EFFECTS OF NITROGEN TREATMENTS AND PROCESSING CONDITIONS ON ACRYLAMIDE FORMATION IN POTATO CHIPS OR FRENCH FRIES

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DEDICATION

This thesis is dedicated to my parents

ABSTRACT

The aim of this work was to examine the effect of frying conditions and blanching with different additives on the acrylamide content in potato chips. Furthermore, acrylamide formation in French fries was examined in relation to different nitrogen fertilization strategies and potato strip size. Frying potato slices at 160°C for 7 min mitigated the acrylamide formation in potato chips processed from Atlantic, Snowden and Vigor varieties by 84, 67 and 78%, respectively, compared to potato slices fried at 190°C for the same frying time. The most effective reduction (19-59%) was obtained when potato slices were blanched in distilled water for 5 min at 65°C. This study showed that different nitrogen treatments can affect the formation of acrylamide in French fries and its precursor contents in potatoes. Decreasing the surface-to-volume ratio by creating thicker potato strips reduced acrylamide content in French fries processed from Russet Burbank, Ranger Russet, and Shepody varieties.

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LIST OF ABBREVIATIONS

3- APA = 3- Aminopropamide
ANOVA = Analysis of variance
b.w. = Body weight
CONTAM = Contaminants in the Food Chain
DW = Dry weight
ESN = Environmentally smart nitrogen
FW = Fresh weight
GC = Gas chromatography
IARC = International Agency for Research on Cancer
LC = Liquid chromatography
MB= Medium bound levels
MeCN= Acetonitrile
MOE = Margin of exposure
MS = Mass spectrometry
N = Nitrogen
ppb = Parts per billion
r = Correlation coefficient
SD = Standard déviation
SPE= Solid phase extraction
SVR = Surface -to-volume ratio
WHO = World Health Organization

CHAPTER 1: INTRODUCTION

1.1. Background

In April 2002, acrylamide was first discovered by Swedish researchers in starch-rich foods processed at high temperature (>120°C) (Viklund et al. 2008a; Amrein et al. 2003). Based on its carcinogenicity in rodents, acrylamide has been classified as 'probably carcinogenic to humans' (Group 2A) by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) (Yuan et al. 2011; Lingnert et al. 2002; Exon, 2006). Acrylamide has generated widespread public concern due to safety issues, as it has been shown to be a neurotoxic, genotoxic, and is a potential carcinogenic compound (Viklund et al. 2008a). At this time, detrimental effects of acrylamide to human health via the diet are uncertain as data from human studies were insufficient for dose-response assessment (Powers et al. 2017). Despite the absence of a firm conclusion regarding the relationship between acrylamide intake from food and the adverse health effects, the global scientific community has encouraged food manufacturers to reduce acrylamide levels in food as low as reasonably achievable (Loaëc et al. 2014).

As asparagine and reducing sugars are the two main precursors for acrylamide synthesis under the Maillard reaction at temperatures above 120°C, minimization of acrylamide precursors and the improvement of processing conditions would have a huge impact on acrylamide reduction in processed foods (Xu et al. 2016). A number of preparation and process factors such as frying temperature and time, blanching methods, pH values and treatments of potato with processing additives (e. g. antioxidants, amino acids, cations or enzymes, etc.) can significantly influence acrylamide synthesis in potato processed products (Shojaee-Aliabadi et al. 2013).

Several reports (Claeys et al. 2010; EFSA 2015; Sirot et al. 2012) revealed that fried potato products are among the food commodities that contribute the highest dietary acrylamide exposure. Detailed consideration is given to potato chips and French fries because of the high acrylamide concentrations and their high consumption rates as popular salty snacks worldwide (Pedreschi et al. 2005; Viklund et al. 2008a). Moreover, the reduction of acrylamide content in potato processed products has been the subject of great interest to many researchers and the food industry because potato tubers have naturally high concentrations of asparagine and reducing sugars, the precursors for acrylamide formation in potato chips and French fries (Amrein et al. 2003).

In this study, three French fry cultivars (Russet Burbank, Ranger Russet and Shepody) and three chipping cultivars (Atlantic, Snowden, and Vigor) commonly used in the potato processing industry (e.g., McCain, McDonald's, Old Dutch). were employed. Examination of the impact of additive treatments on acrylamide synthesis in real food systems under real processing conditions is required for optimization of these treatments, as desired and undesired reactions take place in complex food systems during processing (Shojaee-Aliabadi et al. 2013). Most of the previous research in this area was conducted in asparagine/sugar Maillard model systems and has provided little insight on the effects on asparagine and reducing sugars before and after the treatments (Mestdagh et al. 2008d; Zhu et al. 2016; Yuan et al. 2011). Moreover, acrylamide formation is considered primarily as a surface reaction (Gökmen et al. 2006). Products with high surface- to-volume ratio (SVR) are among those high temperature processed foods that are most prone to acrylamide formation (Taubert et al. 2004). Thus, scrutinization of the size of potato strips on acrylamide formation in French fries will be important as it has not been studied extensively to date. Investigation of the effect of frying temperature and time on

both the synthesis of acrylamide and the content of asparagine and reducing sugars before and after frying of potato chips will be important to enhance our knowledge of the dynamics of both precursors and acrylamide.

The concentrations of acrylamide precursors in potato tubers can vary significantly among different cultivars, tuber storage conditions and field cultivation practices such as fertilizer management (Muttucumaru et al. 2013; Silva et al. 2016). The primary measure employed by the industry is to reduce the concentrations of the precursors in the raw material, as this would generate less acrylamide in potato based snacks and reduce the need to modify processing conditions (Amrein et al. 2003). The nitrogen (N) fertilizer regime can directly influence the asparagine content and indirectly influence the reducing sugar content of the potato tuber (Muttucumaru et al. 2013). Thus, optimizing agricultural practices would be a more promising and low tech approach to minimizing concentrations of these substances.

Previous studies on N application in potato cultivation showed the importance of meeting potato N demand by regulating the time, location, source and rate of N additions, and considering the N supply capacity of the soil (Davenport et al. 2005; Zebarth and Rosen 2007). This can be achieved via the split application of fertilizer, with some applied to the field at planting and the remainder at hilling or via fertilization with irrigation water (fertigation) (Errebhi et al. 1998). As only a small volume of research exists on the effect of N fertilization strategies such as split application, source of N fertilizer and fertigation or a combination of these, it is valuable to investigate the relationship between N treatments and precursor contents and to explore the effects of different N strategies on acrylamide formation in processed potato products.

Therefore, this study aims to characterize the acrylamide formation in potato processed foods under different processing conditions and different agronomic approaches (N fertilization), and to identify potential approaches to reduce the acrylamide level in potato chips and French fries.

1.2. Research objectives

- Determine the acrylamide formation in potato chips under different frying times, temperatures and additive treatments, and in French fries under different N fertilization strategies and for different potato strip sizes.
- Evaluate the relationship between acrylamide and its precursors, asparagine and reducing sugars, and the effects of different N fertilization strategies, additive treatments, potato strip size and frying conditions on acrylamide formation in potato chips or French fries.

1.3. Thesis organization

The thesis is prepared in manuscript style in accordance with Faculty of Arts and Science – University of Lethbridge guidelines. There are 5 chapters with 2 stand-alone manuscripts (Chapters 3 to 4).

Chapter 1: Introduction

Chapter 2: Literature review

Chapter 3: Impact of frying conditions and additive treatments on contents of reducing sugars, asparagine and acrylamide in potato chips

Chapter 4: Effect of nitrogen fertilization strategies and surface area –to-volume ratio on acrylamide formation in French fries

Chapter 5: Summary and future directions

CHAPTER 2: LITERATURE REVIEW

2.1. The history of acrylamide and the discovery of acrylamide in food

Acrylamide (2-propenamide) is an organic compound which is formed by hydration of acrylonitrile (Friedman 2003). It is an odorless, white solid with the chemical formula C_3H_5NO (**Figure 2.1**) (Dearfield et al. 1988). Acrylamide has several applications in industries including water purification, treatment of sewage and wastewater, cosmetics, soil stabilization, special grouting applications and it is also found in tobacco smoke (Elbashir et al. 2014).

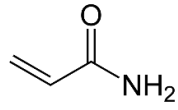

Figure 2.1: Chemical structure of acrylamide

Acrylamide is a neurotoxin and various toxicological studies have demonstrated that it is a probable human carcinogen and genotoxicant (Stadler and Scholz 2004). Neurological impacts have been observed in humans exposed to high levels of acrylamide in the workplace (e.g. numbness of the limbs, weakness and lack of coordination in the legs) (Lingnert et al. 2002; Bjellaas et al. 2007). The IARC classified it as possibly carcinogenic for humans (group 2A) in 1994 (Yuan et al. 2011; Lingnert et al. 2002). During a study investigating the health effects of acrylamide exposure at a railway tunnel construction site, heamoglobin adducts of acrylamide and its metabolite glycidamide were detected in both the control group and the workers who had been occupationally exposed to acrylamide. Thus, the detection of acrylamide in the control

group has led to further research, ultimately leading to the discovery of acrylamide in food (Amrein et al. 2003).

In 2002, the Swedish National Food Administration and the University of Stockholm reported that acrylamide is formed in carbohydrate–rich foods, processed or cooked at high temperatures (> 120°C) such as potato chips and fries (Stadler and Scholz 2004). An extensive variety of cooked foods contain acrylamide at levels ranging from a couple of ppb (parts per billion) to in excess of 1000 ppb, including potato chips, French fries, roasted coffee and bakery products such as bread, crisp bread, biscuits, crackers and breakfast cereals (Anese et al. 2009a). The levels of acrylamide detected in these processed foods were considerably higher than the levels recommended by the World Health Organization (WHO) for drinking water (0.5 µg/L) (Elbashir et al. 2014).

Concerns were raised worldwide because of the risk associated with acrylamide intake, and food chemists, food scientists and food companies began investigating changes in the entire production process in order to reduce acrylamide levels in food products (Bethke and Bussan 2013). The European Food Safety Authority (EFSA) has issued indicative levels for acrylamide in the most relevant food categories. The benchmark levels of 0.75 mg/kg for potato chips and 0.50 mg/kg for French fries have been issued by the European Commission (Powers et al. 2017). Food and Drink Europe have built up a ‘toolbox’ containing measures which can be applied as a guide to reduce the acrylamide levels in food (FoodDrinkEurope 2013).

2.2. Dietary exposure to acrylamide

Non-smokers are exposed to acrylamide by consuming heated starch-rich foods such as processed potato products. In contrast, heated protein-rich foods such as fried meat contained low contents of acrylamide, while undetectable levels are present in unheated or boiled foods (Lingnert et al. 2002; Bethke and Bussan 2013). In addition to potato fried products, acrylamide was found at significant levels in coffee, cereals and crispbread. Based on more than 43 000 analytical results of food commodities, EFSA (2015) reported that solid coffee substitutes, coffee, and potato fried products contained the highest levels of acrylamide. The panel estimated that the 95th percentile dietary acrylamide intake ranges between 0.6 and 3.4 μg/kg body weight (b. w.) per day across age groups and 0.4 to 1.9 μg/kg b.w. per day across surveys. Average medium bound levels (MB) of acrylamide reported in 'Coffee substitutes (dry)' and 'Coffee (dry)' were 1499 μg/kg and 522 μg/kg, respectively. However, lower contents of acrylamide are expected in 'Coffee beverages' and 'Coffee substitute beverages' due to dilution effects. Furthermore, higher acrylamide levels of 389 μg/kg and 309 μg/kg were found in 'Potato crisps and snacks' and 'Potato fried products', respectively. Lower levels were obtained in 'Processed cereal-based baby foods' (average MB level of 73 μg/kg), 'Soft bread' (average MB level of 42 μg/kg) and 'Baby foods, other than cereal-based' (average MB level of 24 μg/kg) (EFSA 2015).

Groups with relatively high levels of exposure to dietary acrylamide include infants, toddlers, children and adolescents, which may be due to their higher caloric demand relative to their body weight and their different dietary patterns (Wilson et al. 2006; Dybing et al. 2005). Research indicates that the dietary exposure of children is, on average, between 0.5 and 1.9 μg/kg b.w. per day and the 95th percentile is between 1.4

and 3.4 µg/kg b.w. per day (EFSA 2015). Since the earliest findings in 2002, extensive data have been collected for the acrylamide content in products such as potato chips, French fries, coffee, crackers and biscuits (Bethke and Bussan 2013). Wide variations in the acrylamide content of potato chips were observed within each year, from year to year, between chip brand, within brands over time and between lots of the same brand (Becalski et al. 2010).

2.3. Health risks and risk assessment

Acrylamide is a well-known neurotoxin and various toxicological studies have shown it to be a probable human carcinogen and genotoxicant (Rice 2005; Friedman 2003). The IARC classified it as a possible carcinogenic for humans (group 2A) in 1994 on the basis of positive bioassay results in laboratory animals (Lingnert et al. 2002). Acrylamide is absorbed by animals and humans via ingestion, through the skin and through the mucosa if inhaled. If taken by the oral route, acrylamide is widely diffused throughout the body since it is a small and hydrophilic molecule (Friedman 2003). In 2015, EFSA expert committee on Contaminants in the food chain (CONTAM) stated that acrylamide in food items has the potential to increase the risk of developing cancer in all age groups (EFSA 2015). According to LoPachin and Gavin (2012) acrylamide appears to cause cumulative neurotoxicity in exposed humans and laboratory animals through reduction of neurotransmission at central and peripheral synapses by disturbing signalling pathways and inhibition of presynaptic function. Moreover, acrylamide can interact with unsaturated aldehydes leading to diabetes, Alzheimer's disease, atherosclerosis, spinal cord trauma and stroke due to cellular oxidative stress (Butterfield et al. 2010; Grimsrud et al. 2008; Hamann et al. 2008; Uchida 2003a; Zarkovic 2003;

Uchida 2003b). The genotoxic action of glycidamide, which is the major metabolite of acrylamide, is regarded as the mechanism of carcinogenicity in cancer risk assessments (Gamboa da Costa et al. 2003; Ghanayem et al. 2005). There is a wide range of case-control and cohort studies investigating the possible association between dietary acrylamide intake and cancer risk (Rice 2005; EFSA 2015).

Most cancer research conducted to date has not been conclusive with regard to dietary acrylamide exposure (Burley et al. 2010; Wilson et al. 2010a; Hogervorst et al. 2007). Increased risks were reported for renal cell, endometrial and ovarian cancer, yet the studies present certain uncertainties due to limited and inconsistent evidence. At this time, detrimental effects of acrylamide to human health via the diet are uncertain as data from human studies were insufficient for dose-response assessment (EFSA 2015). For the risk characterization for acrylamide intakes, the margin of exposure (MOE) approach was put forward, defined as the ratio between a dose leading to tumors in experimental animals and the human intake. A lower value of MOE represents a greater risk (Mestdagh et al. 2009). Based on available results from human studies, the CONTAM panel concluded that acrylamide is not a human carcinogen. However, based on animal evidence the margins of exposure for dietary acrylamide indicate a concern for neoplastic effects (EFSA 2015).

2.4. Pathways for acrylamide formation

2.4.1. Formation from the Maillard reaction

Shortly after the discovery of acrylamide in cooked foods, scientists reported that acrylamide formed from asparagine and reducing sugars via the Maillard reaction (**Figure 2.2**) (Mottram et al. 2002; Stadler et al. 2002). This non-enzymatic browning

reaction generates a plethora of important compounds which are responsible for several aspects of food quality such as flavor, color and aroma formation (Anese et al. 2009b). Mass spectral studies using ^{15}N-labled asparagine and ^{13}C labeled glucose revealed that the N of the amide group and the three carbon atoms originated from asparagine (Mottram et al. 2002; Stadler et al. 2002; Mestdagh et al. 2009). In principle, asparagine alone can form acrylamide due to thermally initiated decarboxylation and deamination (Elbashir et al. 2014). However, a significant increase in the acrylamide content has been observed in the presence of reducing sugars, indicating that the formation of acrylamide is closely linked to the Maillard reaction (Yaylayan et al. 2003). During the first step of the Maillard reaction the carbonyl compound and the alpha-amino group of the free asparagine react following heating at temperatures above 120˚C, forming the corresponding N-glycosyl conjugation and the decarboxylated Schiff base (Becalski et al. 2004; Stadler et al. 2004). The decarboxylated Schiff base may decompose directly to acrylamide and an imine or may hydrolyse to 3- aminopropamide (3-APA) and carbonyl compounds. Furthermore, as the subsequent elimination of ammonia from 3-APA can yield acrylamide, 3-APA is also believed to be an acrylamide precursor (Granvogl and Schieberle 2006).

