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EFFECT OF VACUUM FRYING ON TOXIN FORMATION, RETENTION OF QUALITY ATTRIBUTES, AND CONSUMER ACCEPTABILITY AS A MEANS TO ELIMINATE RECONDITIONING AFTER LONG TERM COLD STORAGE IN WHITE-, RED-, AND PURPLE- FLESHED POTATO CHIPS

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ABSTRACT

Potatoes (*Solanum tuberosum L.*) are the largest fresh market vegetable crop in the United States. Americans consume, on average, 111.2 pounds/ person/ year with 17.8 pounds being consumed as potato chips. Color-fleshed potato chips are receiving more attention from consumers and producers as they are a rich source of phenolic compounds which have been shown to work as antioxidants and function as anti-inflammatory agents. In order to maintain a constant supply of potatoes year-round, potatoes are stored at low temperatures (2-10 °C). During cold storage, the starch in potatoes is converted to sugars. Accumulation of free reducing sugars produces more browning in fried potato products due to the Mallard reaction. To counter the accumulation of reducing sugars, potatoes are reconditioned at higher temperatures (15-20 °C) for up to three weeks prior to processing where as much as 80% of the reducing sugars are converted back to starch. Reconditioning is costly as it requires an additional storage room and additional energy input to maintain the controlled environment. Additionally, reconditioning causes a break in the cold-induced dormancy of potatoes which induces sprouting, resulting in a high economic loss. White-fleshed potatoes have been bred to withstand sprouting during reconditioning. However, color-fleshed potatoes have not, and are underutilized in potato processing.

Deep-fat (i.e., conventional) frying of potato chips at high temperatures increases the rate of the Maillard reaction resulting in the formation of toxins such as acrylamide, 5-hydroxymethylfurfural, and advanced glycation end-products. Therefore, there is a need to develop new technologies to reduce the formation of these toxic compounds.

This study investigated vacuum frying as an alternative processing technique to deep fat frying so that potatoes can be fried directly from cold storage, without reconditioning, which has the potential to remove the cost of reconditioning and reduce Maillard reaction products, all while maintaining chip quality parameters resulting in the production of an acceptable chip similar to a conventionally fried chip that has been reconditioned.

Vacuum frying (130 °C for 4.5 minutes) of Atlantic, Mountain Rose, and Purple Majesty potatoes, with and without reconditioning, produced chips that were more similar in color, texture, and moisture to commercially available samples, as compared to conventional fried (170 °C for 2.6 minutes), with and without reconditioning, as determined by industry used instrumental techniques. Acrylamide levels were found to be between 1,500-1,800 ppb (tolerable daily intake = 2.6 μg/kg). Reconditioning resulted in lower levels of acrylamide regardless of the frying method. Vacuum frying did not lead to significant different levels of acrylamide than conventional frying regardless of the presence or absence of a reconditioning step. Conventional frying without reconditioning resulted in significantly higher levels of 5-hydroxymethylfurfural as compared to conventional frying with reconditioning and vacuum frying with and without reconditioning, levels were found in the range of 20-125 ppb (tolerable daily intake= 540 μg/person). 5-hydroxymethylfurfural levels for vacuum frying, with and without reconditioning, were not statistically significant. Advanced glycation end-product concentrations were not statistically significant between samples and concentrations were found in the range of 20-30 ppb (tolerable daily intake = not reported).

In blinded sensory evaluations, consumers preferred chips that were vacuum fried without reconditioning over the conventional fried chips, with and without reconditioning. Additionally, consumers were willing to purchase colored chips at a higher price than white chips, once they were told that there was a potential health benefit associated with these chips.

The results of this study indicate that vacuum frying without reconditioning does not reduce the overall levels of toxins as expected which suggests that reconditioning is still necessary regardless of frying method. Even though vacuum frying did not lower the levels of toxins, the amounts found in the tested potato chips were all below levels of concern. Finally, consumer testing indicated that there was an overall preference for vacuum fried chips without reconditioning compared to conventional fried chips, with and without reconditioning. This indicates vacuum frying without reconditioning could be utilized as an alternative to conventional frying, either with or without reconditioning, to produce a high-quality chip.

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1 REVIEW OF LITERATURE

1.1 Abstract

Potatoes (*Solanum tuberosum L.*) are the largest fresh market vegetable crop in the United States with over 1 million acres harvested annually [1]. The average American consumption of potatoes is 111.2 pounds/ person/ year with 17.8 pounds of that being consumed as potato chips and shoestrings. In order to keep a constant supply of potatoes year-round, potatoes are stored at low temperatures (2-10 °C) to extend dormancy. During low-temperature storage, potatoes undergo cold-induced sweetening where the starch is converted to sugar. The accumulation of sugar increases the occurrence of the Maillard reaction, which causes excessive browning and produces toxins such as acrylamide, 5-hydroxymethylfurfural, and advanced glycation end products. To reduce the occurrence of the Maillard reaction, potatoes are reconditioned at higher storage temperatures (15-20 °C) for 2 weeks to convert the sugar back into starch. Reconditioning requires additional space as well as additional energy. The break in dormancy causes color-fleshed potatoes to sprout, causing economic loss.

Pretreatment strategies have been studied to reduce the formation of acrylamide. However, there is a lack of mitigation strategies for other toxic Maillard reaction products. There is a critical need to evaluate frying directly from cold storage without the accumulation of Maillard reaction products. One potential mitigation strategy is to vacuum fry the potato chips directly from cold storage; however, current literature has

not looked at the reduction of all three toxins nor the resulting changes to the sensory perception of the potato chips.

1.2 Introduction to the Potato

1.2.1 History of the Potato

Potatoes (*Solanum tuberosum L.*) were first cultivated in the central Andes, in present-day southern Peru and northern Bolivia, approximately 6,000 years ago [2]. Farmers in the Andes Mountains were able to begin to domesticate the potato in the unforgiving terrain of the mountains. The warm days and cool nights allowed for the equal growth of the above-ground plant and the underground tuber, which favored different temperatures [3].

As the Spanish began conquering South America in the early 16th century, the potato was introduced to the European conquistadors. The first written European records depict the potato as egg sized and shaped, colors as white, purple, and yellow, and having a pleasant taste [3]. When the potato was introduced to Europe, it was met with mixed reactions. Many thought it was the same as the already popular sweet potato (*Ipomoea batatas)* so it was quickly spread although some believed they were poisonous and not supposed to be eaten because potatoes were not mentioned in the Bible [4]. As time passed, war and resulting famines in Western Europe caused the potato to be adopted by aristocrats, soldiers, and peasants. In the present day United Kingdom, potatoes were grown for animal, and human consumption and potato farming could support large families. In North America, the potato was introduced in the late 17th century but became more popular when European immigrants brought the potato over almost a century later.

In the mid-19th century, potato blight began affecting the potato crops of North America, the Netherlands, France, Germany, Russia, England, Scotland, and Ireland [5]. As potato farmers waited for the potato blight to stop affecting their crops, they found that some cultivars of potatoes were more resistant to potato blight [5]. The potato was finally introduced to Africa and Asia in the 20th century [5].

1.2.2 Potato Composition

The potato is a nutrient-dense vegetable. Raw potatoes contain 80% water and 20% dry matter. A serving of a potato, a medium sized tuber (148 g or 5.3 oz.), contains 100 calories, 0 g of fat, and 0 mg of both cholesterol and sodium. There are 26 g of total carbohydrates, including 3 g of dietary fiber and 3 g of sugar, and 4 g of protein. Exact sugar composition is highly dependent on cultivar. However, the most predominant sugar is glucose followed by fructose [6]. Potatoes contain 720 mg of potassium which is more than cooked spinach, bananas, and broccoli. Potatoes contain 30 mg of vitamin C which is 45% of the recommended daily value [7]. When potatoes are boiled, they retain many of the same nutritional aspects as the raw potato. However they lose some vitamin C. Upon baking, potatoes have higher levels of all nutrients and 25% more solids [2]. Potatoes are also a good source of Vitamins B1, B3, and B6, magnesium, phosphorous and folate [7].

1.2.3 Potato Biodiversity

Potatoes are a leading vegetable crop worldwide. In 2016, the top producers of potatoes were China, India, Russia, Ukraine, and the United States [8]. In 2016

Americans consumed on average 111.2 pounds/ person/ year [7]. In 2014 and 2015, the total potato consumption was 112.8 and 115.4 pounds/person/year, respectively [7]. 70% of potatoes harvested are processed of which 12% are consumed as potato chips or shoestrings [7].

There are seven cultivated species of potatoes: *Solanum ajanhuiri, Solanum chaucha, Solanum curtilobum, Solanum juzepczukii, Solanum phureja, Solanum stenotomum,* and *Solanum tuberosum* [2]. Each species will be favorable to grow in different environments and to protect against different predators and diseases.

In 2016 in the United States, red and blue/purple potatoes accounted for 7% of the total acreage planted in the fall where 19% were white potatoes, 3% yellow, and 71% Russet [9]. In 2014 and 2015, 6% of the total acreage in the United States were red and blue. However, three of the states did not begin estimates for red and blue until 2016 [9]. Top producers of red and blue potatoes are Minnesota and North Dakota with red and blue potato acreage at 20% and 23%, respectively [9].

1.2.3.1 Breeding History

Potato breeding can be done to create new cultivars with higher yields, increased nutritional qualities, higher overall quality, and resistance to pesticides and pests [10]. Potato breeding has looked at breeding new cultivars which are neither susceptible to the sprouting nor to the cold-induced sweetening [11].

