experiments . Means with superscripts are significantly different from corn oil control ([a] indicates lower).

Each of the tested acidulants, except gluconic acid in chips, significantly reduced acrylamide levels in the potato model compared to the control. Tartaric, phosphoric, and fumaric acids were particularly effective in both chips and fries and had a pH value close to 2. One possible explanation for the ineffectiveness of gluconic acid may be related to the conversion of gluconic acid to δ-gluconolactone at high temperature (Mestdagh et al, 2008).

Table 6.5: The Effects of Various Preservatives on Acrylamide Formation (Park et al., 2005)

Preservative	Chips Acrylamide (% of control)	Chips Solution pH	Fries Acrylamide (% of control)	Fries Solution pH
Benzoic acid (1%)	$57^a \pm 18$	2.85	$55^a \pm 3$	2.87
Propionic acid (1%)	$78^a \pm 10$	2.68	$65^a \pm 22$	2.88
Sorbic acid (1%)	n.d.	2.75	$88^a \pm 4$	3.36
Methyl hydroxybenzoates (0.025%)	$177^b \pm 18$	4.16	$73^a \pm 1$	4.16
Ethyl hydroxybenzoates (0.025%)	$60^a \pm 20$	4.23	$88^a \pm 31$	4.15
Propyl hydroxybenzoates (0.025%)	$65^a \pm 4$	4.51	107 ± 40	4.16

[a] See Table 2 legends for experimental conditions. Numbers are mean of two independent experiments. Numbers with superscripts are significantly different from corn oil control ([a] indicates lower, [b] indicates higher). n.d.: not determined.

Among the tested preservatives, benzoic, propionic, and sorbic acids lowered acrylamide

formation in chips and fries. Only ethyl hydroxybenzoates consistently resulted in acrylamide reduction among the derivatives of hydroxybenzoates. The inconsistencies of the hydroxybenzoate derivatives is not clear, but may be due to the elevated pH level of the solutions (above pH 4).

Acrylamide formation and elimination in phosphate and citrate buffers at various pH values as a function of temperature and time showed four distinct observations (De Vleeschouwer et al., 2006). Maximum acrylamide concentrations are achieved by elevating processing temperatures for shorter periods of time. Acrylamide content decreases after prolonged heating of temperatures above 160°C, followed by elimination at final stages of heating at temperatures around 180-200°C. Increased acidity increases the temperature dependence of the reaction rate constants, and the reaction rate constant at 160°C decreased 10-fold by decreasing the pH from 8 to 4 (De Vleeschouwer et al., 2006).

Other studies conducted by Rydberg et al. (2003) tested the effect of pH on acrylamide formation. In the pH range of 6 to 10, the rate of acrylamide formation increased until the pH value of 8, then declined. By lowering the internal pH of potatoes from 5.72 to 2.96 the acrylamide content was decreased by 70%. One study showed that using citric acid caused a concentration-dependent decrease of up to 50% (Biedermann et al., 2002). The effect of phosphoric, fumaric, and tartaric acid on acrylamide formation in French fries was investigated by Park et al. (2005). Acrylamide levels were reduced by 25% with phosphoric acid, 28% with fumaric acid, and 29% with tartaric acid.

The above studies indicate that lowering the pH below 6 can decrease acrylamide formation in potatoes. The ensuing decrease in acrylamide levels may be due to the protonation of the reactive free α-NH_2 group of asparagine to the nonreactive α-NH_3^+ form as well as an acid-catalyzed hydrolysis of asparagine to aspartic acid and of acrylamide to acrylic acid. Although a low pH system shows promising results for acrylamide mitigation, lowering the pH of the food matrix may have adverse effects on the flavor and taste of the food.

Stadler et al. (2003) demonstrated that lowering the pH of the system can potentially reduce acrylamide formation in food and model systems. By lowering the pH of the food system, the α-amino group of asparagine is protonated which consequently is unable to engage in nucleophilic addition reactions with carbonyl sources (Jung et al., 2003). Cook and Taylor (2005) reported that the effect of citric acid alone was a 23.5% reduction in acrylamide at pH 4.48 (product pH lowered by 1.05 units) and 47% reduction at pH 3.93 (product pH lowered by 1.6 units). The correlation between pH decrease and acrylamide reduction may vary among products due to different initial pH values of the products as well as multiple other factors.

Use of Amino Acids, Proteins and Their Hydrolysates

Addition of the free amino acids cysteine, glycine, and lysine to potatoes before processing has shown to reduce acrylamide levels in potato chips. When potatoes are heated in an oven at 180°C for 25 minutes, the addition of non-asparagine amino acids noticeably reduce acrylamide formation, which is most likely due to competitive consumption of precursors and/or improved elimination/degradation (Rydberg et al., 2003). The study showed that adding 140

mmol/kg of the following acids resulted in the corresponding percentage decreases: glutamine, 70; alanine, 50; lysine, 88; and glycine, 91. When asparagine was added to the potatoes, acrylamide formation jumped by 290%. When cod meat is added to grated potatoes, acrylamide levels decreased by 70% compared to grated potato patties without cod meat (Rydberg et al., 2003). The decrease may be due to a protective protein action, perhaps via a reaction with nucleophilic groups on amino acid side chains.

Other researchers have investigated the effects of lysine, glycine, cysteine on acrylamide formation in potato products. Kim et al. (2005) reported that a 0.5% addition of glycine to potato snack pellets resulted in a 70% reduction in acrylamide. The authors also found that soaking potato slices in a 3% solution of glycine or lysine reduced acrylamide levels in potato chips that were fried for 1.5 minutes at 185°C. The outcome of the study implies that soaking or dipping potato slices in an amino acid solution prior to frying can decrease acrylamide levels.