2.4.2. Formation from acrolein

Acrolein (CH_2=CH-CHO) is an unsaturated aldehyde that has been formed by oxidative lipid degradation or from glycerol leading to the formation of acrylic acid (Lingnert et al. 2002). Acrylic acid can be directly transformed to acrylamide by reaction with ammonia (Gertz and Klostermann 2002). However, Becalski et al. (2003) indicated that in fried potatoes, acrylamide is not principally formed by this pathway. Furthermore,

Mestdagh et al. (2005) reported that oil degradation products such as glycerol did not have any significant impact on acrylamide formation in a potato model system and French fries.

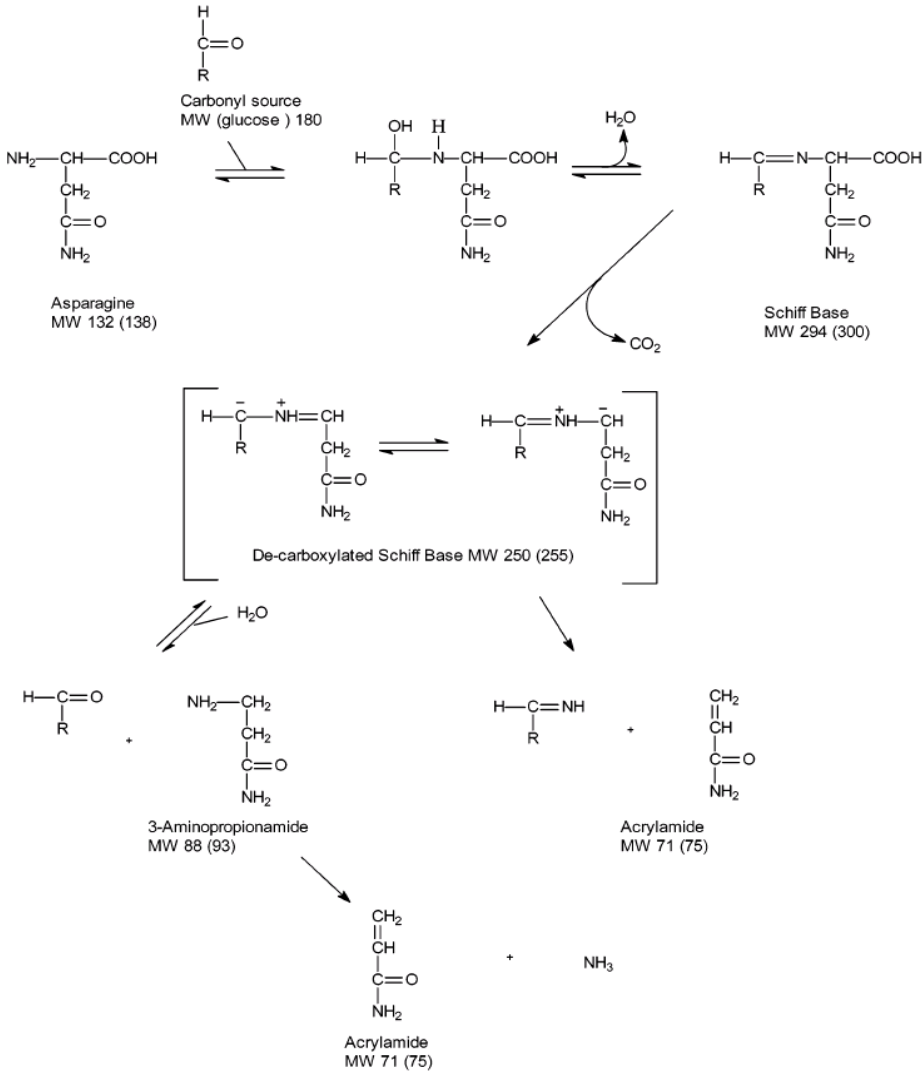

Figure 2.2: Mechanism of acrylamide formation. Source: Anese et al. 2009a

2.5. Analytical methods for acrylamide determination

Numerous analytical methods have been developed to determine acrylamide in different food matrices since 2002. Generally, acrylamide analysis involves three steps: extraction; sample cleanup using solid phase extraction; and chromatographic separation and detection (Troise et al. 2013). The majority of methods employ detection by either gas chromatography (GC) or liquid chromatography (LC) followed by mass spectrometry (MS) (Bagdonaite et al. 2008; Becalski et al. 2005; Mastovska and Lehotay 2006). Assays employing GC techniques are usually based on derivatization of the analyte because of the low volatility and polarity of acrylamide. The commonly employed derivatization procedure is bromination of acrylamide to produce 2,3 – dibromopropionamide (Stadler and Scholz 2004). Direct analysis without derivatization can also be employed, but it is always accompanied by several problems such as additional acrylamide formation due to co-isolation of acrylamide precursors and the high solubility of acrylamide in water compared with organic solvents (Mastovska and Lehotay 2006). LC-MS methods seem to be the preferred method for many laboratories because of simple sample preparation and because the LC-MS/MS offers lower detection limits. Depending on the food matrix, greater consideration and effort need to be invested in sample pre-treatment for high sensitivity and precision (Stadler and Scholz 2004). Furthermore, laboratories still require technical expertise and expensive instrumentation for accurate quantification of acrylamide in potato products. Therefore, an urgent requirement is the development of cheap, rapid screening methods that are accurate and sensitive (Longhua et al. 2012; Zhu et al. 2008).

2.6. Importance of reducing sugars and asparagine

Glucose, fructose and asparagine are the major reactants of acrylamide formation in potato products. Since there is a high concentration of asparagine in potatoes compared to other starchy foods, processed potato products are considered to be high in acrylamide (Amrein et al. 2003; Becalski et al. 2004). The abundance of asparagine in potatoes is one of the reasons that reducing sugars become the rate-limiting factor for acrylamide formation in fried potato products (Williams 2005b). Asparagine content typically ranges between 4 and 25 mg/g^{-}(dry weight, DW), while glucose and fructose typically vary from less than 0.04 to 4.80 mg/g^{-}DW for most potato varieties (Amrein et al. 2003; De Meulenaer et al. 2008; Shepherd et al. 2010). There is a complicated relationship between the levels of acrylamide precursors and the amount of acrylamide formed in the cooked product (Yang et al. 2016; Muttucumaru et al. 2013). Previous studies have demonstrated that acrylamide levels in the processed potato products correlated to reducing sugar concentrations in potato, whereby sucrose and asparagine showed no correlation (Amrein et al. 2003; Elmore et al. 2015; Viklund et al. 2008a; Amrein et al. 2004; Mestdagh et al. 2008a). These findings support the idea of the importance of selecting varieties with low reducing sugar content. Pollien et al. (2003) found that fructose is more efficient than glucose in generating acrylamide when it was heated with asparagine in Maillard model systems. The effectiveness of fructose in acrylamide formation could be attributed to the lower melting point of fructose (126°C) compared with glucose (157°C). In practice, a reducing sugar concentration ranging from 150 to 200 mg per 100g of tuber fresh weight (FW) is used as an indication of their suitability for processing (Paul et al. 2016).

2.7. Agronomical factors affecting the formation of acrylamide in fried potato products

2.7.1. Potato cultivar

As stated above, the formation of acrylamide in processed potato products depends on the amounts of precursors (glucose, fructose, asparagine) in the tubers (Bethke and Bussan 2013). The tuber chemical composition varies among different cultivars and it depends on genotypic and environmental effects, with both having a significant impact on final acrylamide production of the processed potato products (Bethke and Bussan, 2013; Amrein et al. 2003). Potato cultivars have been bred for low reducing sugars for over a half century to produce light-colored, flavorful processed products. These breeding efforts are highly important in terms of acrylamide mitigation (Douches et al. 1996; Love et al. 1998). Varieties such as Tebina and Quincy contain high reducing sugar contents and they are considered to be unsuitable for processing. Some varieties including Saturna, Panda, Agria and Lady Claire are bred and selected for low sugar content and consequently lower acrylamide formation in the processed product. (Amrein et al. 2003; Amrein et al. 2004; Mestdagh et al. 2009; Morales et al. 2008). It is suggested that potatoes used for roasting and frying should contain less than 1g/kg fresh weight of reducing sugars (Biedermann-Brem et al. 2003; De Wilde et al. 2005).

Molecular approaches have been used to decrease the tuber reducing sugar and asparagine contents in popular cultivars. For instance, silencing genes which encode the proteins for aspargine synthesis (asparagine synthetase) reduced the asparagine content in modified Ranger Russet and Atlantic varieties. Reductions of acrylamide content by 95% were observed in fries processed from transgenic lines of Ranger Russet and for Atlantic, and decreasing asparagine content by 80% led to a 90% reduction of acrylamide in potato

chips (Rommens et al. 2008). Other molecular approaches such as silencing the *R1* gene for starch phosphorylase have been adapted to decrease tuber reducing sugar contents. Starch phosphorylase is responsible for starch breakdown. Reducing the expression of the *R1* gene decreased the reducing sugar content of potatoes in storage and further resulted in a decrease in the acrylamide content of fries processed from transformed lines of Ranger Russet (Rommens et al. 2006). However, the processing industry is not currently using genetically modified varieties as a result of legal constraints and public acceptability (Vinci et al. 2012).

2.7.2. Fertilization and soil

Heavy fertilization of potatoes generally occurs as the crop has high nutrient requirements (Mondy and Koch 1978). Previous studies reported that N fertilization rates have an impact on both asparagine and reducing sugar levels in the tubers. Elevated N fertilization leads to a reduction in tuber sugar concentrations, however some studies reported no influence or sometimes increasing sugar levels depending on the part of the tuber (Amrein et al. 2003; Westermann et al. 1994; De Wilde et al. 2006b). Decreasing N fertilization consequently results in high reducing sugar contents in the tubers and further acrylamide formation in the fried potato products (Mestdagh et al. 2009). However, other factors such as soil type and availability of water will have an impact on nutrient uptake and metabolism by the plant, and the degree of sugar increase varies depending on the cultivar (De Wilde et al. 2006b; Morales et al. 2008; Mestdagh et al. 2009). De Wilde et al. (2006a) reported that reducing sugar content in the tubers and acrylamide formation were not significantly affected by the type of soil.

Elevated N fertilization almost always leads to an increase in asparagine in the tubers as asparagine is a N reservoir (Rosen et al. 2018). Another study reported that increased phosphorus nutrition led to an increase in reducing sugars at harvest and after storage (Kolbe et al. 1995). Sulfur deprivation tends to generate low acrylamide, even though the sugar contents were higher in tubers of sulfur-deprived plants (Elmore et al. 2007). According to these studies, it is necessary to find a suitable balance between the level of fertilizers needed to mitigate acrylamide formation, maintenance of yield and quality, and reduction of environmental impacts through legislated fertilization limits (De Wilde et al. 2006b; Mestdagh et al. 2009).

2.7.3. Climatological conditions and harvest

Various climatological conditions significantly influence the reducing sugar and asparagine contents of potato tubers (Mestdagh et al. 2009). Exceptionally warm and dry periods led to lower reducing sugar contents and acrylamide formation upon subsequent frying (De Meulenaer et al. 2008). Chemical maturity stage signifies that the crop is ready for harvest. This stage occurs before vine desiccation and depends on factors such as potato variety, soil moisture, temperature and nutrition (Rosen et al. 2018). When tubers achieve minimum sucrose and reducing sugar concentrations and high dry matter content, they are considered “chemically mature” (Rosen et al. 2018). In contrast, immature and thus smaller tubers contain greater amounts of sugars as the rate of translocation to the tubers surpasses the rate of metabolism (Heltoft et al. 2017).

2.7.4. Potato storage

After harvest, tubers are generally stored at 8-12°C for up to several months since this temperature does not significantly affect the reducing sugar content and further acrylamide formation in fried potato products (Olsson et al. 2004; De Wilde et al. 2005; Paul et al. 2016). Senescent sweetening and cold temperatures can result in high concentrations of sugars during storage. This enzymatic process can ultimately lead to potato sprouting and is enhanced at higher storage temperatures (> 8°C) with long-term storage after harvest (Amrein et al. 2004). Treating potatoes with a chemical sprout suppressing agent can avoid sprouting, although its use is not always desired by the customer (Mestdagh et al. 2009). At low temperatures (< 8°C) potatoes start to mobilize sugars from starch in order to protect themselves from frost, in a process called low-temperature sweetening. This physiological reaction will lead to undesired Maillard browning and increases the acrylamide formation upon subsequent frying (Blenkinsop et al. 2002). However, unlike senescent sweetening, low-temperature sweetening is partly reversible following reconditioning for a period of 3 weeks at 15°C (Biedermann-Brem et al. 2003; Blenkinsop et al. 2002; De Wilde et al. 2005). Asparagine content does not appear to be influenced by the storage temperature and time (De Wilde et al. 2005).

2.8. Effect of potato processing strategies on acrylamide formation

2.8.1. Blanching process

Blanching is effective in the industrial process of French fry production as it gives the fries a uniform colour after frying, inactivates enzymes and forms a layer of gelatinized starch which limits oil absorption and improves texture. Furthermore, blanching also contributes to the reduction of acrylamide content via leaching of

acrylamide precursors from the surface layers of the potato strips (Kita et al. 2004; Pedreschi et al. 2007). It has been reported that blanching can lower the acrylamide formation by 60% via the removal of reducing sugars, although this varies with potato varieties and processing conditions (Pedreschi et al. 2010). Blanching at temperatures of about 70°C for 10-15 min reduced acrylamide formation by approximately 65% and 96% for French fries and potato chips, respectively (Mestdagh et al. 2008c). Blanching temperature and time can be manipulated for efficient extraction of reducing sugars. However, extreme blanching conditions might result in significant losses of nutrients, product colour, texture and taste (Arroqui et al. 2002; Carbonell et al. 2006).

2.8.2. Drying

Drying of blanched or soaked potato cuts prior to frying was proposed as a valuable measure to reduce fat absorption and oil hydrolysis (Choe and Min 2007; Mehta and Swinburn 2001). Moreover, it contributes to the reduction of acrylamide formation in French fries, since shorter frying times were required to obtain the same product qualities such as color and crispiness (Gökmen et al. 2006). Kita et al. (2004) concluded that drying of potato chips at the end of the shorter frying operation would be effective in mitigating acrylamide formation. It was furthermore shown that the addition of a post-drying step after a shorter frying time could result in low moisture potato chips of good sensory quality. However, this was much more time-consuming in comparison to traditional frying (Mestdagh et al. 2009).

2.8.3. Use of additives

2.8.3.1. Amino acids

Some amino acids such as glycine, cysteine and lysine were found to be effective inhibitors of acrylamide formation as they can compete with asparagine to lower the acrylamide amount (Bråthen et al. 2005; Rydberg et al. 2003; Mestdagh et al. 2008d). The addition of 0.1M glycine to the blanching water resulted in a 68% reduction in acrylamide content of potato chips (Mestdagh et al. 2008d). Rydberg et al. (2003) reported that the addition of 35 mmol/L and 140 mmol/L glycine to homogenized potato heated in the oven can reduce acrylamide formation by 71% and 91%, respectively. According to kinetic studies, Zhu et al. (2016) suggested that the reduction of acrylamide formation should be attributed to the elimination reaction of acrylamide with glycine and it cannot be attributed to the competitive reaction of glycine with asparagine. Furthermore, the addition of sulphur containing amino acids can produce unpleasant off-flavours in the food (Claeys et al. 2005).

2.8.3.2. Protective effects of metal ions

The addition of mono and divalent cations (e.g. Na^+ and Ca^{2+}) have also been shown to be an effective means of reducing acrylamide accumulation. For example, dipping potato strips in calcium chloride can reduce acrylamide by 95% in French fries with no adverse effects on product quality (Gökmen and Şenyuva 2007a). Blanching in salts could mitigate acrylamide formation as these ions can interact with asparagine, preventing the formation of the Schiff base, the key intermediate in the pathway of acrylamide formation (Mestdagh et al. 2008b). Gökmen and Şenyuva (2007b) showed that acrylamide formation decreased by up to 59% after increasing Na^+ concentration

from 0 to 5 µmoles, and a further increase of Na^+ led to higher acrylamide formation in a model mixture composed of glucose and asparagine. Similar results were obtained in a model study conducted by Wen et al. (2016), who reported that acrylamide inhibiting efficiency accelerated until minimum acrylamide levels were reached at low Ca^{2+} concentrations, while increasing Ca^{2+} concentrations beyond the maximal (20 µmol/L) led to significantly increased acrylamide formation. Pedreschi et al. (2010) reported that the reduction of acrylamide formation in salt-treated chips is related to changes in the chemical reactions or heat transfer due to reduced oil uptake and it is not related to the acrylamide precursor contents. Addition of calcium chloride can improve product texture but unfortunately can cause a bitter aftertaste (Varela et al. 2007).