Breeding a new cultivar of potato takes 9 to 11 years [10]. Two genotypes are chosen from established cultivars which have the desired pest resistance, tuber and flesh color, or starch composition. These newly cloned cultivars are tested on different fields

for 5 years. Performance tests are done on yield, maturity, and cooking properties. Breeders start with over 200,000 different genetic lines which are narrowed to one [10]. In the field and laboratory, potato breeders evaluate over 50 traits from previous cultivars and make selections on yield, quality, and resistant to environmental conditions [12].

Breeders look at quality parameters that are based on external and internal characteristics. External qualities will influence a consumers' perception of a fresh tuber which include skin color, tuber size and shape, and eyes. Internal qualities include nutritional and cooking properties as well as processing capability. Breeding tubers for the fresh market or for processing creates different needs in tubers, as well as differences in breeding selection.

Extending potato dormancy through breeding allows for the reduction of chemical additives after harvesting to extend the shelf life of potatoes. Breeders can genetically control protein, starch, sugar content as well as reduce the amount of toxic glycoalkaloids [12]. New research in potato breeding is exploring the reduction of asparagine, an amino acid precursor in the Maillard reaction [13]. Breeders continually look at reducing the occurrence of late blight, a potato fungal disease responsible for the potato famines in the 19th century. Currently, resistance to pests and environmental changes in temperature and water during the growth period dominates the breeding industry [12].

1.2.3.2 Color-Fleshed Potatoes

Potatoes come in a variety of flesh and skin colors. Yellow potatoes have tan or golden skin and yellow to gold flesh. They have a mid-level sugar content and are utilized for grilling, roasting, and mashing. Fingerling cultivars can have red, orange,

purple, or white skin and flesh. Red cultivars have red skin and white flesh and are utilized for baking. Specialty potatoes have purple or blue skin and flesh or red skin and red or pink flesh and are primarily used for baking and mashing [14,15].

1.2.3.2.1 Phytochemicals

Potatoes are abundant in phytochemicals which are natural secondary plant metabolites and include phenolics, and carotenoids, and alkaloids [16]. Phytochemicals in fruits and vegetables have been studied for their reduction of the risk of cardiovascular disease, cancer, and diabetes [17–21]. Polyphenols have been identified as the largest group of antioxidants in the diet and potatoes have the third highest amount of polyphenols in the diet after apples and oranges [16].

Factors such as cultivar, field location, the age of the tuber when harvested, storage time and temperature, and cooking influence the amount of phytochemicals present in the potato [16,22,23]. Phytochemicals in potatoes can be found in the skin and in the flesh but are not evenly distributed across the tuber [16,24]. The phytochemicals found in potatoes have been shown to act as antioxidants, reduce breast cancer in rats, and reduce the occurrence of colon cancer *in vitro* [24–27]. Carotenoids are highest in tubes with yellow- and orange-flesh due to the high levels of lutein and zeaxanthin. Alkaloids, such as glycoalkaloids, play a role in the potato's natural defense again disease.

Potato phenolics include flavonoids, coumarins, and tannins. Red-and purple-fleshed cultivars contain 3 to 4 times more total phenolics than white-fleshed cultivars [16]. Flavonoids include a class of compounds called anthocyanins. Purple-fleshed

potatoes have higher levels of anthocyanins than red-fleshed potatoes. Anthocyanins have shown to be natural antioxidants [27]. Three anthocyanins are predominant in purple-fleshed potatoes: petunidin, malvidin, and peonidin [28]. In red-fleshed potatoes, pelargonidin and peonidin are the major anthocyanins [29,30].

1.3 Storage

1.3.1 Storage Evolution

Potatoes can be stored from 2-12 months to keep a constant supply of potatoes, to reduce sprouting, and to reduce waste due to rotting. Storage of potatoes allows for a constant supply of potatoes to the market even in climates where potatoes are grown and harvested during one season. Storage temperatures should be held between 2-10 °C depending on their use. Ideally, potatoes intended for processing into fried products such as potato chips or French fries should be stored between 8-10 °C whereas potatoes sold as fresh tubers can be stored between 4-10 °C [11,31,32]. Storage at 3-4 °C will prolong dormancy and keep tubers for a longer period of time [33]. The relative humidity of the environment is determined by the potato crop upon harvest. An environment of high relative humidity (90%) will maintain the potatoes for longer and reduce weight loss and shrinkage. If the tubers are packed tightly, the relative humidity of the room would need to be decreased [33]. Throughout storage, low carbon dioxide levels are optimal to reduce internal blackening of the tubers [33].

Storage facilities need to be dark, with good ventilation, proper humidity, and temperature controls. The geographical location and the intended use of the tuber will influence how these factors are maintained and the overall design of the facility [34].

Depending on the amount needed for processing, potatoes can be stored in bags, boxes, smaller piles, or large bulk piles. If the storage facility is maintaining potatoes for processing, a secondary, warmer, storage room is needed. Storage equipment facilities have been modernized throughout the years. An emphasis on energy saving storage facilities has kept up with trends for a greener market and lower production costs [34]. Integration of technology allows for storage facilities to be constantly monitored and produce records which can be tracked for specific crops and lots [34]. The traceability of modernized storage facilities can aid in food safety as the crop can be tracked from field to consumer.

1.3.2 Dormancy of Tubers

Post-harvest, tubers undergo two stages of dormancy [35]. During deep dormancy, immediately after harvest, tubers will not sprout, and growth is stopped, the metabolic activity drops significantly and remains low throughout the storage period [36]. After deep dormancy is broken by time or temperature, potatoes can sprout and growth of the sprouting buds can be stopped due to unfavorable external conditions or to physiological changes [35].

The amount of proteins, nucleic acids, and lipids in the tuber slightly decreases during storage [35]. Throughout dormancy, the carbohydrates present in the tuber change; the starch is converted to sugars, a process called cold-induced sweetening [37,38]. The starch present composed 25% of the tubers fresh weight and 75% of the dry weight, making changes to the starch content a major concern [35].

Environmental conditions pre-harvest influence tuber dormancy. High temperature during the growing season, above 35 °C, can induce heat sprouting prior to harvest causing tubers with a low starch content and an abnormal shape and will cause the tuber to break deep dormancy early [36].

1.3.3 Sprouting

During storage, sprouting causes the most economic loss [32]. When sprouting begins, the tuber becomes the source of nutrients for the sprout. Sugars from the potato go into the sprout until the sprout reaches 1 g of dry matter, which causes the potato to shrink rapidly, depending on the number of sprouts.

During storage, potatoes release water due to respiration which can induce sprouting. Cultivar influences the times and temperatures in which the tuber can be stored at as some cultivars can be stored at 30 °C and will not sprout [35]. Wang *et al.* (2016) studied the Russet Burbank cultivar over 15 growing seasons and the resulting changes over storage. The average time to break dormancy, which was described as the onset of sprouting, was 178 days at 5.6 °C, 154 days at 7.2 °C, and 136 days at 8.9 °C [32]. More developed cultivars of potatoes have been bred to reduce sprouting. However, newer cultivars that have been bred for other properties, (i.e., low starch, colors, higher yield) still have the tendency to sprout.

Temperatures between 0-4 °C promote an unfavorable condition for sprouting and can prolong dormancy [35]. In addition to cold temperatures, dormancy can be extended by chemical additives. Pre-harvest, potatoes are treated with maleic hydrazide or chlorpropham [33]. Chlorpropham prevents cell division at the start of a sprout and

prevents it from further growth. In the United States, chlorpropham is used in conjunction with diisopropylnaphthalene to reduce sprouting. Natural caraway seeds extracts, clove oil, and hydrogen peroxide are undergoing trials to determine the efficacy of extending dormancy [33].

1.3.4 Cold Storage

1.3.4.1 Cold-Induced Sweetening

When potatoes are stored at lower temperatures, the starch is converted into sugars in the plant tissue through a process known as cold-induced sweetening. Research suggests that the cold temperatures damage the amyloplast membrane making the membrane more susceptible to enzymatic damage. Low temperatures inactivate phosphofructokinase and glycolysis in the potato is no longer regulated [40,41]. The ratio of amylose to amylopectin can influence the rate of cold-induced sweetening [40]. The starch to sugar conversion happens within the first few days of low-temperature storage and fluctuates throughout the course of storage [42].

The accumulation of sugar in the tubers produces more browning in fried products due to the Maillard reaction. This browning is undesirable to consumers due to taste and dark brown color formation. To reduce the effects of cold-induced sweetening, tubers which will be processed are reconditioned by warming the tubers for 1-3 weeks at higher temperatures, between 12-15°C [40]. Through the reconditioning process, the sugar can be converted back to starch. Reconditioning breaks dormancy which induces sprouting and weight loss of the tuber.

Cultivars of potatoes have been bred to resist cold-induced sweetening. Cultivars such as the White Pearl is resistant while Atlantic and Yukon Gold are susceptible [40]. Blenkinsop *et al.* (2002) studied four cultivars from four growing seasons and how the reducing sugars, fructose, glucose, and sucrose concentrations change throughout storage at 4 °C [42]. In all cultivars and growing seasons, the amount of sugars steadily increased over the first 15 days of storage and remained constant until the termination of the study at 70 days. The amount of reducing sugars accumulated was dependent on both cultivar and growing season [42].

Ohara-Takada *et al.* (2005) looked at a two week storage period at 2 °C and a 4 week storage period at 2 and 18 °C [43]. The tubers stored for four weeks at 2 °C showed significantly more reducing sugars than the chips that were only stored for 2 weeks. There was a 3 day lag period where sucrose, fructose, and glucose all remained at the initial value. The tubers stored at 18 °C had significantly less sucrose, fructose, and glucose than those stored at 2 °C for the same amount of time [43].