It is proposed that glycine and lysine compete with asparagine for the carbonyl groups of the reducing sugar and/or form adducts with acrylamide after it has been formed (Friedman, 2003). In theory, the sulfur groups of cysteine or other thiols can lower acrylamide by forming an adduct with acrylamide or go through heat-induced H_2S elimination to form dehydroalanine [CH_2=CH(NH_2)COOH]. The NH_2 group of asparagine can participate in addition reactions with the double bond of dehydroalanine, as it would with acrylamide (Friedman, 1978).

Schabacker et al. (2004) studied the reduction of acrylamide uptake by dietary proteins in the Caco-2 gut model. The Caco-2 gut model utilizes heterogeneous epithelial coleorectal

adenocarcinoma cells that, when cultured correctly, resemble human enterocytes in the small intestine (Hidalgo et al., 1989). It was discovered that although acrylamide monomers easily diffuse through Caco-2 monolayers, acrylamide absorption from food in the human intestine may differ from these experimental conditions. Molecular acrylamide contains an active double bond, which may interact with a variety of food ingredients, including proteins, DNA and RNA. *In vitro* incubation of acrylamide together with glutathione at pH 8 significantly reduces the amount of acrylamide monomers to 81%. A higher availability of cysteins (molar ration 1:10; acrylamide/glutathione) led to 48% reduction of acrylamide because acrylamide is most likely covalently bound to glutathione via Michael addition of cysteine residues to the terminal double bond (Schbacker et al., 2004). The addition of glycine or glutamine during blanching of potato crisps reduced the amount of acrylamide by about 30% compared to the control (Claeys et al., 2005c). Cui et al. (2005) confirmed similar results via in vitro scavenging reactions of acrylamide with glutathione.

The degradation mechanism of acrylamide in vitro by glutathione is mainly via the decomposition of glycine fragment of glutathione. The glycine moiety can be removed more readily by the cleavage of peptide bonding in the presence of acrylamide. The glycine in the solution is degraded by Strecker degradation to aldehyde, ammonia, and carbon dioxide. This degradation product, such as formaldehyde, reacts with acrylamide, and leads to the decomposition of acrylamide to small molecular fragments. Acrylamide can then be transformed to acrylate which is subject to consecutive decarboxylation and further total oxidation by the

catalytic process of degraded products of glycine (Cui et al., 2005). This scavenging reaction is similar to the catalytic total oxidation of acrylamide in the presence of water via the formation of acrylate (Hawrylak and Szymanska, 2004). As for plant-derived proteins, soy protein hydrolysate can also be used to reduce acrylamide due to the fact that soy protein hydrolysate is believed to reduce acrylamide by introducing additional amino acids to compete with asparagine for key reaction intermediates (Cook and Taylor, 2005).

Effect of Antioxidants

Over the past few years, many correlative tests have been executed on the relationship between acrylamide formation and various antioxidants and both positive and negative effects on acrylamide reduction have been reported. Taeymans et al. (2004) reported decreased acrylamide levels in potato slices that were fried in oil the contained added rosemary extracts. Zhang et al. (2005a) demonstrated that acrylamide levels in a variety of heat-treated foods were effectively reduced when the antioxidant of bamboo leaves was added to the foods. The antioxidant of bamboo leaves is a new flavones-rich extract and newly certified as an innovative type of natural antioxidant for the application of food additives by the Ministry of Health in P.R. China. Soon after such findings, the results from similar tests demonstrated that the antioxidant of bamboo leaves could also reduce acrylamide to a different extent when combined with other plant-derived extracts such as ginkgo biloba extracts, tea extracts, grape seed extracts, etc (Zhang et al., 2005b). The chosen plant-derived extracts also included some antioxidants such as rosemary and liquorice extracts (Zhang et al., 2005b).

Fernandez et al. (2003) reported lower acrylamide levels after adding Flavomare®, a mixture of flavanoids, to potato slices. The flavanoids mix was added to potato slices prior to frying and a powder mix was added to the slices after frying. The acrylamide levels were analyzed after four days and the levels were reduced by up to 50% (Kurppa, 2004). Biedermann et al. (2002b) found a relatively weak reduction of acrylamide formation by the addition of ascorbic acid to potato slices. Similar results were obtained by Levine and Smith (2005) when using ascorbate as the additive. It is still difficult to obtain confirmatory conclusions on either positive or negative relationship between the addition of antioxidants and the reduction of acrylamide.

Chapter 6: Future Research and Concluding Remarks

The Maillard reaction is a flow of successive and parallel reaction steps and the intricacies of the reaction have been delineated in publications (Martins et al., 2001). Henceforth, elemental studies, particularly kinetic research during the procession of the Maillard reaction, should be further investigated. Important information regarding the formation and elimination of acrylamide can be derived from the relevant kinetic parameters and insight into reaction mechanisms can be obtained through such studies. The system via azomethine ylides and decarboxylated Amadori products clarifies the propensity in acrylamide formation found experimentally. However, more research needs to be completed to better understand the role of water and the physical state of the food matrix (amorphous versus crystalline) in acrylamide formation (Blank, 2005).

Conversely, the presence and chemical reactivity of precursors combined with optimized processing parameters are important factors for minimizing acrylamide formation (CIAA, 2005). Achieving substantial reduction while maintaining necessary product attributes, namely flavor and color, that are developed during the Maillard reaction and similar pathways will be a great challenge. Even though many acrylamide reduction techniques have been identified, the consequential effects on sensory attributes in most reduction studies have not been reported. Hence, food products containing reduced acrylamide levels and acceptable texture, flavor, and color has yet to be developed and the relationship between acrylamide reduction and product attributes should always be considered.

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