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2.8.3.3. Blanch and soak in acid solution

The pH is an important determinant of the acrylamide content in food because the rate of the Maillard reaction is dependent on the pH of the reaction environment (Paul et al. 2016; Elbashir et al. 2014). Lowering the pH below 7.0, which is the optimum for the formation of acrylamide, results in lowering of acrylamide levels (Baardseth et al. 2006; Paul et al. 2016). The addition of some acidifying compounds like citric acid into the matrix at a low pH were found to be an effective mean of reducing acrylamide formation (Elbashir et al. 2014). Blanching in 0.05M and 0.01M citric acid at 65°C for 5 min reduced the acrylamide formation of potato chips by 93% and 90%, respectively, in a study conducted by Ismial et al. (2013). Jung et al. (2003) also reported the reduction of acrylamide content in French fries by lowering the pH with citric acid. Blanching in organic acids (e.g. citric acid) could mitigate acrylamide formation by lowering the pH and leaching asparagine and reducing sugars from potato strips (French fries) into the

solution. When exposed to a lower pH, asparagine becomes protonated, which blocks further reaction with a carbonyl to form acrylamide (Jung et al. 2003; Xu et al. 2016; Yuan et al. 2011; Low et al. 2006). Acidification may have an impact on the desirable flavours and colours of the product and moreover can result in a sour product taste. However, this effect can vary with the concentration and type of the acid and applied soaking or blanching treatment (Kita et al. 2004; Franke et al. 2005).

2.8.3.4. Effect of Antioxidants

Food antioxidants have been reported to modulate acrylamide formation with widely varying effects, due to the various types of antioxidants used (Yuan et al. 2011; Zhang et al. 2007; Tareke and Stockholms 2003). For instance, the addition of ascorbic acid has a complex impact on acrylamide formation in an asparagine-glucose model system. Yuan et al. (2011) observed that a concentration of 0.5% ascorbic acid decreased acrylamide formation by approximately 58%, but an increase of acrylamide formation was observed at 1.5% ascorbic acid. Furthermore, potato slices fried in corn or olive oil with rosemary herb reduced the acrylamide formation by about 25% (Becalski et al. 2003). Zhang et al. (2007) found that antioxidants from bamboo leaves could effectively mitigate the acrylamide formation in potato chips and French fries by 74.1 % and 76.1% when the addition ratio was 0.1% and 0.01% (w/w), respectively. However, the reduction could be attributed to other parameters that also change with the addition of antioxidants, such as pH or amino acids present in the extracts (Mestdagh et al. 2009).

2.8.4. Fermentation and enzymatic treatment

Fermentation before the food processing step is an effective way of reducing asparagine, reducing sugars and hence acrylamide formation in bakery products and potatoes by means of precursor consumption and pH reduction (Sadd et al. 2008; Kamkar et al. 2015). For instance, lactic acid fermentation of potatoes by *Lactobacillus plantarum* prior to deep-frying reduced the acrylamide formation by 48-70% in the finished product. Lactic acid fermentation in the presence of glycine or combining blanching with fermentation was effective in decreasing acrylamide formation by 70-94% with good sensory properties of the deep-fried potatoes (Anese et al. 2009a; Baardseth et al. 2006). Furthermore, lactic acid fermentation in the preparation of wholemeal rye bread reduced acrylamide content in the final product (Baardseth et al. 2006). During fermentation, reducing sugars were metabolized, while lactic acid lowered the product pH. Since most of these studies were carried out on model systems at a laboratory scale, pilot and full-scale experiments on an industrial level are needed. For instance, the temperature and pH need to be controlled in order to optimize the activity of the microorganism (Bartkiene et al. 2013). The use of the enzyme asparaginase, which hydrolyses asparagine to aspartic acid and ammonia, is also considered to be effective in reducing the asparagine content of the raw material and hence lower acrylamide formation in the cooked product (Boegl 2006). Pedreschi et al. (2008) reported that acrylamide content in French fries decreased by up to 60% by combining blanching with an asparaginase soaking treatment. However, high concentrations of asparaginase are needed in order to achieve a significant reduction of acrylamide content in fried potatoes. There is little opportunity for regular industrial use of such high concentrations due to the high cost of the asparaginase enzyme (Anese et al. 2009b).

2.8.5. Cutting

The size and cut shape of the potato product are particularly important as the majority of acrylamide forms on the surface and near surface regions of the product, where the maximum temperatures are achieved during frying (Gökmen et al. 2006). In the frying process, the conditions favourable for acrylamide formation develop in the surface layer of the product due to simultaneous drying (Gökmen et al. 2006; Haase 2006). Fine-cut strips (8×8 mm) with a SVR of 5.4 cm^{-1} resulted in significantly higher acrylamide content in French fries than coarse-cut strips (14×14 mm) with a SVR of 3.3 cm^{-1} at a constant frying time. Accordingly, in thinner and smaller strips dehydration occurs more quickly and thus tends to increase acrylamide formation (Matthäus et al. 2004). A thin cut potato crisp would require less thermal input for the same fry time to achieve the same moisture endpoint, which can lead to lower rates of acrylamide formation (FoodDrinkEurope 2013). Taubert et al. (2004).examined the influence of processing parameters on acrylamide formation during the frying of potatoes and reported that there is a consistent increase in acrylamide buildup with increasing temperatures in shapes with low SVR, whereas in samples with higher SVR, acrylamide amounts increased with increasing frying temperatures reaching maximum levels at 160-180°C. Moreover, acrylamide content decreased in higher SVR preparations with higher temperatures and longer processing times.

2.8.6. Effects of different frying oils

Previous reports noted that the type of frying oil used to fry potato products could influence the rate of acrylamide formation. Zhang et al. (2015) reported that frying oils with higher heat transfer coefficients can lead to higher levels of acrylamide in French

fries. Another report (Becalski et al. 2003) showed that chip samples processed with olive oil exhibited higher rates of acrylamide formation compared to corn oil. Gertz and Klostermann (2002) reported higher levels of acrylamide in French fries following deep-frying in palm oil. However, Matthäus et al. (2004) did not find a significant influence of oil type on acrylamide concentration in deep-fat fried potato products.

2.8.7. Frying

The definition of frying can be stated as the immersion of a food material in edible oil to a temperature exceeding the boiling point of water with the development of colour, texture and flavour (Hubbard and Farkas 1999; Yang et al. 2016). Frying is a complex process due to two mass transfers in opposite directions within the frying sample; for starchy products, oil enters the food while water and some soluble material escapes from the products (Ziaiifar et al. 2008). Frying temperature and time are important determinants of the amount of acrylamide and the browning, texture, and flavour development caused by the Maillard reaction (Mottram et al. 2002; Stadler et al. 2002). Longer frying temperatures and times tend to form high acrylamide levels (Matthäus et al. 2004). Reducing cooking temperature and shortening the processing time can be effective in reducing acrylamide, but this could affect the sensory properties in cooked food as the Maillard reaction is responsible for the desirable taste, smell and colour (Foot et al. 2007). However, the same study reported that frying at low temperatures (below 140°C) for longer processing times results in higher fat uptake and poorer texture. A proper choice of temperature and time could be an effective way of mitigating acrylamide formation during frying. (Anese et al. 2009b).

At the end of the frying process, when the surface temperature of the fried product reaches the oil temperature, an exponential increase in the rate of acrylamide formation can be observed (Paul et al. 2016). The frying temperature should be below 175°C and the frying time necessary to achieve the desired quality of the fried product should not be exceeded (Mestdagh et al. 2009). It has been suggested to decrease the temperature at the end of the frying operation in order to lower the acrylamide formation. Fryers that allow a controlled oil temperature profile from a high initial (when the moisture content is high) to a lower final (When the moisture content reduced) frying temperature should be implemented to mitigate acrylamide formation (Grob 2007). Moreover, prolonged heating at high temperatures produces lower acrylamide levels due to elimination or degradation reactions (Granda and Moreira 2005). Such intense heat treatments can lead to greater development of non-enzymatic browning reactions and furthermore could have a negative effect on sensory attributes, such as color, flavor and texture of most foods. These changes can be desirable for other food types, e.g. coffee, barley, cocoa (Anese et al. 2009b). Vacuum frying under reduced pressure can reduce the acrylamide formation by about 94% as lower temperatures can be used during vacuum frying to gain a product with the desirable yellow golden color and texture (Granda et al. 2004).

Palazoğlu et al. (2010) evaluated the effect of frying vs. baking on acrylamide formation in potato chips. According to their results, baking at 180 and 190°C produced lower acrylamide levels in the chips in comparison to their fried counterparts, whereas baking at 170°C resulted in higher acrylamide levels when compared to frying at the same temperature. The effect of microwave frying on the acrylamide content of potato chips and French fries has also been investigated and the results indicated that microwave frying can reduce the acrylamide content in French fries by between 37 and 83% in

comparison to deep-oil frying (Sansano et al. 2018). However, acrylamide levels in microwave-fried potato chips increased significantly with increasing microwave power level, frying temperature and time (Elfaitouri et al. 2018; Sansano et al. 2018).

CHAPTER 3: IMPACT OF FRYING CONDITIONS AND ADDITIVE TREATMENTS ON CONTENTS OF REDUCING SUGARS, ASPARAGINE AND ACRYLAMIDE IN POTATO CHIPS

3.1. Abstract

Potato chips are a significant contributor to the dietary intake of acrylamide, a probable carcinogen in heat-processed foods. The present study aimed to determine the effects of frying conditions and additive treatments on reducing sugars, asparagine levels, and acrylamide formation in fried potato chips. We conducted our experiments with three commonly used chipping varieties (Atlantic, Snowden, Vigor) under different frying times (3, 5, 7 min) and temperatures (160, 170, 180, 190°C). Acrylamide formation in potato chips increased with the increasing frying time and temperature. Additionally, acrylamide formation was accompanied by significant decreases in the levels of reducing sugar and asparagine. The variety exhibiting the lowest acrylamide levels in the processed potato chips was Snowden, while the conditions most conducive to acrylamide formation in all varieties were frying at 190°C for 7 min. Using a 7 min frying time, decreasing the frying temperature from 190 to 160°C mitigated the acrylamide formation in potato chips processed from Atlantic, Snowden and Vigor by 84, 67, and 78%, respectively. We also examined the effects of additives during blanching prior to frying. Blanching in distilled water led to the greatest decreases (19-59%) in acrylamide formation. Glycine (0.1M) significantly reduced the acrylamide formation in potato chips processed from Atlantic (49%) and Vigor (27%) potatoes, while 1% citric acid lowered

the acrylamide content of fried Atlantic potato chips (38%) and 1% acetic acid was effective at lowering the acrylamide content in Vigor potato chips (31%).

3.2. Introduction

Acrylamide is a hazardous compound with the chemical formula C_3H_5NO (Elbashir et al. 2014). Based on its carcinogenicity in rodents, acrylamide has been classified as 'probably carcinogenic to humans' (Group 2A) by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) (Yuan et al. 2011; Lingnert et al. 2002; Exon, 2006). In April 2002, food science professionals, the food industry and consumers were astonished by the accidental discovery that many heated foods contained unexpectedly high levels of acrylamide (Anese et al. 2009b; Tareke et al. 2002; Gökmen et al. 2006). Further research revealed that acrylamide formation principally occurs via the Maillard reaction between reducing sugars (glucose, fructose, etc.) and the amide group of asparagine, at temperatures above 120°C and low moisture conditions (Xu et al. 2016; Yuan et al. 2011; Kim et al. 2005).

The authority of European Food Safety (2015) estimated that the 95th percentile dietary acrylamide intake ranges between 0.6 and 3.4 μg/kg body weight per day across age groups. Previous reports (Chain 2015; Claeys et al. 2010; Sirot et al. 2012) revealed that fried potato products are among the food commodities that contribute the highest dietary acrylamide exposure. Recently, the benchmark levels for potato chips (0.75 mg/kg) and French fries (0.50 mg/kg) have been issued by the European Commission (Powers et al. 2017). Detailed consideration was given to potato chips because of their high acrylamide contents and their consumption rate as a popular snack worldwide (Pedreschi et al. 2005; Viklund et al. 2008a). Importantly, potato tubers contain vast

amounts of acrylamide precursors which can lead to the synthesis of the high levels of acrylamide in potato chips (Amrein et al. 2003).

The minimization of acrylamide precursors, asparagine and reducing sugars, and the improvement of processing conditions is a promising strategy to mitigate acrylamide generation in processed foods (Xu et al. 2016). The amounts of acrylamide precursors in potato tubers vary significantly among different varieties, tuber storage conditions and field cultivation practices (Silva et al. 2016; Muttucumaru et al. 2013). Additionally, the relationship between the levels of acrylamide precursors and the amount of acrylamide formed in processed foods is complex (Yang et al. 2016; Muttucumaru et al. 2013). Previous studies indicate that the reducing sugar content has a remarkable influence on acrylamide formation, especially in processed potato products (Viklund et al. 2008a). A number of processing factors, including frying conditions, additives, oil type and uptake, can significantly influence acrylamide synthesis (Ismial et al. 2013; Yang et al. 2016; Low et al. 2006). Among these factors, frying time and temperature are the most critical to the magnitude of acrylamide formation in potato chips (Kita et al. 2004).

Food additives have been extensively investigated in the context of their effects on acrylamide levels in the food industry. Some amino acids, such as glycine, have been found to effectively inhibit acrylamide formation as they can compete with asparagine to react with reducing sugars (Zhu et al. 2016). The addition of divalent cations has also been shown to be an effective means to diminish acrylamide accumulation. For example, dipping potato strips in calcium chloride prior to heating can reduce acrylamide by 95% in French fries with no adverse effects on product quality (Gökmen and Şenyuva 2007a). In addition, food antioxidants have been reported to modulate acrylamide formation with widely varying effects (Yuan et al. 2011). Several studies have reported a decrease of

acrylamide formation using compounds such as citric acid (Xu et al. 2016; Yuan et al. 2011; Low et al. 2006). Thus, there may be a practical way to use additives during the blanching process to reduce or prevent acrylamide formation. In the present study, we took a multipronged approach to minimize acrylamide generation in potato chips. We explored the effects of the following variables on the levels of reducing sugars, asparagine and acrylamide: potato variety, frying time, frying temperature and additive treatment during blanching.

3.3. Materials and methods

3.3.1. Potato materials

Three chipping potato varieties were used in this study: Atlantic, Snowden and Vigor. Tubers of Atlantic and Snowden were collected from the research farm at the Vauxhall Research Substation in Alberta, Canada. Tubers of Vigor were obtained from a commercial farm near Taber, Alberta, Canada. All tuber samples were harvested in September, 2017, and stored until use at 10°C in darkness and 90% relative humidity without application of sprout inhibitors.

3.3.2. Potato chip preparation

Potato samples (about 100 medium-sized tubers of each variety) were hand-washed and peeled and cut in half longitudinally. One of the halves of each tuber was sliced and stored at -20°C until analysis for reducing sugars and asparagine. The other half was sliced into 1.5 mm-thick sheets for processing into potato chips. The fresh slices were immediately rinsed in tap water for 1 min to eliminate any sugars and starch adhering to the surface, and then dried at 60°C in an oven for 3 min per side before frying.

3.3.3. Chemicals and solvents

Acrylamide (99.9%) and chloroform (99.8%) were acquired from Caledon Laboratories Ltd. (Georgetown, ON, Canada) and methacrylamide (98%) was purchased from Acros-Organics (NJ, USA). Acetonitrile (99.9%), hexane (95%) and formic acid (>98%) were from BDH Chemicals (Mississauga, ON, Canada). Phenol (99%) was supplied by Anachemia Sciences (Montreal, QC, Canada), and acetone (99.7%) by Fisher Scientific (Janssen Pharmaceuticalaan, Belgium). Ultrapure water was generated by a Milli-Q®-IQ 7000 system (Millipore Ltd., Etobicoke, ON, Canada). All other reagents used in this study were of analytical grade.

3.3.4. Additive treatments

Before frying, seven samples of potato slices (~150 g) of each variety were blanched in 500 ml of one of the following solutions: 1) distilled water; 2) 0.1 M calcium chloride; 3) 1% acetic acid; 4) 0.1 M sodium chloride; 5) 0.5% ascorbic acid; 6) 1% citric acid; 7) 0.1 M L-glycine. The blanching of potato slices was carried out at 65°C for 5 min to imitate industrial conditions during potato chip processing (Mestdagh et al.,

2008b). Unblanched potato slices were used as controls. After blanching, the potato slices were blotted and fried for 5 min at 180°C, which is the most common temperature used in potato chip production (Pedreschi and Moyano, 2005). About 100 g of randomly selected potato slices were collected from each treatment and stored at -20°C for analysis of reducing sugars and asparagine levels. The experiments were replicated at least three times for each treatment.

3.3.5. Frying conditions

All frying experiments to produce potato chips were conducted using a 3×4 factorial design (3, 5, 7 min × 160°C, 170°C, 180°C, 190°C). Dried slices (~10 from each group) were fried in a 3.5 L T-fal fryer (Tefal, Rumilly, Haute-Savoie, France) using canola oil. The fried chip samples were cooled to room temperature and then stored at -20°C until analysis of the acrylamide and residual reducing sugars and asparagine levels. All experiments were performed in triplicate, each being repeated at least three times.