1.3.4.2 Changes to Phenolics during Storage

Storage at low temperatures alters the phytochemicals, especially phenolics, found in potatoes. Reconditioning the potatoes at higher temperatures could allow for the loss of total phenols during short-term cold storage to be reduced. Retaining the health benefits of phenolics is essential in maintaining color-fleshed potatoes as a functional food.

Külen *et al.* (2012) studied changes to small molecules throughout storage in yellow- white- and color-fleshed potato cultivars [44]. Potatoes were stored at 4 °C and

samples were taken at 2, 4, 6, and 7 months post-harvest. The total phenolic content of the color-fleshed potatoes was significantly higher in gallic acid equivalence than the white- and yellow-fleshed cultivars. The total phenolic content was the highest immediately after harvest, decreased after 2 months, increased at 4 months, and increased to the level observed immediately post-harvest at 7 months. Two cultivars of white-fleshed potatoes had higher levels of total phenols after 7 months of storage [44]. The antioxidant capacity of the potatoes was determined across the cultivars. The color-fleshed had higher antioxidant capacity than the white- and yellow- fleshed cultivars. All cultivars had a fluctuation in the antioxidant capacity throughout storage, however, at the end of storage, color-fleshed cultivars had a higher than the initial harvest values and white- and yellow-fleshed had lower than harvest values [44]. The vitamin C content of the cultivars decreased throughout storage. A rapid decrease at the beginning of storage was observed and a gradual decrease towards later in storage [44].

Similar results were reported by Madiwale *et al.* (2012) where cultivars were processed and were determined for their total phenolic content, anthocyanin content, antioxidant activity, and human colon cancer cell anti-proliferative and pro-apoptotic activity after 60 and 90 days of storage at 3 °C [45]. Total phenolic content and antioxidant capacity of raw samples were similar to the results described above. The anthocyanin content increased throughout storage similarly to the total phenolic content. Storage decreased the antiproliferative properties associated with the color-fleshed potatoes [45].

1.3.5 Reconditioning

Reconditioning of potatoes is optimum at temperatures of 15-20 °C for up to three weeks at 95% relative humidity [11,46]. Up to 80% of the reducing sugars are converted back to starch, and the remaining 20% is lost due to respiration [47]. By reconditioning the potatoes, potatoes can be processed into French fries and potato chips as the amount of browning during frying due to the Maillard reaction is reduced.

1.3.5.1 Changes throughout Reconditioning

Kyriacou *et al.* (2009) studied 4 cultivars over three seasons to track the changes through reconditioning [46]. Sprout growth, fresh weight loss, and sugar levels were monitored after long-term cold storage and subsequent reconditioning. Their results found that reconditioning longer than 30 days did little to affect the resulting sugar content. There was a greater percent decrease in the first 15 days of reconditioning than between days 15 and 30. The difference within the cultivar between growing year and sugar content as well as sprouting was insignificant. Sprouting rates throughout reconditioning varied between the cultivars with some sprouting after 60 days where others sprouted between 15 and 30 days [46].

1.4 History of the Potato Chip

George Crum, of Saratoga Springs, New York, is accredited with the accidental creation of the potato chip during the summer of 1853 [48]. Crum was a chef at an upscale restaurant who was known to have a short temper, especially if plates were sent back. One customer, returned a plate of fried potatoes, rumors of his complaints range from they weren't crunchy, salty, or thin enough. Crum then sliced a new potato very

thin, deep fried them, and heavily salted them [48]. Many sources will cite the story of Crum and the picky customer as the creator of the potato chip. However, Crum's obituary in 1914 fails to mention anything more than "Saratoga Chips" and stories of Crum's creation of the potato chips appears around 1940's [48]. Different members of the Crum family have taken credit for creating the chip. One story is Crum's sister accidentally dropped a slice of potato into the fryer [48]. At the time of Crum's death, the potato chip was a new product and not widely popular, so there was no written record of how it was first made.

Potato chips were first sold within the community where they were produced. They were sold in baskets, bins, and paper bags with no marketing names on them. From New York, the potato chip began to spread out across the Eastern United States. As stores sent out their potato chips, they stored them in barrels or glass cases and sold them by weight [48]. By the early 1930's, companies began to mass produce potato chips. The earliest recorded potato chip factory was in Ohio creating the Ohio Chip Association. Pennsylvania soon after became a major producer of potato chips and remains one to this day [48].

1.4.1 White Fleshed Potato Chip

1.4.1.1 American Consumption and Amount of Potatoes Used

In 2016, 60,226,000 cwt of potatoes grown in the United States were processed into chips and shoestrings, a 6% increase from 2015 and an 18% and 0.4% decrease from 2014 and 2013, respectively [9]. In the United States, there are 96 potato chip and shoestring plants [9]. 19% of the potato chip and shoestring plants are in the Eastern

United States including Pennsylvania, Delaware, Maryland, New Jersey, New York, Virginia, and the District of Columbia [9].

In 2016, the average annual consumption of potato chips and shoestrings per American was 17.8 pounds/person/year [7]. Previously, in 2014 and 2015, the average annual consumption was 19.9 and 19.8 pounds/ person/ year, respectively [7]. In the 2016/17 market year, as of May 2017, the United States exported 54,629,000 pounds of potato chips and imported 16,961,000 pounds [7]. The 2015/16 market year saw 110,968,000 pounds exported and 36,278,000 pounds [7]. In 2016, potato chip production in the United States, both plain and flavored, was valued at $8,856,402,000 [49].

1.4.2 Color- Flesh Potato Chips

1.4.2.1 Increase in Potato Chip Consumption

Color-fleshed potato chips are receiving more attention from consumers and producers. Colored potatoes are a rich source of phenolic compounds which have been shown to work as antioxidants [50]. The antioxidant potential of the phenolic compounds found in colored potatoes has been reported to promote beneficial anti-carcinogenic and anti-inflammatory properties [51]. Deep-fat frying has been shown to reduce the amount of total phenolics, anthocyanins, and carotenoids where air frying retained more of these compounds [45,52]. Cold storage has been found to elevate the phenolic compounds and antioxidant activity present in white- and purple-fleshed potato cultivars [22]. Because of the vast differences between cultivar, generalizations of processing effects cannot be made. However, frying retains some of the total phenolic compounds as well as the

beneficial properties. The potential health benefits associated with color-fleshed potatoes may explain the increase in colored potato chip consumption.

1.4.2.2 Color-Flesh Potatoes Storage

Prior to processing, colored potatoes undergo the same storage conditions as white potatoes. Blue and purple potatoes were found to have a 4% weight loss after 4 months of storage [53]. Weight loss in potatoes occurs through respiration and evaporation and is physically indicated by sprouting [53]. Higher temperatures associated with reconditioning cause colored potatoes to sprout more rapidly than white potatoes. The sprouting of potatoes, specifically color-fleshed potatoes, poses the need to fry colored potatoes without breaking dormancy through reconditioning.

1.5 Frying

1.5.1 Atmospheric Deep Fat Frying

Atmospheric deep-fat frying utilizes high heat with dehydration to produce a crisp texture. Potato chips are fried to reach a dehydrated state of less than 2% moisture content by frying at temperatures ranging from 160-180 °C for 2-4 minutes. This rapid dehydration produces a favorable chip. However, it increases the uptake of oil. A study by Baumann and Escher (1995) studied the oil uptake with different chip thickness, frying temperatures, and times [54]. Tubers of equal weights and specific gravity were used, but they found variation in one tuber depending if the slices were taken from the ends or the center of the tuber. It was found that a higher temperature reduces the amount of oil uptake. However, it increases Maillard reaction products (MRP) formed through non-enzymatic browning.

Oil type and quality influences the final potato chip product. Large-scale potato chip production is commonly is done as continuous frying as batch frying is more costly and produces lower volumes [55]. The continuous fryer starts with a potato at one end, and a finished product is collected at the other. Frying oil can be periodically added, and can be automated to account for the type of food, the heat needed, the oil filtration, and the time to change the oil. Since this can be automated, a more consistent product can be produced [55].

Oil quality can be measured by color, flavor, or smoke point. However, they have low sensitivity to slight changes. The composition of free fatty acids and total polar compounds provide a more comprehensive measurement of the oil degradation [55]. Bou *et al.* (2012) studied the consistency of the oil quality of 3 industrial potato chip processing plants and found their oil quality to be consistent and having a low percentage of the total polar compounds, indicating higher quality oil [55]. On the contrary, Sebastian *et al.* (2014) studied the oil quality of restaurants which used batch frying for different products (fried meats, cheeses, and potato chips). The study found that the frying oil was highly oxidized and past the acceptable value of total polar compounds [56].

1.5.1.1 Health Concerns

The health impact of fried foods, especially in the Western diet, has been a growing concern. Atmospheric frying has advantages in retaining heat-sensitive vitamins, such as vitamin C, compared to baking and boiling [57]. However, atmospheric frying shows a 50% reduction of lipid soluble vitamins such as vitamins A and E [57].

Prolonged atmospheric frying caused an increase in lipid-oxidation products which have been studied for their toxicity, carcinogenic, and inflammatory properties [58]. Frying oil fumes generate large amounts of polycyclic aromatic hydrocarbons and carbonyl compounds which can pollute the air [59]. The polycyclic aromatic hydrocarbons give rise to human health concerns as research has shown to cause carcinogenic and mutagenic effects [59].