3.3.6. Acrylamide analysis

Acrylamide formed during the frying of the potato samples was determined by the gas chromatographic (GC) method described by Weijun (2015), using several modifications. The stock solutions of acrylamide and methacrylamide (internal standard) were prepared in acetonitrile (MeCN). Each analytical batch included a spike sample for recovery measurements. A 0.5 g representative sample was placed into a 50 ml conical centrifuge tube (Fisher Scientific, Mississauga, ON, Canada), and then 4.8 ml MeCN, 2.5 ml hexane, 5 ml Milli-Q water and 10 µl methacrylamide were added. After the addition

of 0.25 g NaCl and 2 g $MgSO_4$, the sample tube was immediately sealed, vigorously shaken, and centrifuged at 4000 rpm for 10 min. A 2 ml aliquot was transferred from the middle, MeCN phase to a 2 ml microcentrifuge tube (Fisher Scientific) and centrifuged at 13000 rpm for 10 min to remove any residue. A 1 ml aliquot of supernatant was loaded into a solid phase extraction (SPE) cartridge (CarboPrep® 200, 3 ml, 250 mg, conditioned with 3 ml acetone and 3 ml MeCN) and allowed to pass through the tube by gravity. The analyte was eluted from the SPE cartridge with 1 ml acetone. The eluate was transferred into a vial and stored at 4°C for evaluation of acrylamide levels by GC with flame ionization.

3.3.7. Reducing sugar analysis

The levels of glucose and fructose were evaluated using the Megazyme D-Fructose/D-Glucose Kit (Megazyme, Chicago, IL, USA), which is an enzyme-based, UV absorption method. A 5 g sample of blanched, non-blanched, or fried homogenate was mixed with 10 ml distilled water in a 50 ml conlical tube (Fisher Scientific). The tube was shaken linearly on a MaxQ2506 shaker for 1 h. After centrifugation at 4000 rpm for 5 min, a 1 ml aliquot of the supernatant was purified using a standard phenol/chloroform method to remove proteins. The purified sample solutions were analyzed at 340 nm using a BioTek® Epoch 2 Microplate Reader and Gen5 software (v2.06; BioTek® Epoch 2, BioTek Instruments, Inc., Winooski, VT, USA). The total amount of reducing sugar was calculated by summing the levels of glucose and fructose and expressed in milligrams per 100 g of dry weight (DW).

3.3.8. Asparagine analysis

Free asparagine was measured using the Megazyme L-Asparagine/L-Glutamine/Ammonia Kit (Megazyme), an enzyme-based, UV absorption method, with some modifications. A 1 g sample of blanched or non-blanched homogenate, or a 0.5 g sample of fried homogenate, was mixed with 10 ml 33% (v/v) ethanol in a 15 ml conical tube (Fisher Scientific). The tubes were shaken linearly on a MaxQ2506 shaker for 16 h. After centrifugation at 2800 rpm for 10 min, the supernatant was collected and analyzed at 340 nm as described above. The quantity of asparagine in milligrams per 100 g of DW was calculated.

3.3.9. Statistical analysis

Analysis of variance (ANOVA) for the acrylamide, reducing sugar, and asparagine content in the potato samples was conducted using PROC MIXED ANOVA in the SAS 9.1 statistical software package (SAS Institute, Cary, NC, USA). Differences between the mean values were evaluated using the Tukey test at a 95% significance level. Pearson's correlation analysis was carried out to study the relationships between variables.

3.4. Results and Discussion

3.4.1. Acrylamide precursors in untreated fresh tubers

We first analyzed the naturally occurring levels of reducing sugars and asparagine in untreated fresh tubers **(Table 3.1).** The reducing sugars (combined glucose and fructose) varied from 222.5 ± 4.96 mg/100 g in Vigor to 585.2 ± 1.60 mg/100 g in Atlantic (2.6-fold difference), whereas the asparagine content ranged between 542.9 ± 1.22 mg/100 g in Atlantic to 1183.3 ± 19.34 mg/100 g in Vigor (2.2-fold difference). Interestingly, the cultivar with the lowest level of reducing sugars (Vigor) had the highest asparagine content among all three potato genotypes tested (222.5 ± 4.96 mg/100 g sugars and 1183.3 ± 19.34 mg/100 g asparagine). Conversely, the cultivar with the highest level of reducing sugars (Atlantic) showed the lowest asparagine content (585.2 ± 1.60 mg/100 g sugars and 542.9 ± 1.22 mg/100 g asparagine), whereas the third cultivar, Snowden, contained intermediate levels of the acrylamide precursors. No correlation between the level of reducing sugars and asparagine was observed in these varieties, although the mean values of acrylamide contributors were genotype specific.

Table 3.1: Reducing sugar and asparagine contents of the chipping potato cultivars

Variety	Reducing sugars (mg/100g DW)	Asparagine (mg/100 g DW)
Atlantic	585.2 ± 1.60a	542.9 ± 1.22c
Snowden	326.6 ± 3.90b	775.4 ± 0.38b
Vigor	222.5 ± 4.96c	1183.3 ± 19.34a

Values are expressed as means ± standard deviations (SD). Mean values with different letters are significantly different from each other within columns, following one-way ANOVA and Turkey's pairwise comparisons ($p < 0.05$).

3.4.2. Frying conditions and acrylamide formation in potato chips

We detected acrylamide in the fried chips of all three cultivars. Overall, the observed range of acrylamide content was similar to the values reported for other chipping potato varieties (Viklund et al. 2008), with a concentration ranging from 0.47 to 5.81 mg/kg. The acrylamide levels depended on frying conditions, precursor levels, and potato genotype. Acrylamide levels increased markedly with higher frying temperature and prolonged time; however, the absolute amount of acrylamide formed varied greatly among the cultivars **(Figure 3.1)**. The differences in the rate of acrylamide formation were anticipated, and likely resulted from variabilities in the levels of precursors (Yang et al. 2016).

No acrylamide was detected in potato chips after 3 min of frying at 160°C or 170°C in any of the three varieties tested. Additionally, no acrylamide was detected after 3 min of frying Snowden potato slices at 180°C or after 5 min of frying Atlantic or Vigor at 160°C. Despite the lowest reducing sugar levels in the Vigor variety, high rates of acrylamide formation were observed. This may be due to the high asparagine content in this potato, compared with Atlantic and Snowden. Overall, the potato chips processed from Snowden had the lowest acrylamide content, even though the level of reducing sugars was significantly higher than in Vigor. However, Snowden had an intermediate level of asparagine. Together, these findings confirm that low levels of both asparagine and reducing sugars are required to minimize the formation of acrylamide from potato tubers. This conclusion is in agreement with results presented by Viklund et al. (2008) showing that potato varieties with lower amounts of both asparagine and reducing sugars can considerably mitigate acrylamide formation in potato chips.

In our present study, the frying temperature and time interaction was significant ($p < 0.01$), indicating that the amount of acrylamide formed at a given temperature depends on the duration of frying time. This corresponds well with previous studies (Williams 2005; Brathen and Knutsen 2005). While the acrylamide content was comparatively low at 160°C, 170°C, and 180°C in Vigor tubers, it increased rapidly when the temperature increased to 190°C at all frying times and such a 10°C increase led to acrylamide contents increased significantly by 4.9-, 3.6-, and 2.8-fold at 3, 5, and 7 min, respectively.

Similar to Vigor, a significant increase in acrylamide content in the other two varieties was also found after increasing the frying temperature from 180°C to 190°C. Matthäus et al. (2004) also reported a dramatic increase in acrylamide formation when the temperature increased from 180°C to 190°C in fried potato products. Interestingly, the formation of acrylamide in the Atlantic and Snowden potatoes was more apparent when temperatures increased from 160°C to 170°C and the frying time was 7 min. Kim et al. (2005) found that acrylamide can be synthesized rapidly at temperatures above 160°C with increasing frying temperature and time. A possible explanation for these phenomena was investigated in French fries by Gökmen et al. (2006), who asserted that the majority of acrylamide accumulation is on the surface, where the highest temperatures are reached. Relatively high oil temperatures of 170°C and 190°C lead to surface temperatures of greater than 120°C, which can be sufficient for both the moisture evaporation and temperature increase that favour acrylamide formation, In contrast, the energy input gained at the low frying oil temperature of 150°C is insufficient for the surface temperature to exceed 120°C within 9 min.

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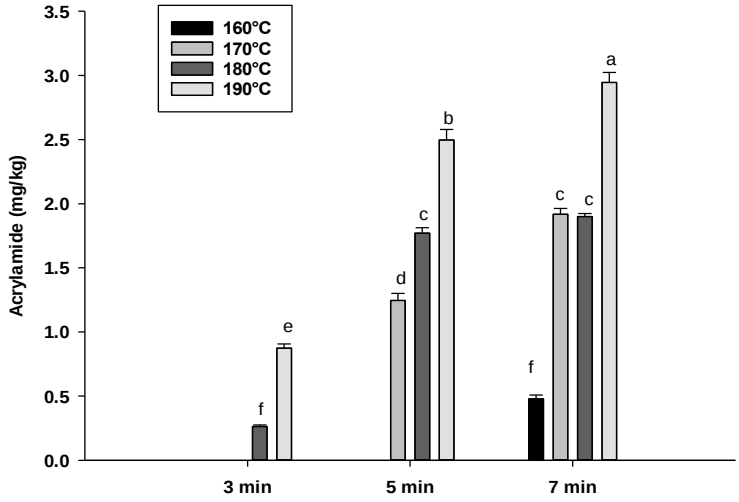
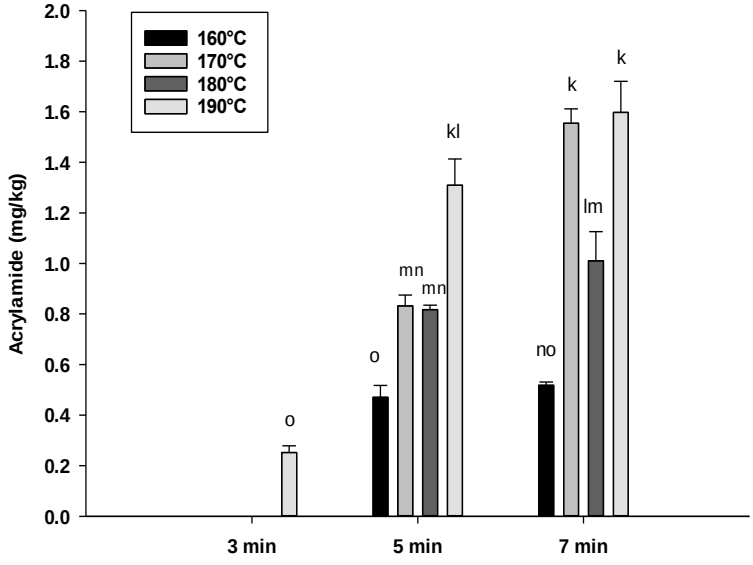
(A)


160°C
170°C
180°C
190°C
Acrylamide (mg/kg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f
e
d
c
b
f
c
c
a
3 min
5 min
7 min

(B)


160°C
170°C
180°C
190°C
Acrylamide (mg/kg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
o
o
mn
mn
kl
no
k
lm
k
3 min
5 min
7 min

(C)

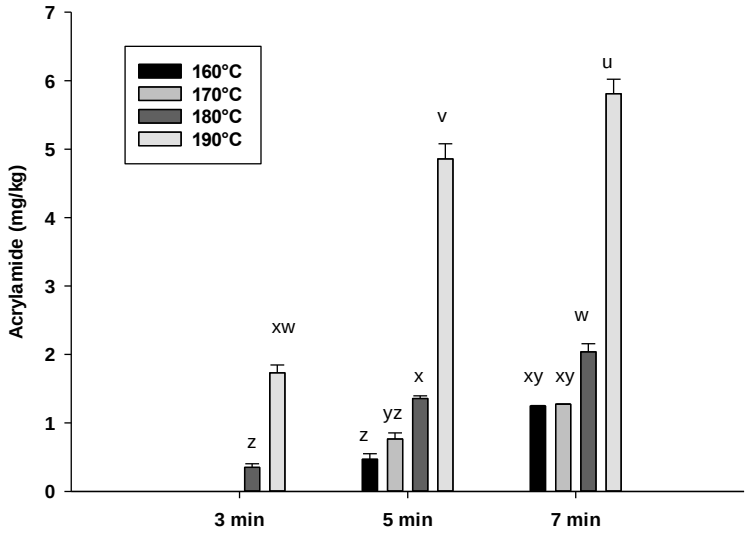


Figure 3.1: Acrylamide levels in potato chips of Atlantic (A), Snowden (B) and Vigor (C) processed under different times (3, 5, 7 min.) and temperatures (160°C, 170°C, 180°C, 190°C). Means with different letters are significantly different ($p < 0.01$) in the same variety, Atlantic (a-f), Snowden (k-o), Vigor (u-z).

When the frying temperature was lowered from 190°C to 160°C, the acrylamide formation in potato chips after 5 min of frying was decreased by 64 and 90% in Snowden and Vigor potatoes, respectively, while the acrylamide formation in potato chips after 7 min of frying was decreased by 84, 67 and 78% in Atlantic, Snowden, and Vigor, respectively. This is in agreement with other studies (Pedreschi et al. 2004; Wicklund et al. 2006) that have shown that frying at lower temperatures plays an important role in mitigating the rapid accumulation of acrylamide in fried potato products. Frying at 190°C for 3 min increased acrylamide content in comparison to frying at 160°C for 7 min in chips made from Atlantic and Vigor by approximately 81 and 38%, respectively. This is in agreement with the observations by Shojaee-Aliabadi et al. (2013) that frying at high temperatures for short periods of time increased acrylamide content in chips in

comparison to frying at lower temperatures for a longer time. However, this was not always consistent within our examined temperature range of 160-190°C, as acrylamide contents were lower after frying at 170°C for 3 min vs. 160°C for 5 and 7 min, 180°C for 3 min vs. 170°C for 5 and 7 min, and 190°C for 3 min vs. 180°C for 5 and 7 min **(Figure 3.1)**. For the Atlantic and Snowden varieties, the reduction in potato chip acrylamide was significant at all frying temperatures.

At a constant frying temperature, the acrylamide increase as after the frying time from 3 to 5 min was higher than that after the frying time from 5 to 7 min in all three varieties, which is in in agreement with the study by Williams (2005). The same behaviour was observed by Granda and Moreira (2005) in which, at the initiation of frying, there was no degradation of acrylamide, but after some time, acrylamide formation and degradation occurred simultaneously. Moreover, Elmore et al. (2005) also reported that the decrease in acrylamide content during prolonged heating could be due to secondary reactions between acrylamide and other food components or evaporation of acrylamide from the potato surface.

3.4.3. Effects of frying conditions on residual reducing sugars and asparagine in potato chips

Because little is known about the residual acrylamide precursors in fried potato products, we evaluated the reducing sugar and asparagine contents in chips processed under different frying conditions (**Figure 3.2** and **Figure 3.3**). Degradation rates of reducing sugars and asparagine increased with frying time and temperature, with only 0.6-2.0% of reducing sugars and 14.7-34% of asparagine remaining after frying at 190°C for 7 min in all three varieties. The reducing sugar and asparagine contents in potato

chips were significantly influenced by the interaction of temperature and time. The decrease in both precursors during frying was accompanied by an increase in acrylamide in all three potato varieties, thereby confirming the roles of reducing sugars and asparagine as essential contributors to acrylamide formation in potatoes (Elmore et al., 2005).

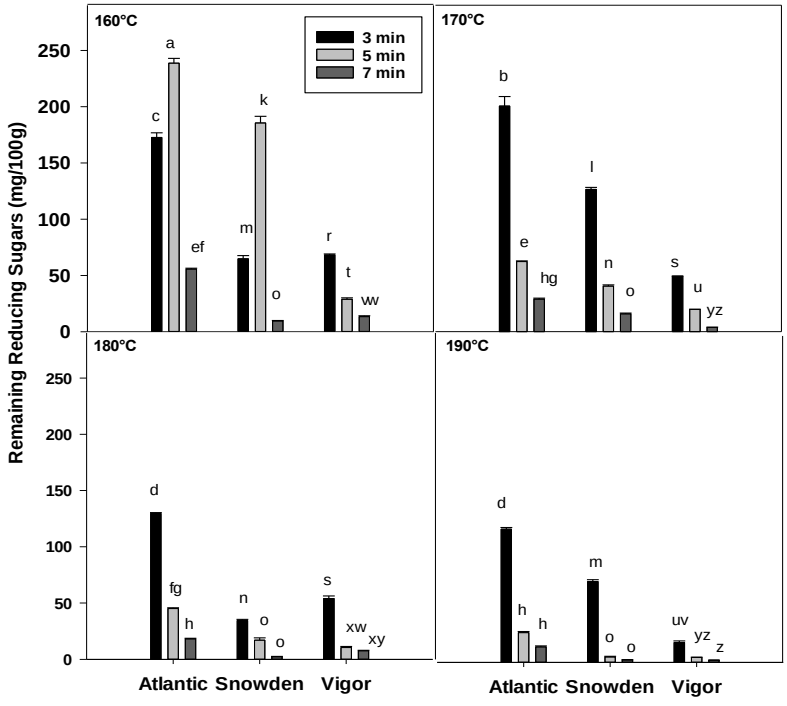


Figure 3.2: Effects of frying time on the amount of residual reducing sugar in potato chips prepared at the indicated temperatures. The means with different letters are significantly different ($p < 0.01$) within the same potato variety, Atlantic (a-h), Snowden (k-o), Vigor (r-z).