1.5.2 Vacuum Frying

Vacuum frying has been proposed as an alternative to atmospheric frying to produce a fried product with low oil content, but with the same desirable texture and flavor characteristics, consumers expect in a fried product. Vacuum frying is typically done at a pressure below that of atmosphere, typically 6.65 kPa [60]. By lowering the pressure, the boiling point of the water present in the food is lowered [60]. The fried products can be fried at lower temperatures (110- 140 °C) [60–62]. The lower temperatures could reduce the occurrence of Maillard reaction products which rapidly form at temperatures above 120 °C.

Vacuum fryers are typically electric pressure cookers modified to be the vacuum frying vessel (Figure 1-1). The vessel sits on a heating plate and can measure and control the temperature of the oil. The pressure cooker lid is fitted with a pressure gauge and a rod that the frying basket is attached to and can be raised and lower to submerge the frying basket in the oil. The vessel is connected to a condenser which rapidly cools the released moisture and prevents moisture from reaching the vacuum pump which prolongs

the life of the vacuum pump oil. Finally, a vacuum pump is fitted to the condenser so that vacuum can be pulled [60,61,63,64].

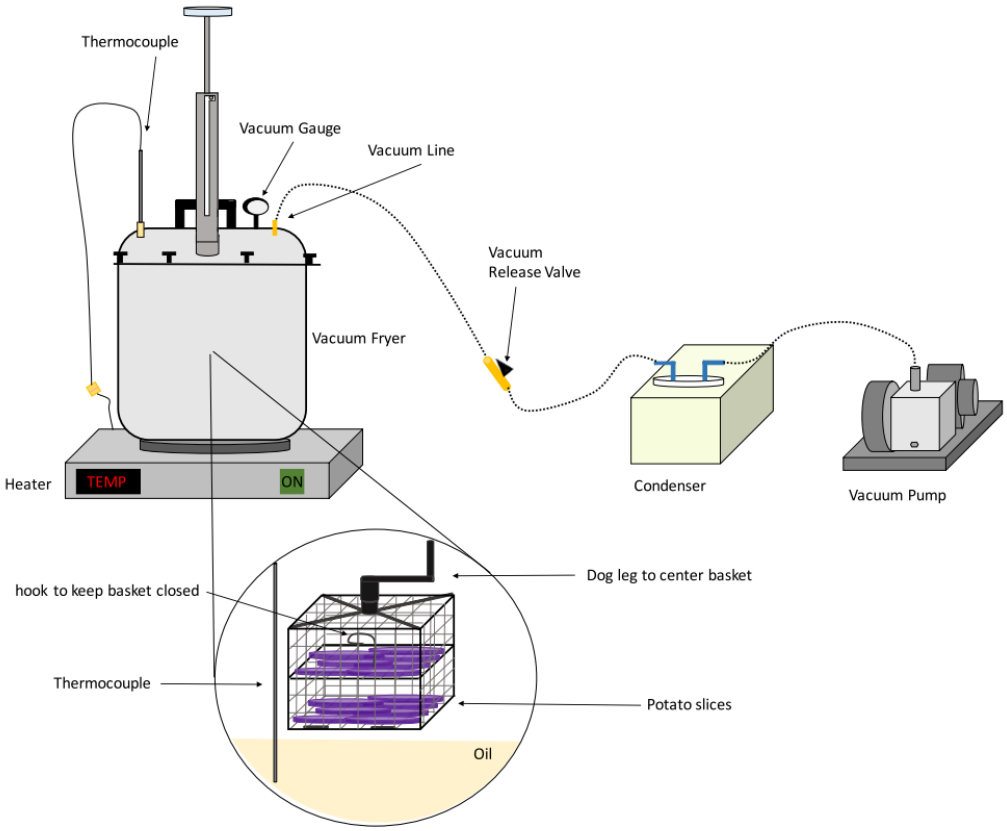

Figure 1-1 Vacuum fryer apparatus, dotted lines represent hosing [65].

Dueik *et al.* (2010) vacuum and atmospheric fried carrot chips finding a 50% reduction of oil, an 86% retention of β-carotene, and preservation of the color of the vacuum fried chips compared to the traditional atmospheric deep fat fried [62]. Garayo and Moreira (2002) compared vacuum and atmospheric frying in potato chips and found that as vacuum pressure increased the oil absorption rate increase [60]. It was also determined that the vacuum fried potato chips were significantly lighter in color than

those fried at atmospheric pressure and that there was no significant difference in texture [60].

Crosa *et al.* (2014) studied the changes in oil quality of a vacuum fryer [66]. By measuring the total polar compounds, oxidative stability, fatty acid composition, and α-tocopherol content, the vacuum frying oil deteriorated slower than the atmospheric oil [66]. This allows for oil in a vacuum fryer to be utilized for longer periods of time before being replaced which decreases downtime of operation due to cooling and heating oil, costs of oil, and waste.

Anthocyanins, present in colored potatoes, degrade in the presence of oxygen and at high temperatures. Da Silva and Moreira (2008), found the anthocyanin content of blue potatoes that were vacuum fried to be 60% higher than those fried at atmospheric pressure [67]. Vacuum frying's reduction of oil uptake, preservation of anthocyanins, and a decrease in Maillard reaction products makes it an attractive alternative to conventional deep-fat atmospheric frying.

1.6 Maillard Reaction

1.6.1.1 Mechanism

The Maillard reaction (MR) is a form of non-enzymatic browning between reducing sugars and amino acids. The excessive accumulation of reducing sugars during cold storage and frying of potatoes at high temperatures increases the occurrence of the MR in potato chips. The chemical reaction was first proposed in 1912, and the mechanism was later described by John Hodge in 1953 [68]. The MR starts with a glycation reaction of a carbonyl group of a reducing sugar with a free amino group

(Figure 1-2). This forms an intermediate, unstable, Schiff base which rearranges to a ketoamine, which is known as an Amadori product. The Amadori product can follow different pathways and form furfurals, reductones, and carbonyl and hydroxycarbonyl products. Many intermediates can have antioxidant activity as they can catalyze redox reaction if certain transition metals are present [68].

Sugars that are present react with amino acids via Strecker degradation to form Strecker aldehydes of the amino acids which give the aroma associated with the MR. In the last stages of the MR, the major color products are formed as the furfurals, reductones and aldehydes react with aldols to form the final compounds [68].

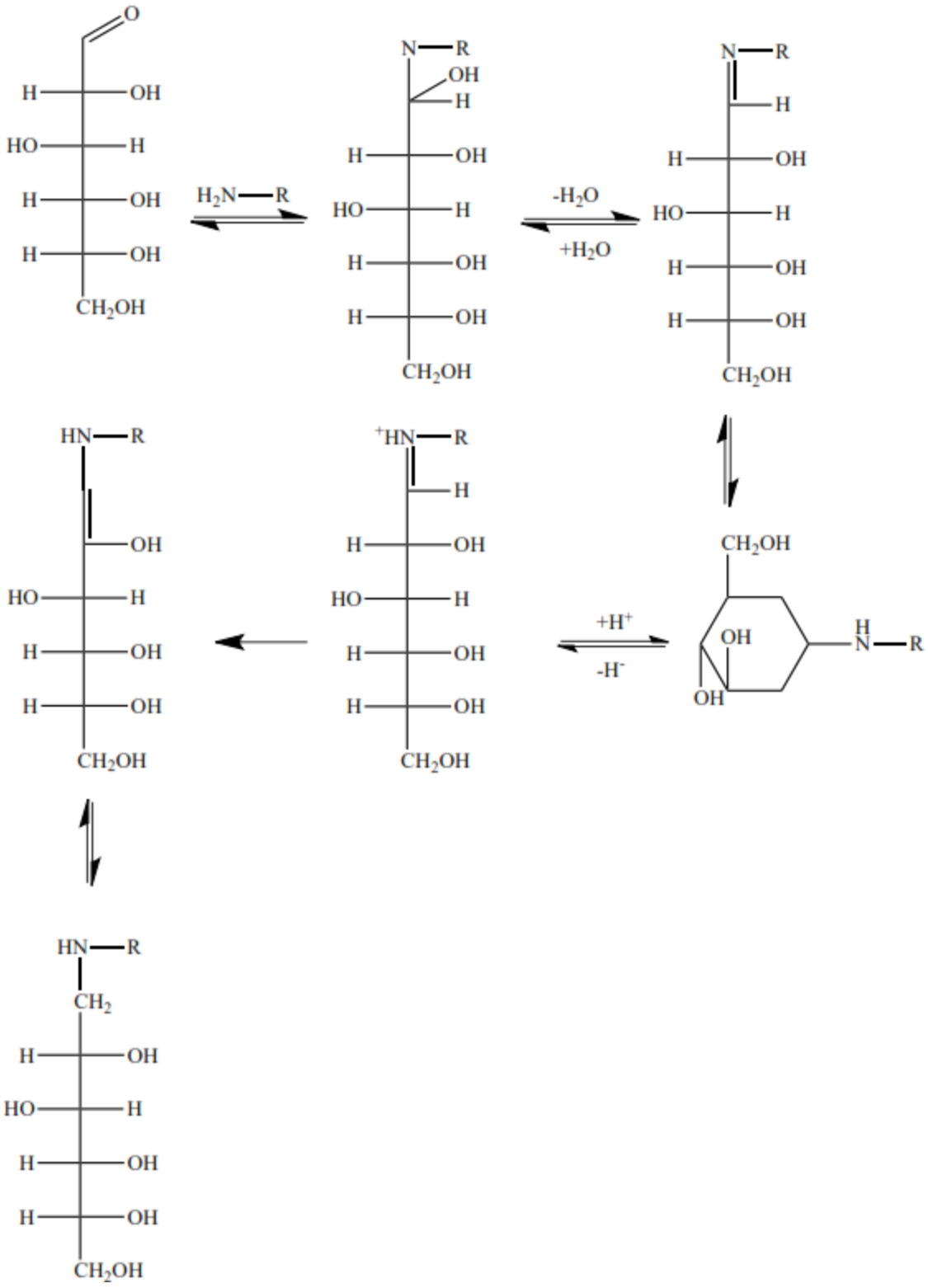

Figure 1-2 Maillard reaction mechanism of reducing sugar and amino acid to Amadori product [69]

1.6.2 Beneficial Compounds

The MR has been studied for the changes in flavor, color, texture, and nutritional quality of foods. More recent research has focused on toxins formed, metal interactions with Amadori products, and the changes associated with protein cross-linking [68].

1.6.2.1 Color and Flavor

Millard reaction products (MRPs) give distinct flavors and aromas to their cooked products. Liu *et al.* (2012) looked at the sensory profile of MRPs in a trained panel and found characteristics such as bitter and umami correlated to high concentrations of MRPs [70]. Wong *et al.* (2008) found that factors such as temperature pH, time, and the ratio of precursors influenced the flavor notes [71]. Aromas such as flowery and fruity were used to describe the initial aromas; as the MRPs were heated, aromas such as caramel were more pronounced. Panelists described the MRP formed from the amino acid methionine to have a fried potato odor [71]. The study found that most amino acids produced a pleasant odor, however, if an unpleasant sulfur odor was described, the unpleasantness became more pronounced with increased heating [71].

1.6.2.2 Antioxidant

MRPs have been studied for their antioxidant potential in food systems. MRPs have been suggested to have radical scavenging activity. Yu *et al.* (2012) studied MRPs from different reducing sugars and amino acids and their radical scavenging activity and their reducing power [72]. Amino acids with glucose and fructose had similar radical scavenging activities which increased as the system as heated, indicating that MRPs have higher antioxidant potential [72]. Osada and Shibamoto (2006) found a synergistic effect

on the antioxidant potential of all MRPs and suggested that retaining some of the MRPs is beneficial in the food system [73].

1.6.3 Toxicants

1.6.3.1 Acrylamide

1.6.3.1.1 History

In 2002, the Swedish National Food Administration found high levels in acrylamide (AA) (2-propenamide, CAS 79-06-1) (Figure 1-3) in carbohydrate-rich heat-treated foods, adding it to the list of food-borne toxicants [74]. In 2006, the United States Food and Drug Administration published results from a 2002-2006 survey on acrylamide in food [75]. The survey data presented acrylamide content in foods such as baby food, coffee, snack foods, meat products, cereals, as well as samples from fast food establishments [75]. From this survey data, the FDA presented recommendations. However, it is not regulated in the food product.

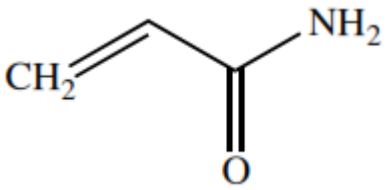

Figure 1-3 Chemical structure of acrylamide

1.6.3.1.2 Formation

The amino acid asparagine was found to be the amino acid responsible for AA formation. Asparagine is predominant in potatoes and bakery products. Zyzak *et al.* (2003) proposed the mechanism for the formation of AA using LC/MS peak identification to determine the products at each step. The amino group in asparagine

reacts with the carbonyl group on a reducing sugar, usually glucose, to form the Schiff base. When heated, the Schiff base decarboxylates and can form two products. It can hydrolyze to form 3-aminopropionamide which when heated, the ammonia is eliminated and acrylamide is formed, or the Schiff base can form acrylamide with the elimination of an imine group (Figure 1-4) [76]. AA formation is influenced by pH, temperature, time, moisture content, and precursor concentration. AA rapidly forms at temperatures above 120 °C [77].

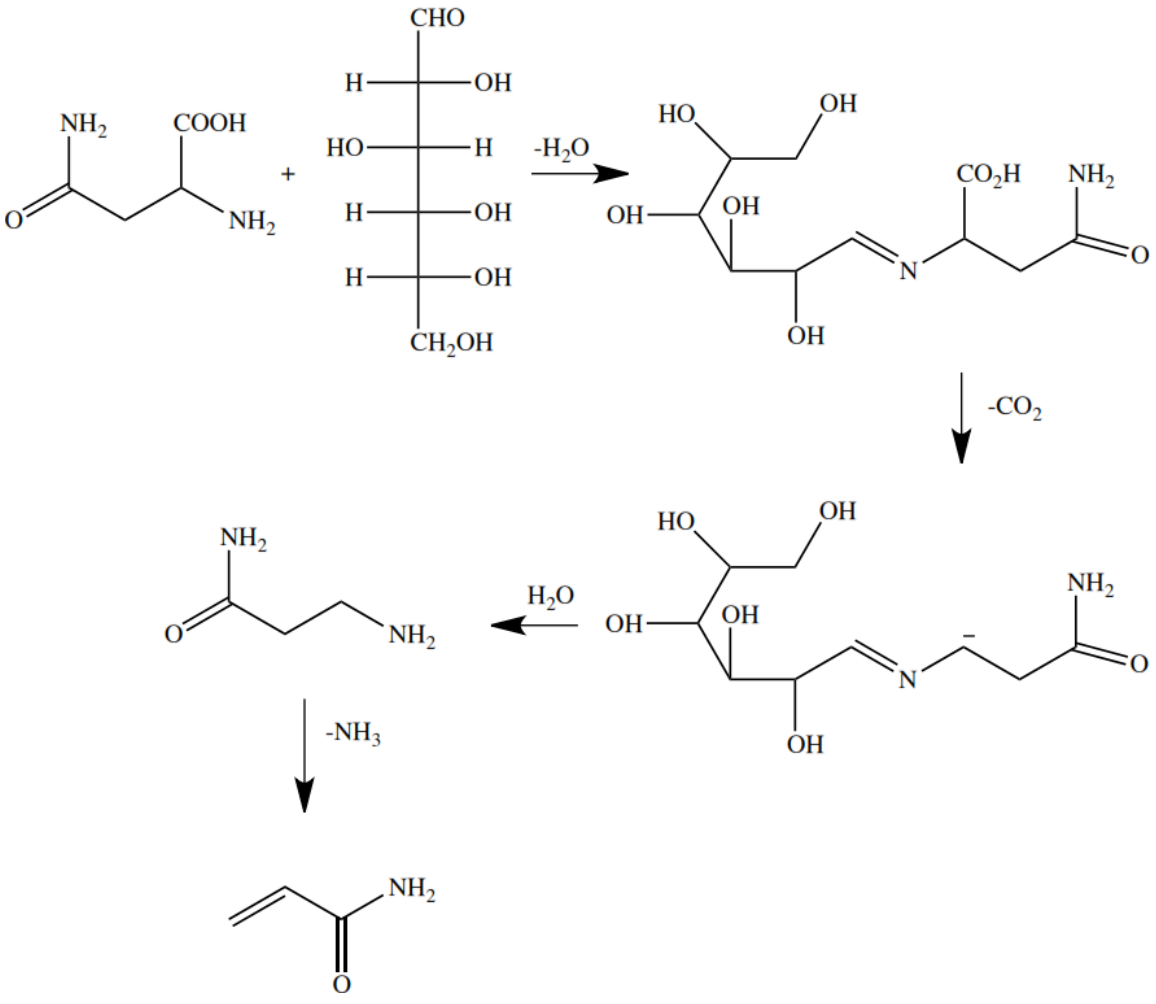

Figure 1-4 Mechanism for acrylamide formation from asparagine and glucose [76].

1.6.3.1.3 Extraction and Detection

Sample preparation and extraction of AA widely varies depending on the food matrix. AA is highly polar and water soluble. Solvents such as water, ethyl acetate, methanol, and cyclohexane have been utilized to extract AA from the food matrix [78–81]. Food samples are usually defatted with n-hexane, prior to extraction [79]. Solid phase extraction C18 cartridges have been used for both LC and GC analysis methods [78]. Extraction solvent and method influences the amount of AA found in the sample matrix, so there is a need for an addition of internal standards and matrix recoveries [82]. AA has previously been derivatized, via bromination, to form the heavier, more easily identified 2, 3-dibromo-2-methylpropionamide. Bromination is typically done overnight at low temperatures. Bromination has an extensive cleanup step where the brominated acrylamide is further extracted from the matrix.

AA can be analyzed using a variety of analytical techniques. Liquid chromatography-mass spectrometry (LC-MS) and LC-MS/MS have been utilized as well as gas chromatography-mass spectrometry (GC-MS) and GC-MS/MS. Monitoring for the ions m/z of 71 and 55 are characteristic of AA [83]. When the ion passes through the collision cell, AA is fragmented into m/z 55 which is due to the loss of the amine group [84]. By utilizing MS/MS detection, multiple reaction monitoring (MRM) can be used, and transitions of m/z of 71-55 and 71-27 can be monitored simultaneously which allows for quantification without derivatization [78].

1.6.3.1.4 Toxicity and Biological effects

1.6.3.1.4.1 Humans

The European Food Safety Authority (EFSA) found the mean and 95th percentile of daily AA exposure levels to be 0.4 to 1.9 μg/kg body weight and 0.6 to 3.4 μg/ kg body weight, respectively [85]. EFSA found infants, toddlers, and children to be the most exposed group and found potato snack products and coffee to be the food contributing most to adult's exposure [85]. Majority of American's food exposure to acrylamide occurs from potato chips, French fries, cereals, salty snacks, and coffee. The World Health Organization and the Food and Agriculture Organization of the United Nations estimated that the average daily exposure to AA is 0.3-0.8 mg/kg a day [86] and the USDA estimated 0.24-1.06 mg/kg a day. The tolerable daily intake for neurotoxicity and carcinogenesis to be 40 μg/kg-day and 2.6 μg/kg-day, respectively [86]. Studies have shown long-term exposure to AA due to environmental factors to cause nervous system damage and oxidative stress [87].