At constant frying temperatures of 170°C, 180°C and 190°C, the amounts of reducing sugar decreased significantly in all three varieties as the frying time increased from 3 to 5 and 7 min (**Figure. 3.2**). A similar, significant reduction was found at 160°C in Vigor; however, this effect was not apparent in Atlantic and Snowden where a

significant increase in reducing sugars was observed at 160°C as the frying time increased from 3 to 5 min. Overall, as a result of the high degradation rates, a remarkable loss of reducing sugars was observed by prolonging the frying time from 3 to 5 min at a given temperature in all three varieties, which correlates with the high rates of acrylamide formation during the early stages of frying (the first 3-5 min). Interestingly, such a relationship was not observed in the levels of residual asparagine during preparation (**Figure. 3.3**).

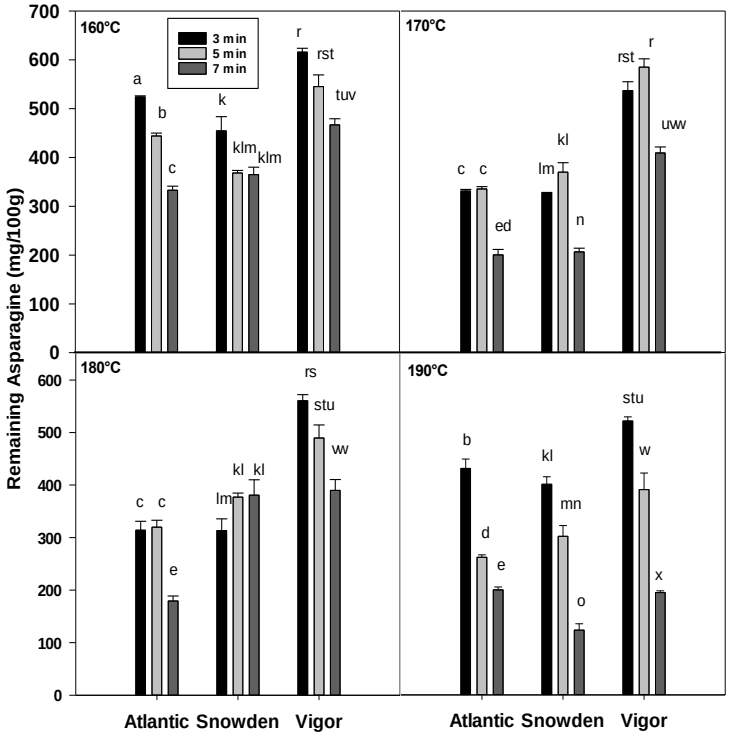


Figure 3.3: Effects of frying time on residual asparagine in potato chips prepared at the indicated temperatures. The means with different letters are significantly different ($p < 0.01$) within the same variety, Atlantic (a-e), Snowden (k-o), Vigor (r-x).

Our results indicate that the percentage change in asparagine content (1-60%) under increasing frying time at a constant temperature was generally lower than the

percentage change in reducing sugars (28-94%). Thus, the rate of decomposition of reducing sugars was higher than that of asparagine as frying time increases. This is in agreement with Amrein et al. (2003) and Williams (2005), both of whom reported that the reducing sugar content is more important than the asparagine content in the formation of acrylamide in potato products. Furthermore, 87-90% of reducing sugars, and only 39-54% of the asparagine molecules, were converted from their original levels in the Atlantic and Vigor varieties to produce nearly 0.5 mg/kg of acrylamide. Elmore et al. (2005) found that only 0.29% of asparagine molecules were converted to acrylamide at 180°C, while only 7.5% of reducing sugars remained in the potato after 20 min of cooking at the same frying temperature. That study also found a linear relationship between acrylamide formation and residual levels of reducing sugars and asparagine.

We found significant negative correlations between the levels of acrylamide and the remaining reducing sugars ($r = -0.488$, $p < 0.01$) and asparagine ($r = -0.429$, $p < 0.01$) in potato chips. As for the individual varieties, the strongest negative correlations were obtained between the remaining reducing sugars and acrylamide ($r = -0.7$ $p < 0.01$) in Atlantic tubers and the remaining asparagine and acrylamide ($r = - 0.892$, $p < 0.01$) in Vigor tubers. It is interesting to note that the correlation was much stronger between the residual fructose and acrylamide than between the residual glucose and acrylamide in all three varieties. Consistent with this finding, Pollien et al. (2003) found that fructose is more efficient than glucose in generating acrylamide when the former is heated in the presence of asparagine in Maillard model systems.

There was a strong positive correlation ($r = 0.905$, $p < 0.01$) between the residual glucose and fructose amounts; significant correlations were found within the three varieties tested. However, the residual asparagine levels showed no correlation with the

total residual reducing sugars in the potato chips. The correlation between the residual asparagine and fructose levels was stronger than that between the residual asparagine and glucose amounts in all three varieties. The asparagine and fructose correlation was significantly positive in the Atlantic ($r = 0.802$, $p < 0.01$) and Vigor ($r = 0.761$, $p < 0.01$) varieties. This is consistent with other studies in which heated asparagine decomposed readily when fructose was added to potato samples and then heated at 103°C, 150°C, and 180°C (Pollien et al. 2003; Elmore et al. 2005; Rydberg et al. 2003).

3.4.4. Effect of additive treatments on acrylamide, reducing sugars and asparagine contents

It has been reported that blanching can lower the acrylamide formation by 60% via the removal of reducing sugars, although this varies with potato varieties and processing conditions (Pedreschi et al. 2010). We examined the effects of blanching in distilled water or additives on acrylamide formation in potato chips (**Figure 3.4**). Compared to the unblanched control, blanching in water or additives effectively decreased not only the acrylamide but also the reducing sugar and asparagine levels in potato slices. The reducing sugar and asparagine levels in potato slices after blanching in the various additives are shown in **Figure 3.5 and Figure 3.6 ,** respectively. For all three varieties, the greatest decrease in acrylamide was obtained for potato chips blanched in distilled water (19-59%). This effect of distilled water could be attributed to the leaching of reducing sugars and asparagine from the surface layer of potato slices during blanching. These decreases ranged from 26 to 76% in three varieties.

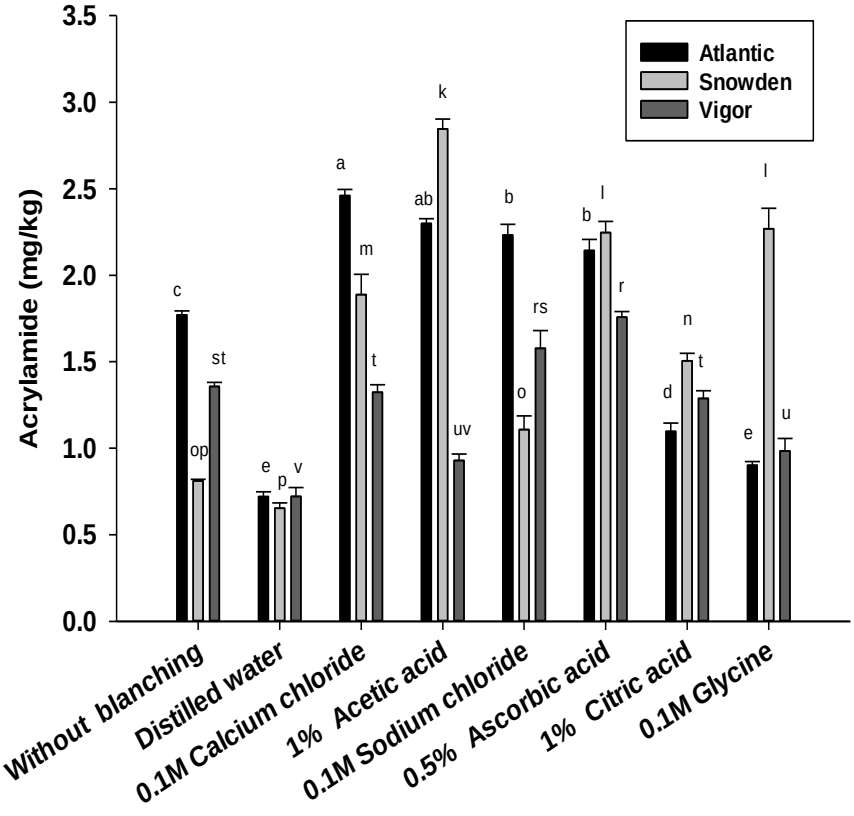


Figure 3.4: Effects of blanching in additives on acrylamide formation in potato chips. The means with different letters are significantly different ($p < 0.01$) within the same variety, Atlantic (a-e), Snowden (k-p), Vigor (r-v).

The blanching of Vigor potatoes in 1% acetic acid or Atlantic potatoes in 1% citric acid lowered the acrylamide formation by 31 or 38%, respectively. In a study conducted by Ismial et al. (2013), blanching in 0.05 M or 0.01 M citric acid at 65°C for 5 min decreased the acrylamide formation in potato chips by 93 or 90%, respectively. Blanching in organic acids can mitigate acrylamide formation by lowering the pH and leaching asparagine and reducing sugars from the potato to the solution. When exposed to lower pH, asparagine becomes protonated, which blocks further reaction with a carbonyls to form acrylamide (Jung et al. 2003). Blanching with 1% acetic acid

decreased the reducing sugar and asparagine contents in Vigor potato slices by approximately 25 and 54%, respectively, while blanching in 1% citric acid decreased the reducing sugar and asparagine contents in Atlantic potato slices by approximately 30 and 49%, respectively.

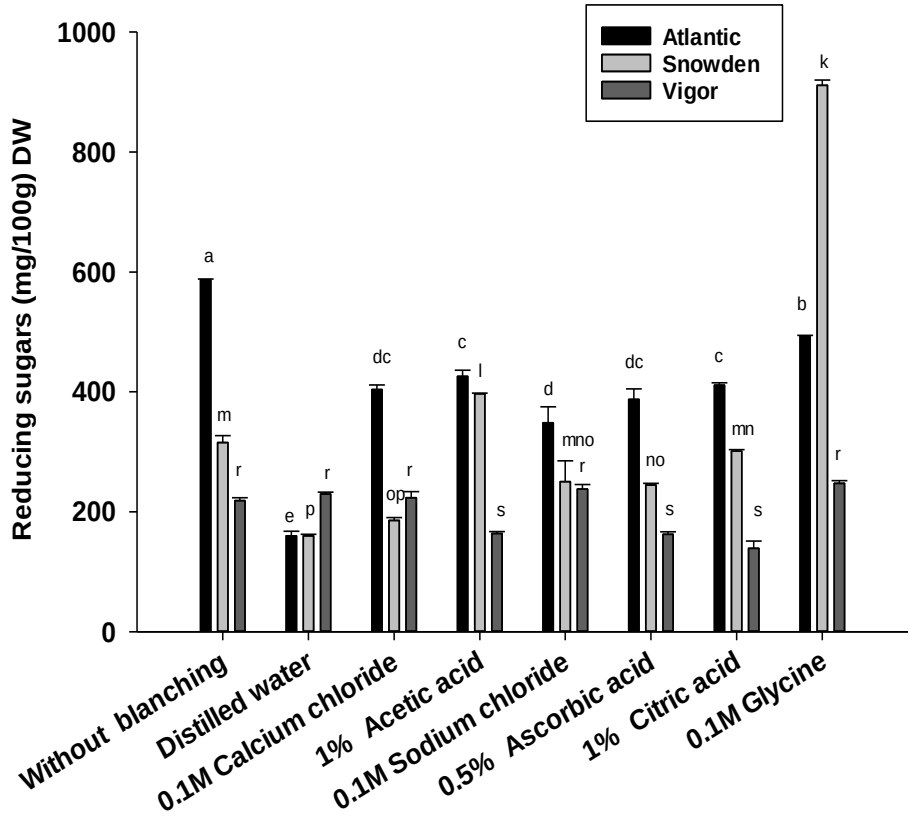


Figure 3.5: Residual reducing sugar levels in potato slices blanched with the indicated additives. The means with different letters are significantly different ($p < 0.01$) within the same variety, Atlantic (a-e), Snowden (k-p), Vigor (r-s).

In contrast to previous studies (Ismial et al. 2013; Mestdagh et al. 2008b; Gökmen and Şenyuva 2007a), we observed an increase in acrylamide formation in some of the potato samples following blanching in organic acids or salts. Pedreschi et al. (2004)

found that the addition of organic acids can decrease the amount of acrylamide in potato chips fried at 150°C, however this effect was not observed at higher temperatures of 170°C and 190°C. Furthermore, Gökmen and Şenyuva (2007b) showed that acrylamide formation decreased by up to 59% after increasing the Na^+ concentration up to 5 µmol/L; a further increase in Na^+ led to higher acrylamide formation in a model mixture composed of glucose and asparagine. Similar results were obtained in a model study conducted by Wen et al. (2016) where acrylamide inhibiting efficiency accelerated until a minimum acrylamide level was reached at low Ca^{2+} concentrations, while increasing Ca^{2+} concentrations beyond 20 µmol/L led to significantly increased acrylamide formation.

Interestingly, the increase in acrylamide content we observed after blanching in the presence of salts does not agree with the apparent decline in reducing sugars and asparagine levels in the potato slices. It was previously shown that cations can decrease acrylamide formation by preventing the formation of the Schiff base of asparagine, a key intermediate in the acrylamide synthesis (Gökmen and Şenyuva 2007a). Pedreschi et al. (2010) reported that the decreased acrylamide formation in salt-treated chips is related to changes in chemical reactions or heat transfer due to diminished oil uptake and it is not related to the acrylamide precursor contents.

Ascorbic acid (0.5%) significantly promoted acrylamide formation in chips processed from all three varieties. Previous studies have reported that the addition of ascorbic acid has a complex impact on acrylamide formation in an asparagine-glucose model system. For instance, Yuan et al. (2011) observed that a concentration of 0.5% ascorbic acid decreased acrylamide formation by approximately 57.76%, but an increase of acrylamide formation was observed at 1.5% ascorbic acid. This increase is likely a result of the thermal decomposition products of ascorbic acid acting as reducing sugars,

which may then react with amino groups to enhance the acrylamide-forming Maillard reaction (Vernin et al. 1997).

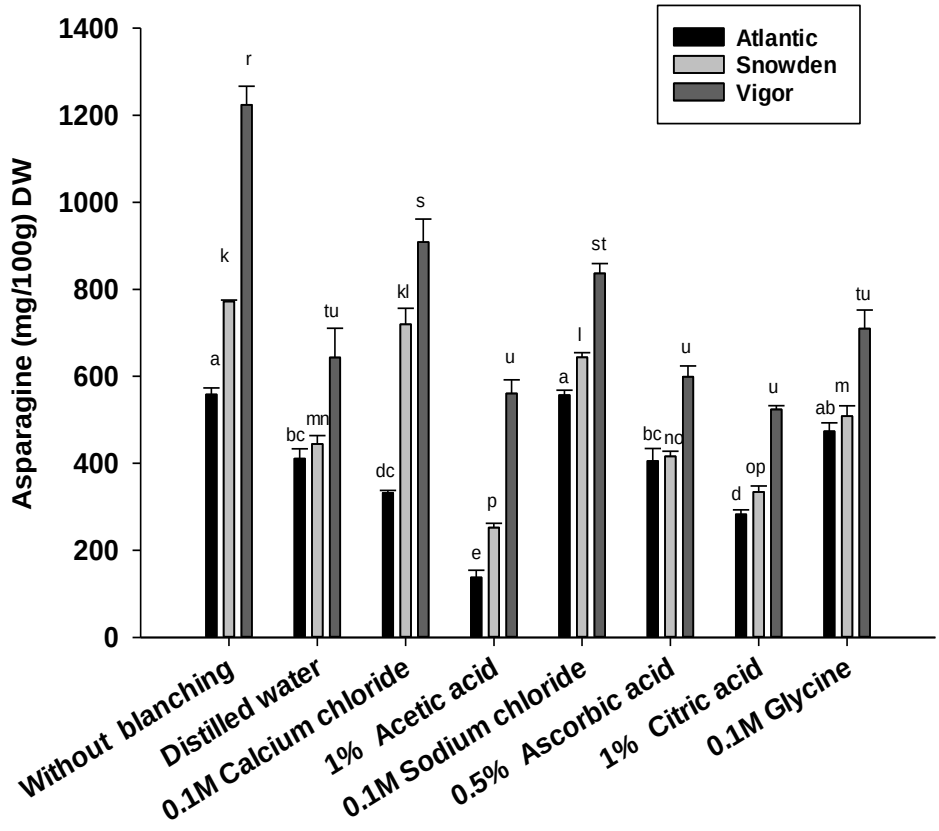


Figure 3.6: Residual asparagine levels in potato slices blanched with the indicated additives. The means with different letters are significantly different ($p < 0.01$) within the same variety, Atlantic (a-e), Snowden (k-p), Vigor (r-u).