1.6.3.1.4.2 Animals

Exposure to AA in animals has shown to affect the nervous and reproductive systems. In mice, the oral LD_{50} was found to be 107 mg/kg [88]. Yang *et al.* (2016) found a dose of 5 mg/kg body weight to have high toxicity in rats by disrupting lipid metabolism and inducing oxidative stress [89]. In rat models, AA metabolites were the highest in the blood followed by the skin, spleen, lungs and fat, 24 hours after exposure to acrylamide. In mice, after 24 hours, the AA metabolites were highest in the skin, fat, testes, blood, liver, lung, spleen, brain, and kidneys [89,90]. Friedman *et al.* (1995) found rats treated with 3 mg/kg a day of AA had peripheral nerve degeneration and cancerous

cells in their thyroid and either mammary glands or testes [91]. Maronpot *et al.* (2015) exposed rats to various doses of AA for two years and found an increase of cancer cells in the mammary glands and the thyroid [92].

1.6.3.1.5 Potatoes and Acrylamide

When potatoes are in cold storage, the amount of reducing sugars increases, if potatoes were fried directly from cold storage, the fried products would have an increase of MRPs, specifically AA. Because of the growing health concerns of AA, there is a need to recondition the potatoes prior to processing. Some potato cultivars have been bred to be resistant to cold-induced sweetening, which would eliminate the need for reconditioning while having low levels of MRPs [40].

Potato cultivar influences the occurrence of AA. Potato cultivars with increased levels of reducing sugars and asparagine have increased amounts of AA when deep fried into potato chips or French fries [31,93,94]. Yang *et al*. (2016) found Red Pontiac cultivars (red skin/ white flesh) to have significantly higher levels of acrylamide than Agria and Kennebec (white skin/ white flesh) cultivars [95]. The correlation between asparagine and reducing sugar concentration showed that the reducing sugar concentration was the limiting reactant in AA formation [95]. Research by Amer *et al.* (2014) found significant differences between Purple Majesty (purple-fleshed), Atlantic (white-fleshed), and Yukon Gold (yellow-fleshed) cultivars after 90 days of storage and after atmospheric frying with Purple Majesty having the highest followed by Atlantic, and Yukon Gold [22].

1.6.3.1.6 Mitigation Strategies

Reduction of AA by natural and chemical additives and through different processing techniques has been a focus of the food industry. Morales *et al.* (2014) studied the effect that natural extracts had on the reduction of acrylamide in fried potato products [96]. Green tea had a 62% reduction of acrylamide where cinnamon and oregano extracts had a 39% and 17% reduction, respectively [96]. There was no significance between acceptance levels when given to a consumer panel, which could be due to the short time the potatoes were soaked in the extracts [96].

Sansano *et al.* (2015) studied pretreatments, blanching the potato slices or immersion in solutions of citric acid, glycine, calcium lactate, sodium chloride, or nicotinic acid, and the effect of an air fryer on AA levels [97]. Each pretreatment, excluding a 1% solution of sodium chloride, reduced the amount of reducing sugars present in the raw slices. The untreated slices saw a significant reduction in acrylamide levels between the atmospheric deep fat frying and the air frying. However, treatments such as blanching, citric acid, 1% glycine, and 2% sodium chloride, had higher levels of acrylamide in the air fryer than the conventional deep fat fryer [97].

Granda and Moreira (2005) vacuum and atmospheric fried Atlantic potatoes and measure the AA content at different frying times and temperatures [98]. In atmospheric frying, they found that their potato chips with a low surface-to-volume ratio had an increase of AA as the temperature increased. Under vacuum, the AA concentration increased as the time and temperature increased. In comparison, they found that atmospheric frying at 150 °C vs. vacuum frying at 140 °C led to a 63% increase in AA concentration [98].

Studies have suggested that high temperatures for extended periods of time could degrade acrylamide. Mottram et. Al (2002) found acrylamide levels to be the same when potato chips were heated to 160 °C and 200 °C but elevated at 180 °C [99]. Taubert *et al.* (2004) found that as processing time increased, acrylamide formation decreased [100].

1.6.3.2 Advanced Glycation End-products

1.6.3.2.1 History

The glycation of proteins depends on the rate of Amadori product formation and its rate of conversion to other products. Intermediate glycation products react with proteins and form stable, irreversible crosslinks called advanced glycation end-products (AGEs). These AGEs can form adducts to proteins such as N^{ε}-(carboxymethyl)lysine (CML)(Figure 1-5) [68]. AGEs form in human tissue and during cooking at high temperatures.

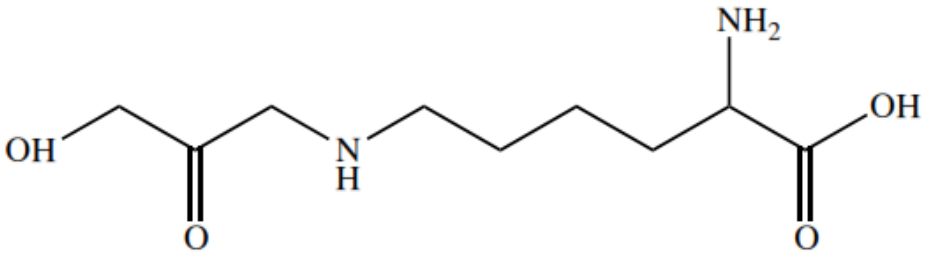

Figure 1-5 Structure of N^{ε}-(carboxymethyl)lysine, a common marker for advanced glycation end-products in food.

1.6.3.2.2 Formation

Glucose reacts with the amino group on lysine. The product is hydrolyzed resulting in the Schiff base. The Amadori product is oxidized, and an amino group is removed (Figure 1-6) [101]. The formation of AGEs is irreversible and is dependent on the age of the tissue [102].

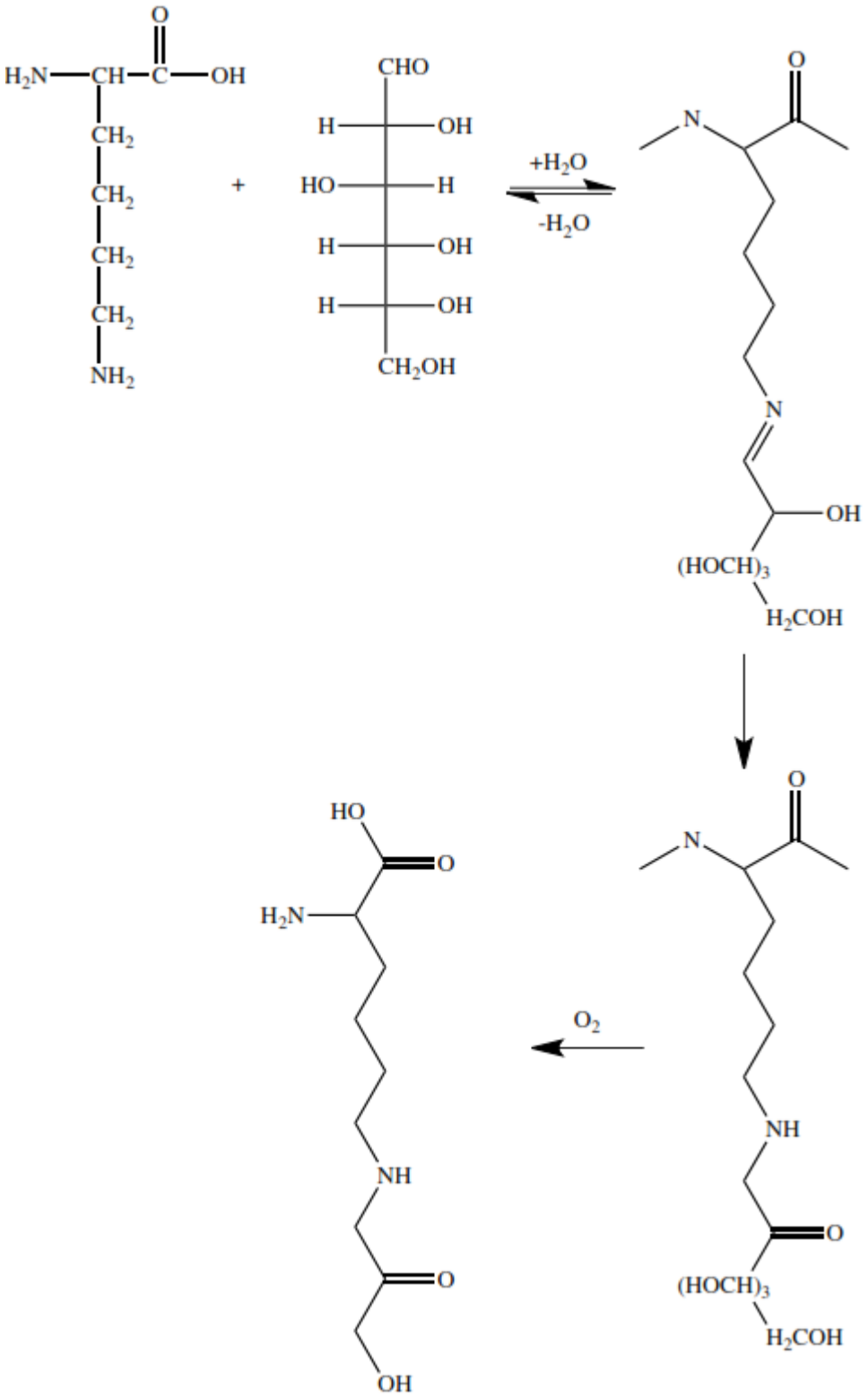

Figure 1-6 Mechanism of the formation of N^{ε}-(carboxymethyl)lysine, a common marker for advanced glycation end-products in food [101].