We found that blanching in 0.1 M glycine decreased acrylamide synthesis by 49 and 27% in Atlantic and Vigor potato chips, respectively. This is in accordance with a previous report (Mestdagh et al. 2008b) where the addition of glycine to the blanching water reduced acrylamide by 68% in potato chips. Another study reported a 39% decrease in acrylamide content as the result of glycine competing with asparagine to react

with carbonyl compounds in the Maillard reaction (Mestdagh et al. 2008d). Based on kinetic studies, Zhu et al. (2016) suggested that the decrease in acrylamide formation may be attributed to the elimination reaction of acrylamide with glycine and not the competitive reaction of glycine with asparagine. In our study, blanching in 0.1 M glycine led to a significant decrease (16%) in reducing sugar levels in Atlantic potato slices as well as a significant decrease in asparagine content in the Atlantic and Vigor varieties (5 and 42%, respectively). Interestingly, glycine (0.1 M) enhanced the formation of acrylamide in the Snowden potatoes by 79%, which could be attributed to the high levels of reducing sugars in the potato slices after adding glycine to the blanching solution. The latter may be explained by the previous finding that the hydrolysis of sucrose is more efficient in the presence of glycine when the sample is heated at 55°C (Buera et al. 1987).

3.5. Conclusions

Acrylamide formation in potato chips increases with high frying temperature and long frying time and it is accompanied by significant losses of reducing sugars and asparagine in all three potato varieties tested. Lower acrylamide levels were observed at high temperatures for short frying times compared to low temperatures for long frying times. At a constant frying temperature, acrylamide formation accelerated rapidly when frying time was increased from 3 to 5 min and the formation rate decreased after 5 min of frying. As for additive treatments, blanching in distilled water had the greatest inhibitory effect on acrylamide formation (19-59%). Blanching in 1% citric acid, 1% acetic acid or 0.1M glycine resulted in less acrylamide generation in potato chips. Blanching in most additive treatments diminished both reducing sugars and asparagine contents in the potato chips.

Our results demonstrate that numerous, simple modifications to the processing steps can mitigate acrylamide formation in potato chips. As the pathway of acrylamide formation is complex and many other factors (e.g., oil uptake) are involved in determining the final amount of acrylamide, additional kinetic studies and quality assessments (texture, colour) should be considered in the implementation of acrylamide mitigating measures on a large scale.

CHAPTER 4: EFFECT OF NITROGEN FERTILIZATION STRATEGIES AND SURFACE AREA –TO-VOLUME RATIO ON ACRYLAMIDE FORMATION IN FRENCH FRIES

4.1. Abstract

Acrylamide formation during the production of French fries results from the Maillard reaction, which occurs between the amino acid asparagine and reducing sugars. Approaches to reduce acrylamide primarily include the improvement or modification of processing conditions. Efficient management of N fertilizer in potato production is another approach that can affect asparagine and reducing sugar contents and acrylamide formation in French fries and other fried potato products. The effects of different N fertilizer treatments on asparagine, reducing sugar and acrylamide contents in potato variety Russet Burbank were examined in this study. The effects of different N fertilizer strategies including split N application, source of N fertilizer, fertigation or a combination of these strategies on acrylamide precursors in fresh tubers and acrylamide formation in French fries were investigated. The amount of acrylamide and its precursors varied between N treatments and growing seasons but the effects were not always consistent across two years of this study. Adequate N fertilization tended to lower reducing sugar contents in tubers. No significant differences in total and marketable yield were observed between the N treatments. Three potato strip sizes (0.8, 1.0, 1.2 cm in width) were also evaluated for the processing of French fries in three commonly used potato varieties (Russet Burbank, Ranger Russet, Shepody). Decreasing the SVR by creating thicker potato strips reduced acrylamide content in all three varieties. Compared to the 0.8 cm strips, the 1.2 cm strips resulted in significant reductions of acrylamide

contents by 63%, 61% and 85% for Russet Burbank, Ranger Russet and Shepody, respectively.

4.2. Introduction

In April 2002, acrylamide was first discovered by Swedish researchers in starch-rich foods processed in high temperature (>120^{o}C) (Viklund et al. 2008a; Amrein et al. 2003). Since 2002, the food industry worldwide has been collaborating with scientists to minimize the levels of acrylamide in heated foods. The adoption of starting materials low in acrylamide precursors and the modification of processing conditions and post-process interventions are the main mitigation techniques (Xu et al. 2016). Decreasing the amount of acrylamide precursors can have a significant impact on acrylamide formation because reducing sugars and asparagine play an important role in synthesizing and accumulating acrylamide in processed foods (Paul et al. 2016; Morales et al. 2008). However, the relationship between the contents of acrylamide precursors and acrylamide formation in processed foods is complicated. A reduction in asparagine content can have a greater effect on acrylamide formation if reducing sugars are present in the food at higher levels than asparagine and vice versa (Muttucumaru et al. 2013). Furthermore, the contents of acrylamide precursors in potatoes can differ significantly depending on the variety, field cultivation practices and tuber storage conditions (Silva et al. 2016).

Effective fertilizer management is critical to profitable potato production and adequate nutrient levels can ensure that maximum yield and quality is achieved. However, the effect of plant nutrition on crop composition and the consequences for food quality and safety is still not clear. Being one of the essential elements for plant growth, nitrogen (N) plays an important role in ensuring optimal photosynthate production in

plants, leaf formation, tuber growth and yield (Muttucumaru et al. 2013). Moreover, N fertilization has an impact on both asparagine and reducing sugar levels in the tubers. An increase of N fertilization leads to a reduction in tuber sugar contents, although some studies reported no influence or sometimes increasing sugar levels depending on the part of the tuber examined (Amrein et al. 2003; Westermann et al. 1994; De Wilde et al. 2006b). When the N fertilization rate is elevated, asparagine in potato tubers typically increase as it is a N reservoir (Rosen et al. 2018). Previous studies on N application in potato cultivation showed the importance of meeting potato N demand by regulating the time, location, source and rate of N additions, and considering the N supply capacity of the soil. Furthermore, matching N fertilizer additions to potato N demand should result in maximum N use efficiency and marketable yield increase (Davenport et al. 2005; Munoz et al. 2005; Zebarth and Rosen 2007). Therefore, the application of N fertilizer initially at planting and again later in the growing season once the plants have emerged is recommended to potato growers. This can be achieved via the split application of fertilizer, with some applied to the field at planting and the remainder at hilling or via fertilization with irrigation water (fertigation) (Errebhi et al. 1998).

Recently, fertilizers with enhanced efficiency, such as ESN (Environmentally Smart Nitrogen - controlled release with polymer coated urea) and Super U (slow release urea with urease and nitrification inhibitors), have become available to potato growers (Wilson et al. 2010b). Different N application strategies such as split application, fertigation or combination of these strategies may also have an effect on the content and accumulation of asparagine and reducing sugars in potato tubers and subsequently acrylamide formation in processed French fries.

Previous studies suggested that altering preparation and process methods may reduce or prevent acrylamide formation in French fries (Matthäus et al. 2004; Foot et al. 2007). Investigation of the fry strip size should not be neglected when the influence of processing parameters on acrylamide formation in French fries is considered. Fry strip thickness can affect acrylamide formation through changes to the surface area-to-volume ratio (SVR), as the majority of acrylamide forms in the surface and near-surface regions of a potato strip (Gökmen et al. 2006). In this study, Russet Burbank harvested from different N fertilization strategies and three potato varieties (Russet Burbank, Ranger Russet and Shepody) processed with different fry strip sizes were evaluated to characterize acrylamide formation in their French fries with the objective to identify potential approaches for acrylamide reduction in French fries.

4.3. Materials and Methods

4.3.1. Chemicals and solvents

Acrylamide (99.9%) and chloroform (99.8%) were purchased from Caledon Laboratories Ltd. (Georgetown, Ont., Canada). Methacrylamide (98%) was obtained from Acros-Organics (New Jersey, USA). Acetonitrile (99.9%), hexane (95%) and formic acid (>98%) were from BDH Chemicals (Mississauga, Ont., Canada). Phenol (99%) was obtained from Anachemia Sciences (Montreal, Canada). Acetone (99.7%) was supplied by Fisher Scientific (Janssen Pharmaceuticalaan, Belgium). The Milli-Q water was obtained from a milli-Q® -IQ 7000 system (Millipore Ltd. Ont., Canada). All other solvents and chemicals used were of analytical grade.

4.3.2. Potato sample collection

Potato cultivar Russet Burbank was used and the field trials were conducted on a sandy loam soil at Alberta Irrigation Technology Centre in Lethbridge, AB. The trial was a randomized complete block design with four replicates (blocks). The treatments included the application of urea and ESN in different quantities at the pre-planting stage (**Table 4.1**). Some of the treatments also involved N application at the time of hilling and others included simulated fertigation treatments to reach the same total N application rate. Fertigation was simulated by applying ammonium nitrate and irrigating on three dates. The target rate of N fertilization was approximately 80% of the agronomist recommended rate for Russet Burbank production in southern Alberta (200 kg/ha). All plots received approximately 10 kg N/ha as mono-ammonium phosphate. The plots were irrigated with a centre pivot and low-pressure nozzles as required to maintain soil moisture close to 70% capacity, typically once or twice per week. The potatoes were stored at 10°C and 90% relative humidity without application of sprout inhibitors. Potato samples for 10 different N treatments were collected from potato field trials in 2016 and 2017 to evaluate the effect of N fertilization strategy on asparagine, reducing sugars and acrylamide formation.

Three potato varieties (Russet Burbank, Ranger Russet and Shepody) were collected from Vauxhall Research Substation to evaluate the effect of potato strip size on acrylamide formation. These potato samples were harvested in September 2017 and stored at 10°C and 90% relative humidity without application of sprout inhibitors before the treatments.

Table 4.1: Description of nitrogen fertilization treatments in this study

Treatments	Pre-Plant		At Hilling		Simulated
	Urea	ESN	Urea	ESN	Fertigation
Untreated (Control)	-	-	-	-	-
Urea Pre-Plant Broadcast	100	-	-	-	-
Urea Split	60	-	40	-	-
Urea/ESN Split	60	-	-	40	-
ESN + Fertigation	-	60	-	-	40
ESN Pre-Plant Broadcast	-	100	-	-	-
Pre-Plant Urea + Fertigation	60	-	-	-	40
Urea/ESN Split + Fertigation	30	-	-	30	40
Urea/ESN Pre-Plant + Fertigation	30	30	-	-	40
Urea/ESN at Hilling	-	-	60	40	-

Values are percentage (%) of total N fertilizer added.

4.3.3. Preparation of potato strips for frying

4.3.3.1. Effect of N fertilization

Potato samples from the field trials (about 8 tubers from each replicate) were cut into strips (1cm×1cm) using a French fry cutter. About 100 g of randomly selected potato strips were collected from each treatment and stored at -20°C for subsequent reducing sugar and asparagine analysis. Strips were rinsed immediately after cutting for 1 min in tap water to eliminate any starch adhering to the surface and dried in an oven at 60°C for 3 minutes per side prior to frying. All experiments were conducted in triplicate.

4.3.3.2. Effect of potato strip size

Potatoes (about 30 medium sized tubers from each variety) were cut into three different strip sizes using a French fry cutter (0.8 cm, 1.0 cm and 1.2 cm in width). A ruler and knife were used to cut these strips to the same length of 5 cm. About 100 g of

randomly selected potato strips were collected from each variety and stored at -20°C for subsequent reducing sugar and asparagine analysis. Prior to frying, strips were rinsed and dried as previously described in section **4.3.3.1**. All experiments were conducted in triplicate.

4.3.4. Frying of potatoes

Canola oil was used in the frying and approximately ten dried slices were fried in a 3.5 L T-fal fryer (Tefal, Rumilly, Haute-Savoie, France) at 180°C for 5 min. The French fries were then cooled at room temperature and stored at -20°C for acrylamide analysis.

4.3.5. Asparagine analysis

Free asparagine was determined enzymatically according to the microplate assay procedure of Megazyme's L-Asparagine/L-Glutamine/Ammonia Kit with some modifications. One gram of homogenate was mixed with 10 ml of 33% (v/v) ethanol in 15 ml falcon tubes. The tubes were shaken linearly on a MaxQ2506 shaker for 16 h. After centrifugation at 2800 rpm for 10 min, the supernatant was collected for further analysis. The readings were made at 340 nm using a BioTek® Epoch 2 Microplate Reader and Gen5 software version 2.06 (BioTek® Epoch 2, BioTek Instruments, Inc., Winooski, VT, USA). The results were expressed as mg/100 g fresh weight (FW).

4.3.6. Reducing sugar analysis

Glucose and fructose were enzymatically determined according to the microplate assay procedure of Megazyme's D-Fructose/D-Glucose Kit with some modifications. Five grams of homogenate were mixed with 10 ml distilled water in 50 ml falcon tubes.

The tubes were shaken linearly on a MaxQ2506 shaker for 1 h. After centrifugation at 4000 rpm for 5 min, an aliquot of 1 ml supernatant was purified with the standard phenol and chloroform procedure to remove proteins. The purified sample solutions were subjected to enzymatic analysis of glucose and fructose. The readings were made at 340 nm using the same microplate reader mentioned above. Total reducing sugars were calculated by summing glucose and fructose. The results were expressed as mg/100 g FW.

4.3.7. Acrylamide analysis

The determination of acrylamide was conducted according to the available procedure (Weijun, 2015) with several modifications. The stock solutions of acrylamide and methacrylamide (internal standard) were prepared in acetonitrile (MeCN). Each analytical batch included a spike sample for recovery measurements. Approximately 2 g of a representative sample was weighed into a 50 ml falcon tube, and then 4.8 ml MeCN, 2.5 ml of hexane, 5 ml of milli-Q water and 10 µl of methacrylamide were added. After the addition of 0.25 g of NaCl and 2 g of $MgSO_4$, the sample tube was immediately sealed, vigorously shaken, and then centrifuged at 4000 rpm for 10 min. Two ml aliquot was transferred from the middle MeCN phase to a snap-cap centrifuge tube and centrifuged at 13,000 rpm for 10 min to remove any residue. One ml supernatant was loaded into a SPE tube (CarboPrep® 200, 3 ml, 250 mg, conditioned with 3 ml of acetone and 3 ml of MeCN) for solid-phase extraction and allowed to filter by gravity. Following this, the SPE cartridge was eluted using 1 ml of acetone. The eluate was transferred into a vial and stored at $4^{\circ}C$ for further analysis of acrylamide by gas chromatography (GC) with flame ionization.

4.3.8. Statistical analysis

Analysis of variance (ANOVA) of acrylamide, reducing sugar and asparagine contents as functions of N fertilizer strategy, potato variety, year and potato strip size was conducted using PROC MIXED in SAS 9.1 Statistical software package (SAS Institute, Cary, NC, USA). Differences between mean values were evaluated using the Tukey test at 95% significance level. Pearson's correlation analysis was carried out to examine the relationships between variables.

4.4. Results and Discussion

4.4.1. Effect of nitrogen fertilization on asparagine, reducing sugars and acrylamide formation

4.4.1.1. Effect of nitrogen fertilization on reducing sugars and asparagine

The contents of asparagine and reducing sugars were significantly ($p < 0.01$) influenced by the interaction of N treatment and year. The asparagine content varied between 175.15 and 200.14 mg/100g in 2016 and between 206.48 and 251.14 mg/100g in 2017. These values are consistent with those of previous studies for different varieties, which found that asparagine content ranges between 203 and 321 mg/100g FW (Yang et al., 2016) and 15 and 762 mg/100g FW (Vivanti et al., 2006). In 2017, all treatments showed significantly lower reducing sugar contents (from 181.46 to 249.10 mg/100g) compared to the previous year when these values ranged from 280.10 to 391.32 mg/100g across all N treatments. The low reducing sugar and high asparagine contents observed in 2017 compared with 2016 may be due to variability in climatic conditions during the growing season, chemical maturity, harvest operations or storage conditions. Chemical maturity stage signifies that the crop is ready for harvest. This stage occurs before vine

desiccation and depends on factors such as potato variety, soil moisture, temperature and nutrition (Rosen et al. 2018). When tubers achieve minimum sucrose and reducing sugar contents and high dry matter content, they are considered “chemically mature”. In contrast, immature tubers contain greater amounts of sugars as the rate of translocation to tubers surpasses the rate of metabolism (Heltoft et al. 2017).

Over maturation can also elevate sugar contents (Bussan et al. 2009). In this study, tubers were harvested on 139 days after planting in 2017 and 134 days in 2016. The decrease in reducing sugar contents between 134 and 139 days could be due to the synthesis of starch by reducing sugars in tubers. Similarly, Okoth et al. (2012) reported a decrease in reducing sugar contents in tubers harvested at 120 days after planting in comparison to those harvested at 90 days. As the agronomic practices and storage conditions were the same during 2016 and 2017 growing seasons, variation in climatic conditions between two years may explain the differences in levels of acrylamide precursors. A significant effect of seasonal variation on the reducing sugar, dry matter, total free amino acid and free asparagine contents of potato varieties used for potato chip and French fry production have also been reported in previous studies (Viklund et al. 2008b; De Meulenaer et al. 2008). Average temperatures were higher than normal during the entire 2017 growing season (**Figure 4.1**). Furthermore, the average maximum temperature was higher and number of days with a maximum temperature >30°C was longer during the 2017 growing season in comparison to 2016. Throughout the entire 2017 growing season there were 27 days with maximum air temperature > 30°C compared with only 4 days during the 2016 growing season. De Meulenaer et al. (2008) reported that exceptionally warm and dry summers can lead to a decrease in reducing sugar content in tubers, likely because they reached full maturity earlier. In contrast, several studies reported that high

air temperatures above 25 to 30°C can elevate sugar levels, especially in varieties such as Russet Burbank which are susceptible to the sugar end defect (Thompson et al. 2008; Rosen et al. 2018).

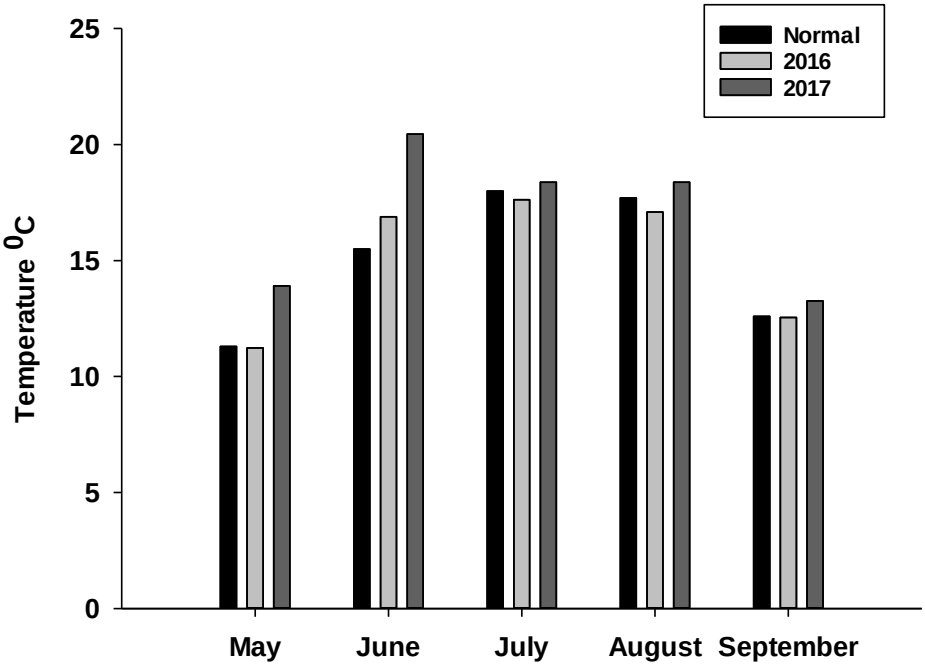
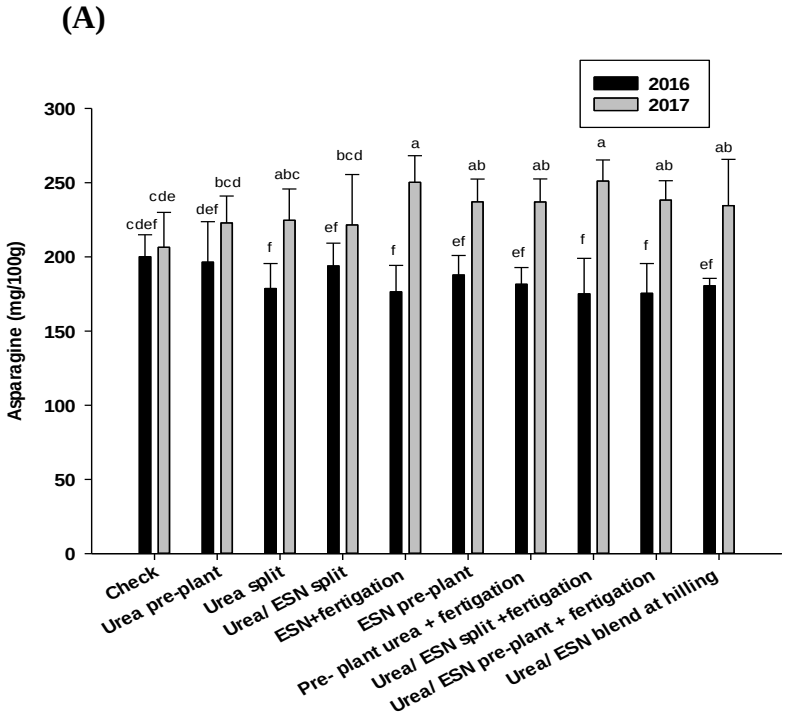


Figure 4.1: Mean temperature in 2016, 2017 and the normal values for the growing location**.**

In 2017 all N treatments resulted in considerable increases in asparagine content compared to the untreated control with no additional N (**Figure 4.2A**). Previous studies have shown that an increase of N fertilization led to a rise in both total amino acid and asparagine contents in tubers (Gerendás et al. 2007). Furthermore, the enhanced N fertilization can cause a decrease in tuber sugar content at harvest as these sugars are used up during biosynthesis of asparagine (Morales et al. 2008; Kumar et al. 2004; De Wilde et al. 2006b). In this study, a significant negative correlation was observed

between asparagine and reducing sugar content ($r = -0.641$, $p < 0.01$). This is likely associated with high asparagine and low reducing sugar contents observed in 2017 growing season. Low N application can enhance reducing sugar contents and leads to a further increase in acrylamide formation (Mestdagh et al. 2009; De Wilde et al. 2006b). This is in accordance with low asparagine and high reducing sugar contents obtained in the previous year. According to some studies (Kumar et al. 2004; Zebarth et al. 2004), N fertilization had no effect on sugar content, although its effects may vary depending on the part of the tuber. While several studies have measured the influence of increasing N application on acrylamide and its precursors, there are very few studies that have measured the influence of different N fertilization strategies on these parameters.



(B)

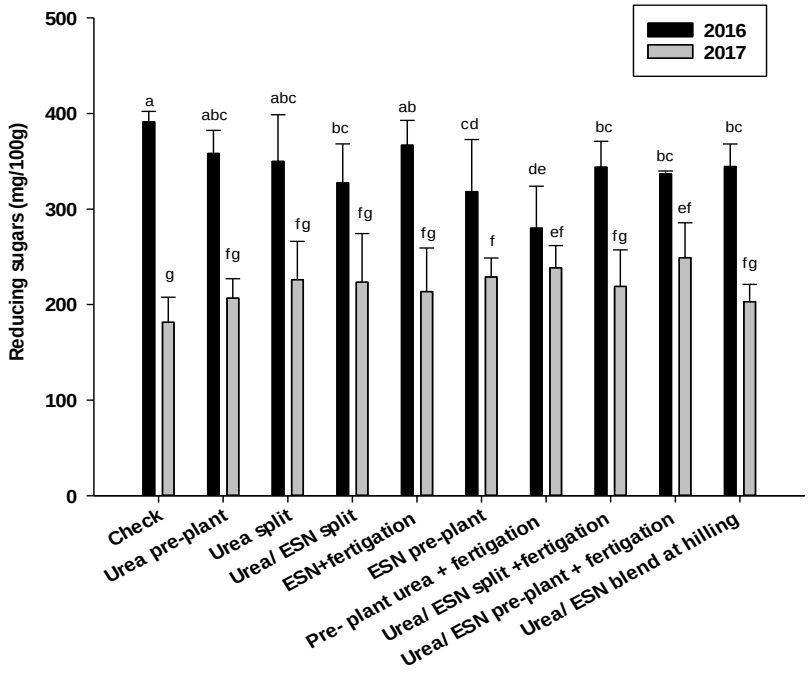


Figure 4.2: Effect of N fertilization strategies on asparagine (A) and reducing sugars (B) contents of Russet Burbank. Means with different letters are significant ($p < 0.01$) in different treatments.

In this study, no significant differences in asparagine content were observed between treatments in 2016 growing season. However, treatments including fertigation resulted in significantly higher asparagine contents during 2017 growing season compared with treatments where the N was applied entirely as urea at the pre-planting stage, where the N was applied as Urea:ESN in a split application and the control. The ESN Plus Fertigation and the Urea: ESN Split Plus Fertigation resulted in the highest asparagine content while it was lowest in the untreated control. The values obtained for ESN Plus Fertigation and Urea-ESN Split Plus Fertigation were significantly higher than

those under Urea Pre-Plant and Urea: ESN Split applications. Moreover, the Urea:ESN blend at Hilling and the ESN Pre-Planting applications led to significantly higher asparagine contents compared to the control. However, no significant differences in marketable and total tuber yield were observed among the treatments in both 2016 and 2017 growing seasons. These results are in agreement with Gao et al. (2018) who showed that various N fertilization strategies combining N source, timing, and fertigation did not affect yield or the N uptake of potatoes. This limited response was likely due to high N application rates, where the amount of N fertilizer provided during the study was sufficient to meet crop demands even though it was 80% of the recommended application rate (Gao et al. 2018). Similarly in this study, the target N was intended to be approximately 80% of the agronomist recommended rate for Russet Burbank production in southern Alberta (200 kg/ha).

With the exception of ESN Plus Fertigation, all of other treatments including fertigation, ESN Pre-Plant and Urea:ESN blend at Hilling resulted in significantly lower reducing sugar contents than the control in the 2016 growing season (**Figure 4.2B**). In contrast, ESN Pre-Plant, Pre-Plant Urea Plus Fertigation and Urea-ESN Pre-Plant Plus Fertigation treatments resulted in significantly higher reducing sugar contents than the control in the following year. However, differences in the acrylamide precursor contents across the ten N treatments were not consistent between the two growing seasons.

4.4.1.2. Effect of nitrogen fertilization on acrylamide formation

Our results indicated that the interaction of N treatment and year for acrylamide formation was significant ($p < 0.01$). Acrylamide levels ranged from 0.12 to 0.86 mg/kg in 2016 growing season and from 0.2 to 0.47 mg/kg in 2017 growing season. These

values are in accordance with previous report for French fries from a survey conducted by the U.S. Department of Agriculture (Wang et al. 2016). The differences in acrylamide content among the growing seasons and N treatments can be attributed to the variations in asparagine and reducing sugar contents (Viklund et al. 2008a). Muttucumaru et al. (2013) reported that the elevated N application gave rise to higher levels of acrylamide, although the effect was variety dependent. In contrast, De Wilde et al. (2006b) reported that N fertilization reduced the formation of acrylamide. However, such effects varied between variety, growing conditions, harvest date and storage conditions (Rosen et al. 2018; Muttucumaru et al. 2013).

The ESN Plus Fertigation treatment led to the lowest acrylamide contents in 2016 and Urea-ESN Split Plus Fertigation led to the lowest acrylamide contents in 2017 (**Figure 4.3**). In 2016 growing season, Pre-Plant Urea Plus Fertigation, Urea:ESN Pre-Plant Plus Fertigation and Urea:ESN at Hilling led to significantly higher acrylamide formation than the control in 2016 and all treatments in 2017. This was probably related to the high reducing sugar contents observed in all N treatments in 2016 compared to 2017. However, the apparent increase in reducing sugar contents in the control , Urea Pre-Plant, Urea Split, Urea-ESN Split, ESN Plus Fertigation and ESN Pre-Plant treatments in 2016 resulted in a limited change in the acrylamide content in French fries.

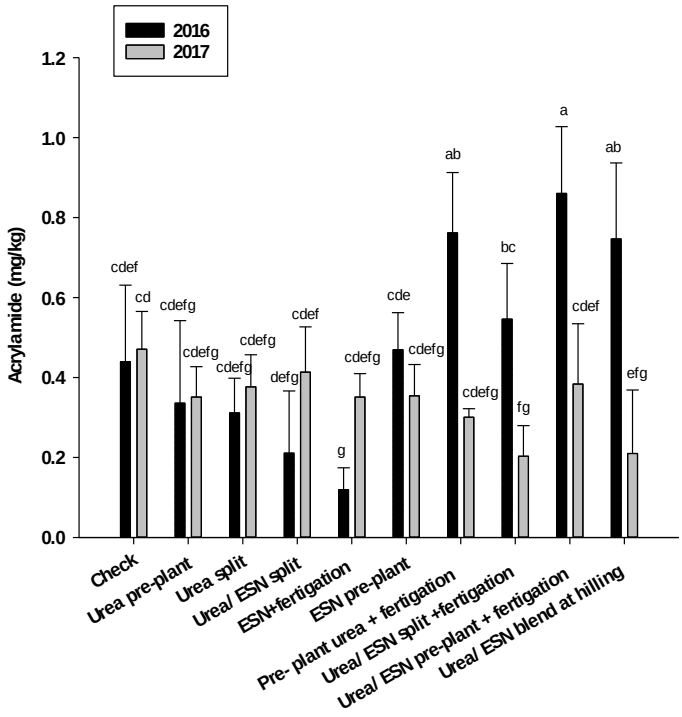


Figure 4.3: Effect of nitrogen fertilization strategies on acrylamide formation in French fries (Russet Burbank). Means with different letters are significant ($p < 0.01$) in different treatments.

Similar acrylamide contents were observed among almost all treatments in 2017 growing season, likely due to similar contents of reducing sugar contents among the treatments for this year. However, no significant correlations were found between acrylamide and precursor contents, which is not in accordance with previous studies that reported significant correlations among these variables (De Wilde et al. 2006b; Amrein et al. 2003; Sun et al. 2018).

4.4.2. Surface to volume ratio on acrylamide formation

Acrylamide contents of French fries were assessed for potato strips of different widths (0.8, 1.0 and 1.2 cm). The reducing sugar and asparagine contents of potato varieties used in this study are shown in **Table 4.2**. The reducing sugar contents of tubers were less than the 3 g/kg fresh weight recommended by FoodDrinkEurope for the production of fried potato products (Mesías et al. 2017). The asparagine contents in tubers were consistent with the values obtained for different varieties (0.15-7.62 g/kg) in a study conducted by Vivanti et al. (2006).

Table 4.1: Asparagine and reducing sugar contents of the French fries varieties

Variety	Reducing sugars (mg/100g FW)	Asparagine (mg/100g FW)
Russet Burbank	84.10±0.98c	226.80±5.35a
Ranger Russet	186.50± 3.38a	179.38±2.72b
Shepody	110.91±0.74b	166.05±1.74b

Values are expressed as means ± standard deviations (SD). Mean values with different letters are significantly different from each other within columns, following one-way ANOVA and Turkey's pairwise comparisons ($p < 0.01$).

The content of total reducing sugars in Russet Burbank was the lowest of three varieties tested, while the asparagine content of Russet Burbank was the highest among these three varieties. Fries processed from Shepody potatoes had the lowest content of acrylamide (varied between 0.025 and 0.168 mg/kg) for all widths, while acrylamide content in fries processed from Russet Burbank and Ranger Russet potatoes ranged from 0.149 to 0.45 mg/kg. Decreasing the SVR (surface to volume ratio) by creating thicker strips of potato reduced the acrylamide content per unit of mass (**Figure 4.4)**. It

decreased significantly as the width size of strips increased from 0.8 cm to 1.0 cm and 1.2 cm in fries processed from all three varieties. Furthermore, changing the width from 0.8 to 1.0 cm caused a reduction in acrylamide of between 55 and 63%. When frying at 180°C for 5 min, changing the width size from 0.8 to 1.2 cm led to a reduction in acrylamide content by about 63%, 61% and 85% in Russet Burbank, Ranger Russet and Shepody, respectively. A possible explanation for this phenomenon has been investigated in previous studies. As postulated in Gökmen et al. (2006), high levels of acrylamide build up on surface and in near-surface regions of the potato strip where the highest temperatures are achieved during frying, suggesting that the formation of acrylamide is mainly a surface phenomenon. Thus, SVR plays an important role in the frying operation (Gökmen and Palazoğlu 2009). During frying, when increasing the SVR, greater amounts of acrylamide precursors become exposed to higher surface temperatures, and the whole volume of the potato strip heats rapidly leading to higher rates of acrylamide formation. Taubert et al. (2004) found a consistent increase of acrylamide level with increasing frying temperature and time in potato shapes with low SVR, due to a large interior pool of saturated precursor content, which can favor the formation of acrylamide over degradation.