1.6.3.2.3 Detection

CML is the most commonly studied AGE due to its stability and ability to be measured via enzyme-linked immunosorbent assays (ELISA) and LC-MS methods. GC-MS, high-performance liquid chromatography (HPLC), and LC-MS/MS methods have also been done. CML concentrations can be expressed as mmol/mol lysine, mg/kg protein or mg/kg food. Because lysine and protein values are specific to the food product and processing, CML content of some food products cannot be reported in these ways [103]. Measurement by ultra-performance LC-MS requires protein isolation, hydrolysis, and cleanup steps and does not fully account for the matrix effect of food products [103].

1.6.3.2.4 Factors impacting formation

Research has found high levels of AGEs in heated foods and in foods that are protein and lipid-rich [104]. Foods that are rich in carbohydrates such as vegetables have the lowest concentrations of AGEs. However, snack foods, such as potato chips, show a significant increase in AGE content [104].

1.6.3.2.5 Toxicity and Biological Effects

1.6.3.2.5.1 Humans

AGEs induce oxidative stress and induce inflammation. Accumulation of AGEs is a marker for diabetes [105]. AGEs affect multiple organs in the body as a person ingests high levels of high heat-treated foods, such as fried foods, including French fries and potato chips. High levels of AGEs can lead to a myriad of other health issues. AGEs in the brain can lead to inflammation and Alzheimer's disease, deposits in the eyes can lead to cataracts, heart and arteries can lead to hypertension, heart failure, or stroke, kidney

deposits increase the occurrence of kidney failure, bones cause stiffness and fractures, and muscles and tendon deposits cause loss of muscle strength [105].

1.6.3.2.5.2 Animals

Patel *et al.* (2012) fed mice high AGE containing diets and found the liver sections to have high steatosis, even though the diet was low in fat, had low liver functions, but no change in the efficacy of glucose metabolism [106].

1.6.3.2.6 Potatoes and AGEs

Mizutani *et al.* (2014) suggested that the high concentration of proanthocyanidins in fruits inhibited the formation of AGEs, specifically CML [107].Goldberg *et al.* (2004) found in varying amounts of AGEs in white potatoes, depending on cooking method [104]. In boiled potatoes, 174 units/gram (U/g) of AGEs were detected. In homemade and fast food French fries, 6,939 and 15,219 U/g were found respectively. Potato chips were found to have 28,818 U/g [104].

1.6.3.2.7 Mitigation Strategies

Current mitigation strategies for AGEs include reducing exposure to foods that contain high levels of AGEs. However, levels of AGEs across food varies on the thermal treatment. There are no current mitigation strategies for the reduction of AGEs in food prior to ingestion, so there is a critical need study reduction of AGEs in food products.

1.6.3.3 Hydroxymethylfurfural

1.6.3.3.1 History

5-Hydroxymethyl-2-furfural (HMF) (Figure1-6) is an intermediate byproduct of the MR that has been shown as a marker for heat damage. HMF forms more rapidly in carbohydrate-rich foods which are treated at high heat from pasteurization or cooking.

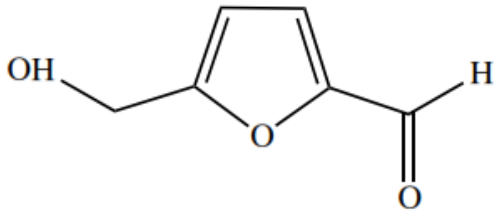

Figure 1-7 Structure of 5-Hydroxymethyl-2-furfural

1.6.3.3.2 Formation

Fructose and glucose follow different mechanistic pathways for the formation of HMF. Fructose (Figure 1-8) is dehydrated forming fructofuranosyl oxocation. A proton is eliminated, and then another water molecule is released, which forms the double bond present in the furan ring. Through a final dehydration step, a carbonyl group forms giving the final structure of HMF [108].

If HMF is formed via glucose (Figure 1-9), the glucose is formed into 1,2-enediol which dehydrates forming a double bond. A second dehydration takes place which forms another double bond and the dicarbonyl compound. Finally, cyclization and a final dehydration occur forming HMF [108]. Glucose can also interact with the amino group resulting in a Schiff base which can be deprotonated and rearranged to form Amadori compounds. The Amadori compounds undergo deamination, dehydration, or fragmentation to form furan derivatives, which then follow the previous glucose conversion to HMF [108].

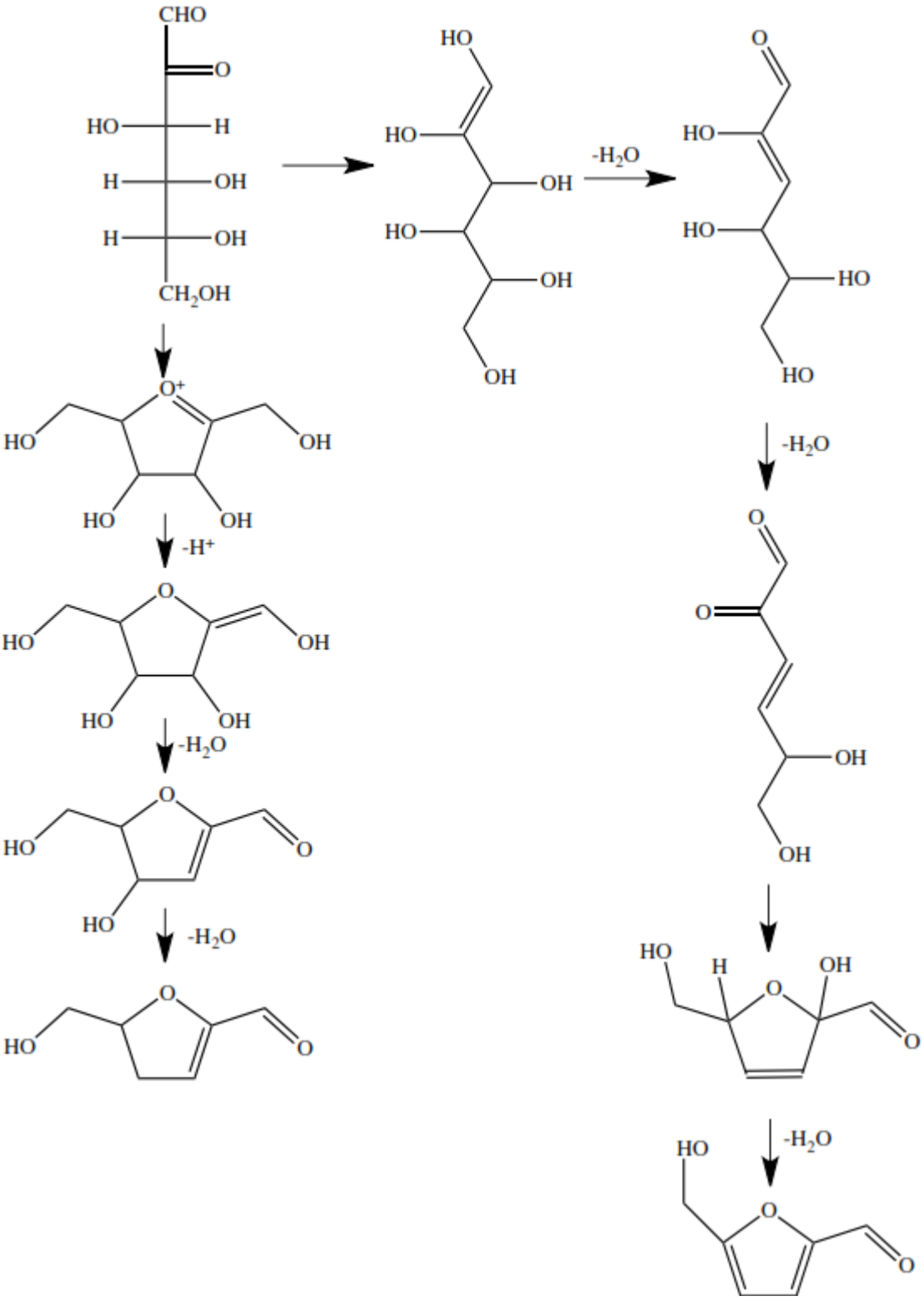

Figure 1-8 Mechanism of the formation of 5-Hydroxymethyl-2-furfural with fructose as the predominant precursor sugar [108].