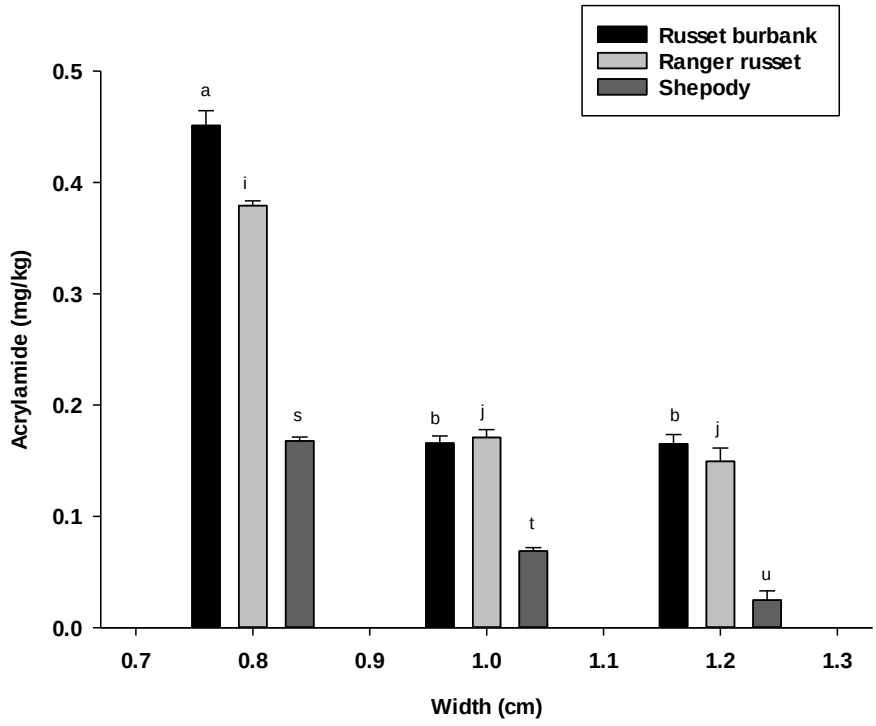


Figure 4.4: Effect of strip size on acrylamide formation in French fries. Means with different letters are significant ($p < 0.01$) in strip sizes at the same cultivar. a-b: Russet Burbank, i-j: Ranger Russet, s-u: Shepody.

In shapes with high and intermediate SVR, maximum acrylamide rates were achieved at 160-180°C and further increasing the frying temperature and time decreased the rate of formation of acrylamide. This is due to continuous depletion of the small interior pool of precursors, where degradation can determine the rate of acrylamide formation during extended frying time. These observations suggest that the SVR dependent variations in acrylamide formation can also be attributed to temperature and time. Further increasing the strip width from 1.0 to 1.2 cm did not result in a significant change in acrylamide formation in fries processed from Russet Burbank and Ranger Russet. A possible explanation for this was reported by Gökmen and Palazoğlu (2009), who attributed this phenomenon to the prior consumption of the lower amount of sugars

originally present in the 1.0 cm strips compared with the 1.2 cm strips, or the rapid degradation of acrylamide due to the higher temperatures experienced much earlier within the 1.0 cm strips, which limited the acrylamide formation.

4.5. Conclusions

Our results show that the reducing sugar and asparagine contents in tubers and subsequent acrylamide formation in French fries were significantly influenced by the interaction of N treatments and the growing season. Adequate N fertilization caused a decline in reducing sugar contents in tubers at harvest. Different N fertilization strategies tended to affect acrylamide content by influencing acrylamide precursor contents in tubers. The timing, source of N fertilizer, fertigation or combination of these strategies had no significant influence on total and marketable yield. No consistent changes in acrylamide or precursor contents were observed between the two growing seasons across the ten N treatments. SVR is an important determinant of acrylamide formation in final potato products. In French fries, reducing the SVR by cutting thicker potato strips resulted in a decrease of acrylamide formation upon frying.

CHAPTER 5: SUMMARY AND FUTURE DIRECTIONS

5.1. Summary

Potato chips and French fries contribute to the dietary intake of acrylamide, a probable carcinogen in heat-processed foods. This study aimed to determine the effects of frying temperature (160, 170, 180, 190°C), time (3, 5, 7 min) and additive treatments on reducing sugars, asparagine levels and further acrylamide formation in fried potato chips processed from Atlantic, Snowden and Vigor varieties. Moreover, the effects of different N fertilizer treatments including split N application, source of N fertilizer, fertigation or a combination of these strategies on asparagine, reducing sugar and acrylamide contents in the potato variety Russet Burbank were examined. We also investigated the relationship between potato strip size (0.8, 1.0, 1.2 cm width) and acrylamide levels in French fries of Russet Burbank, Ranger Russet and Shepody varieties. Acrylamide formation in potato chips increased with increased frying temperature and time and it was accompanied by significant losses of reducing sugars and asparagine. Moreover, lower acrylamide levels were generated at high temperatures for short frying times compared to low temperatures for long frying times. Blanching in distilled water, 1% citric acid, 1% acetic acid and 0.1M glycine were found to be an effective means of reducing acrylamide formation during frying. Blanching in most additive treatments reduced both reducing sugars and asparagine contents in potato slices. The amount of acrylamide and its precursors varied between N treatments and growing seasons but the effects were not always consistent across the two years of the study. Decreasing the SVR by creating thicker strips of potato reduced the acrylamide content in all three French fry cultivars. It was found that the 1.2 cm width size resulted

in the greatest reductions of 63%, 61% and 85% for Russet Burbank, Ranger Russet and Shepody varieties, respectively, compared to the 0.8cm width size. In conclusion, this study has provided valuable information for future researchers as well as potato chip/French fry producers in relation to understanding the factors that may be exploited to reduce acrylamide formation in these cooked products.

5.2. Future directions

Although several possibilities are suggested for acrylamide mitigation in fried potato products, an integrated approach is required to achieve substantial reduction while retaining the corresponding product quality attributes such as flavor, color and texture. One of the practical and efficacious possibilities for reducing acrylamide formation is the optimization of the time-temperature profile during frying. This thesis and other similar studies (Pedreschi et al. 2004; Wicklund et al. 2006) have indicated that lowering the processing temperature and time could minimize acrylamide formation in fried potato products. In order to prevent high acrylamide levels, frying temperature should not exceed 170-175°C and lower temperatures at the end of the frying process may mitigate acrylamide formation (Mestdagh et al. 2009). However, a lower processing temperature will affect fat uptake, moisture and texture of the fried product (Kita et al. 2004). Future lines of research should expand the investigation to search for a time/temperature combination coupled with other treatments and techniques (e.g. vacuum frying) to reduce acrylamide formation while preserving overall food quality. Furthermore, to optimize process conditions kinetic research during the Maillard reaction is needed. For instance, kinetic data offer the advantage of investigating the effect of moisture content on browning and acrylamide formation (Amrein et al. 2006).

Results show that blanching and additive treatments on cut potatoes can reduce the amount of acrylamide precursors and subsequent acrylamide formation in the fried product. However, applied additives and concentration levels, blanching temperature and time should be well chosen so that product quality is not compromised. Moreover, investigations should also examine product optimization techniques such as the use of flavorings (e.g. paprika, sodium salt) in order to cover up any sensory defects resulting from the application of acrylamide-lowering additives (Mestdagh et al. 2008b).

Our present study showed that the different N fertilization strategies tended to affect acrylamide content by influencing acrylamide precursor contents in tubers. However, the effects of N fertilization are complex due to chemical/physiological maturity, cultivar and growing conditions (Rosen et al. 2018). Future research should examine the effect of N fertilization strategies on acrylamide formation in more than two consecutive experimental years by incorporating more sites and varieties. In French fries, reducing the SVR by cutting thicker potato strips resulted in a decrease in acrylamide formation upon frying. Therefore, optimization of cutting practices and screening to remove thinner and smaller cut sizes may contribute to a reduction in acrylamide formation.

Other future lines of research should expand the investigation to include developing varieties that consistently maintain lower levels of reducing sugars and asparagine contents even under prolonged storage. Efforts are also needed to identify measures that can be adopted by consumers to lower acrylamide production in home-prepared foods. Considerations such as feasibility, cost, effluent treatment, safety and consumer acceptability should be considered when implementing an acrylamide mitigation strategy on an industrial level.

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Appendix-1: Glucose and fructose contents of fresh potato samples

Variety	Glucose (mg/100g DW)	Fructose (mg/100g DW)
Atlantic	351.65 ± 1.18	233.56 ± 0.42
Snowden	223.47 ± 1.90	103.16 ± 2.00
Vigor	150.9 ± 1.94	65.56 ± 1.24

Values are expressed as means ± standard deviations.

Appendix-2: Effects of frying time on the glucose and fructose remain in potato chips prepared by 160˚C, 170˚C, 180˚C, 190˚C

Variety	Temperature (˚C)	Time (min)	Glucose (mg/100g DW)	Fructose (mg/100g DW)
Atlantic	160	3	90.73 ± 2.39	82.01 ± 1.71
		5	122.33 ± 1.08	116.32 ± 0.63
		7	21.50 ± 1.04	34.27 ± 0.37
	170	3	112.99 ± 1.38	87.45 ± 1.48
		5	24.24 ± 0.91	38.10 ± 0.40
		7	13.02 ± 0.99	15.90 ± 0.17
	180	3	63.86 ± 0.25	65.69 ± 0.60
		5	16.38 ± 0.53	28.74 ± 0.24
		7	6.86 ± 0.02	11.31 ± 0.57
	190	3	64.43 ± 1.49	53.87 ± 0.81
		5	11.59 ± 0.77	14.82 ± 1.05
		7	5.87 ± 0.30	7.86 ± 0.73
Snowden	160	3	39.05 ± 1.89	25.79 ± 0.71
		5	122.78 ± 4.53	62.74 ± 1.21
		7	3.41 ± 0.41	6.30 ± 0.15
	170	3	96.95 ± 1.11	29.53 ± 0.46
		5	20.53 ± 0.57	19.93 ± 0.59
		7	7.30 ± 0.42	8.54 ± 0.34
	180	3	16.65 ± 0.58	17.91 ± 0.42
		5	5.83 ± 1.04	11.22 ± 1.29
		7	0.47 ± 0.00	1.88 ± 0.27
	190	3	42.21 ± 4.49	29.49 ± 0.21
		5	1.38 ± 0.01	3.42 ± 0.54
		7	0.52 ± 0.04	1.57 ± 0.27
Vigor	160	3	38.41 ± 0.79	29.87 ± 0.26
		5	10.56 ± 0.25	18.40 ± 0.95
		7	4.16 ± 0.48	9.38 ± 0.31
	170	3	23.58 ± 0.41	25.63 ± 0.36
		5	7.40 ± 0.72	12.36 ± 0.54
		7	1.56 ± 0.16	2.18 ± 0.33
	180	3	26.65 ± 1.25	27.41 ± 1.07
		5	4.74 ± 0.39	5.75 ± 0.61
		7	3.63 ± 0.24	4.00 ± 0.29
	190	3	6.57 ± 0.63	11.03 ± 0.76
		5	2.11 ± 0.15	2.29 ± 0.27
		7	0.90 ± 0.00	1.00 ± 0.05

Values are expressed as means ± standard deviations**.**

Appendix-3: Analysis of variance for acrylamide concentration and remaining concentrations of glucose, fructose, total reducing sugar and asparagine as affected by cultivar, temperature, time and their interactions

Source of variation	Degree of Freedom	Acrylamide	Glucose	Fructose	Total reducing sugar	Asparagine
Main effects						
Cultivar	2	<.01	<.01	<.01	<.01	<.01
Temperature	3	<.01	<.01	<.01	<.01	<.01
Time	2	<.01	<.01	<.01	<.01	<.01
Interactions						
Cultivar*Temperature	6	<.01	<.01	<.01	<.01	<.01
Cultivar*Time	4	<.01	<.01	<.01	<.01	<.01
Temperature*Time	6	<.01	<.01	<.01	<.01	<.01
Cultivar*Temperature* Time	12	<.01	<.01	<.01	<.01	<.01

Appendix-4: Pearson correlations between acrylamide and remaining concentrations of, total reducing sugar, glucose, fructose and asparagine as affected by cultivar, temperature and time

Remaining amount	Acrylamide			
	Atlantic	Snowden	Vigor	Overall
Total reducing sugar	-0.7**	-0.553**	-0.668**	-0.488**
Glucose	-0.671**	-0.538**	-0.604**	-0.468**
Fructose	-0.727**	-0.559**	-0.720**	-0.489**
Asparagine	-0.656**	-0.678**	-0.892**	-0.429**

Appendix-5: Glucose and fructose remain in potato slices blanched with additive treatments

Variety	Additive Treatment	Glucose (mg/100g DW)	Fructose (mg/100g DW)
Atlantic	Without blanching	354.80 ± 3.22	231.85 ± 1.73
	Distilled water	82.05 ± 6.75	59.45 ± 1.58
	0.1 M Calcium chloride	244.27 ± 5.92	159.60 ± 1.66
	1% Acetic acid	264.78 ± 6.39	160.94 ± 1.94
	0.1 M Sodium chloride	200.47 ± 5.63	147.93 ± 3.65
	0.5% Ascorbic acid	250.10 ± 4.23	137.18 ± 3.65
	1% Citric acid	230.73 ± 0.58	180.24 ± 4.64
	0.1 M Glycine	298.74 ± 4.10	194.37 ± 3.49
Snowden	Without blanching	216.69 ± 6.86	98.65 ± 2.66
	Distilled water	109.62 ± 1.86	50.23 ± 2.62
	0.1 M Calcium chloride	112.69 ± 4.18	72.96 ± 1.99
	1% Acetic acid	273.39 ± 1.73	123.25 ± 2.90
	0.1 M Sodium chloride	154.11 ± 1.98	96.10 ± 2.66
	0.5% Ascorbic acid	166.64 ± 3.07	77.85 ± 1.74
	1% Citric acid	181.16 ± 2.96	119.84 ± 2.40
	0.1 M Glycine	646.89 ± 11.06	264.30 ± 1.47
Vigor	Without blanching	154.28 ± 3.55	64.22 ± 1.43
	Distilled water	169.29 ± 4.50	60.20 ± 3.53
	0.1 M Calcium chloride	139.11 ± 3.41	84.26 ± 6.92
	1% Acetic acid	96.89 ± 2.57	66.94 ± 1.53
	0.1 M Sodium chloride	159.39 ± 2.09	78.53 ± 1.63
	0.5% Ascorbic acid	103.70 ± 2.45	58.60 ± 2.20
	1% Citric acid	82.44 ± 3.98	56.57 ± 6.96
	0.1 M Glycine	164.34 ± 0.48	82.99 ± 4.35

Values are expressed as means ± standard deviations.

Appendix-6: Effect of N fertilization strategies on glucose and fructose contents of Russet Burbank

Treatment	2016		2017	
	Glucose (mg/100g FW)	Fructose (mg/100g FW)	Glucose (mg/100g FW)	Fructose (mg/100g FW)
Check	273.62 ± 13.11	117.70 ± 5.43	131.84 ± 22.16	49.62 ± 8.86
Urea Pre-Plant	249.06 ± 18.19	109.14 ± 11.14	147.71 ± 15.53	58.96 ± 6.34
Urea Split	219.29 ± 26.53	130.65 ± 23.09	156.66 ± 27.33	69.43 ± 14.85
Urea/ ESN Split	227.74 ± 27.47	99.70 ± 16.42	155.80 ± 21.22	67.48 ± 19.26
ESN + Fertigation	231.47 ± 14.69	135.41 ± 13.89	139.80 ± 26.92	73.75 ± 19.00
ESN Pre-Plant	216.10 ± 12.14	102.17 ± 16.99	160.66 ± 20.11	68.29 ± 6.36
Pre- Plant Urea + Fertigation	186.00 ± 24.47	94.09 ± 19.54	155.35 ± 15.88	83.22 ± 10.64
Urea/ ESN Split + Fertigation	227.17 ± 19.41	116.64 ± 9.20	142.02 ± 26.49	76.94 ± 12.61
Urea/ ESN Pre-Plant + Fertigation	226.70 ± 6.08	110.11 ± 6.71	162.63 ± 18.53	86.47 ± 19.22
Urea/ ESN Blend at Hilling	239.27 ± 12.61	105.33 ± 12.16	135.34 ± 14.38	49.62 ± 8.86

Values are expressed as means ± standard deviations.

Appendix-7: Glucose and fructose contents of fresh potato samples

Variety	Glucose (mg/100g FW)	Fructose (mg/100g FW)
Russet Burbank	48.98 ± 1.21	35.12 ± 0.24
Ranger Russet	133.82 ± 1.88	52.68 ± 0.85
Shepody	74.86 ± 0.40	36.05 ± 0.42

Values are expressed as mean values ± Standard deviations.