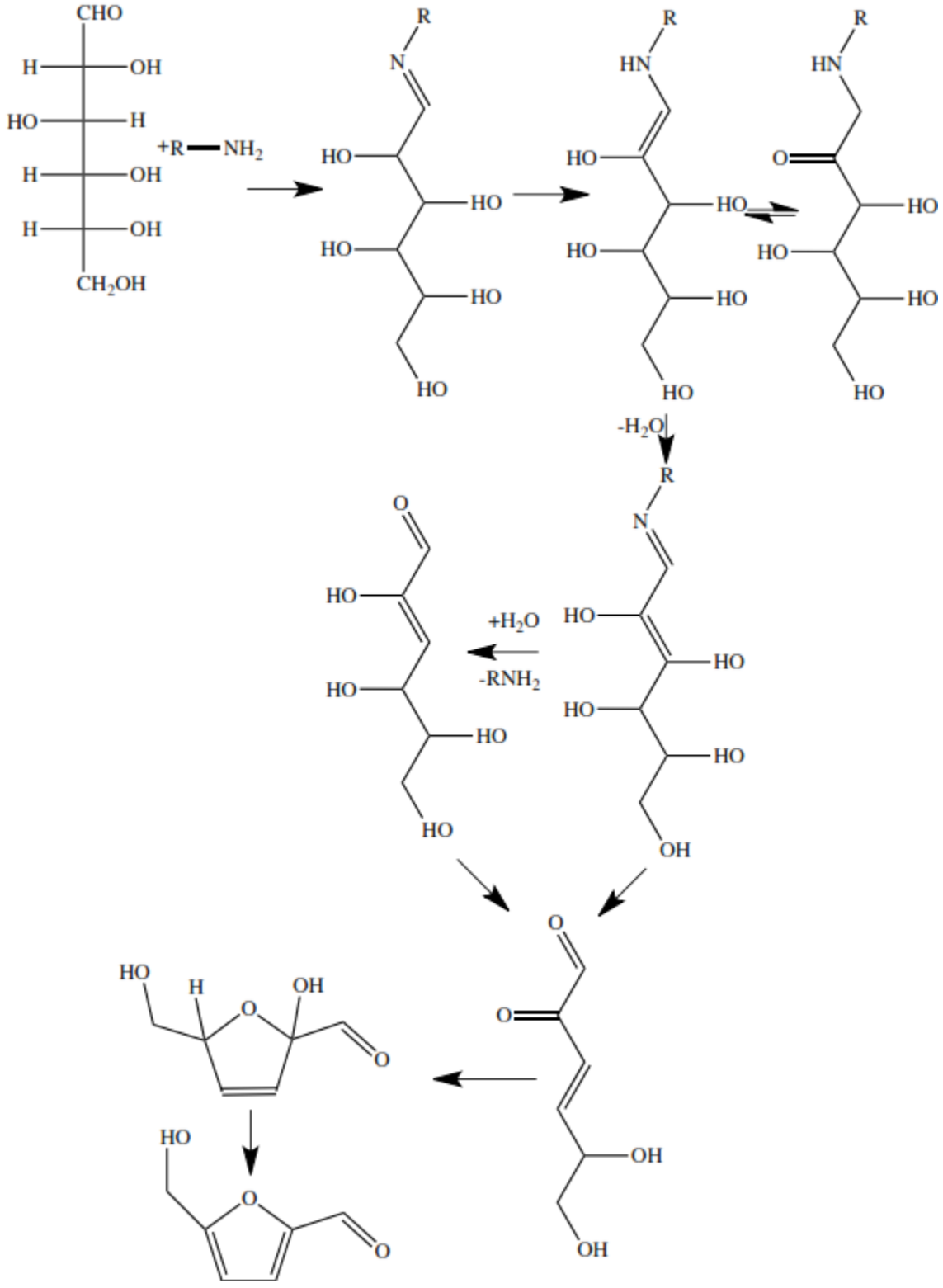

Figure 1-9 Mechanism of the formation of 5-Hydroxymethyl-2-furfural with glucose as the predominant precursor sugar [108].

1.6.3.3.3 Detection

HMF can be characterized via HPLC-MS [109,110]. Taherzadeh *et al.* (2000) proposed different fragmentations of HMF that could be present at different electron impact energies and different fragmentations of the base HMF [110]. GC-MS methods using selected ion monitoring (SIM) have allowed for a more selective MS monitoring which could account for complex food matrices [111]. Derivatization of HMF allows for heavier ions to be analyzed. HMF ions are typically 128 and 97 m/z, however, after derivatization 183 and 109 m/z are the predominant markers for HMF [111]. When HMF is derivatized with silicon compounds and subsequent fragmentation, a methyl radical is lost, and the furan ring opens [111].

1.6.3.3.4 Toxicity and biological effect

1.6.3.3.4.1 Humans

HMF can be metabolized into toxic compounds making the levels of parent HMF consumed of concern. Nassberger (1990) reported HMF to induce cellular damage by inhibiting mitochondria respiration which suggested high HMF concentrations to be problematic for immunocompromised individuals [112]. Daily intake of HMF is estimated to be 30-150 mg per person [113]. Rufian-Hernares and de la Cueva (2008) found the daily intake of HMF in the Spanish diet to come from coffee and white bread and to be 20 times higher than 540 μg/ person/ day, which is the EFSA threshold for concern in foods [114].

1.6.3.3.4.2 Animals

In rats, the oral LD_{50} was found to be 2,500 mg/kg [88]. In rat and dog studies, HMF was found to be toxic at doses of 75 mg/ kg body weight. Effects of HMF *in vitro* and *in vivo* have been widely varied. HMF has been suggested as a therapy for sickle cell disease as it can bind to sickle hemoglobin [115]. Other studies found HMF to induce DNA damage [113]. The variation in the effect of HMF could be due to the differences of how HMF is metabolized and which compounds are formed [116]. Rats and mice that were orally administered HMF saw 80-92% of the administered HMF dose excreted in the urine and feces within 48 hours, with the majority of it being excreted in the first 8 hours [117].

Monien *et al.* (2012) found HMF to induce gene mutation in Chinese hamster V79 cells that were modified for human gene expressions [118]. It was suggested that the HMF undergoes sulfo-conjugation forming 5-suldooxymethylfurfural, which reacts with DNA, causing mutations [118].

1.6.3.3.5 Potato Chips and HMF

HMF is not commonly analyzed in fried products, even though frying creates optimum conditions for the formation of HMF. HMF forms rapidly at temperatures above 150 °C, and fried products have an excess of reducing sugars and free amino acids [119]. Food products such as honey and fruit juices are studied with high concentrations being found in products from plums [113]. Breakfast cereals and biscuits, which brown through the MR have neem found to have HMF concentrations of 7-50 μg/g [120]. Ratios of free asparagine to reducing sugars affect the levels of AA and HMF in biscuits. High levels of reducing sugars and low levels of asparagine led to higher levels of AA and lower HMF

compared to biscuits made with low levels of reducing sugars and high levels of asparagine [121]. HMF concentrations in potato chips have decreased with an increase in water activity which indicates the longer the chips are fried, the higher the concentration of HMF [122].

1.6.3.3.6 Mitigation Strategies

Mitigation strategies for HMF have included decreasing the pH of dough in bakery products prior to heat treatment [123]. HMF concentrations were reduced under acidic conditions. However, the resulting product was not considered acceptable as it had an increase of surface browning [123]. Other mitigation strategies include reducing the precursors or lowering the processing temperature to decrease the rate of formation [124].

1.7 Conclusions

Potatoes are an important vegetable crop worldwide. Potatoes are processed prior to consumption such as frying into potato chips. To maintain the potato supply for yearlong consumption, potatoes are stored at low temperatures to extend dormancy. During low-temperature storage, the starch is converted to reducing sugars. The accumulation of reducing sugars increases the occurrence of the Maillard reaction and associated products. Maillard reaction products produce beneficial compounds that attribute to the overall flavor and aroma associated with fried foods. However, it produces toxins such as acrylamide, 5-hydroxymethylfurfural, and advanced glycation end-products. In order to convert the sugars back into starch and reduce excessive browning, potatoes are reconditioned at higher temperatures for 2 weeks. The move to

higher temperatures is costly in energy and space and causes a break in dormancy which causes cultivars of potatoes, such as color-fleshed potatoes, to sprout.

There is a critical need to evaluate frying directly from cold storage without the accumulation of Maillard reaction products. Changes to the entire frying process by frying below atmospheric pressure could eliminate the need for reconditioning and reduce the occurrence of toxin formation. I propose vacuum frying directly from cold storage as a potential mechanism. No systematic studies have been completed on the reduction of acrylamide, 5-hydroxymethylfurfural, and advanced glycation end-products in the same food product. A need exists to reduce the occurrence of toxins and produce a product that is still acceptable to consumers. I suggest vacuum frying as a mitigation strategy for these goals.

2 RATIONALE AND AIMS

The potato is the largest fresh market vegetable crop in the United States with over 1 million acres harvested annually [1]. The average American consumption of potatoes is 111.2 pounds/ person/ year with 17.8 pounds of that being consumed as potato chips and shoestrings [7]. While white-fleshed potatoes are the most abundant, total acreage of color-fleshed potatoes, such as red and blue/purple, have increased in the past 3 years [9].

After harvesting, potatoes are stored for 2-12 months at cold temperatures (2-10 °C) which allows for a constant supply of potatoes outside of the harvest season [11,31,32]. During cold storage, a majority of potato cultivars undergo cold-induced-sweetening where the starch converts to reducing sugars [42]. Reducing sugars are a precursor to the Maillard reaction which occurs during high-temperature processing, such as frying [71]. Additionally, frying at higher temperatures increases the rate of the Maillard reaction, which rapidly occurs at 120 °C and above [68]. The Maillard reaction causes excessive browning and results in the formation of potential toxins such as acrylamide, advanced glycation end products, and 5-hydroxymethylfurfural [68]. In order to counter the accumulation of reducing sugars, potatoes are reconditioned at higher temperatures (15-20 °C) for up to three weeks prior to processing where as much as 80% of the reducing sugars are converted back to starch [47]. Reconditioning requires an additional storage room and additional energy input to maintain the temperature. Reconditioning also causes a break in the cold-induced dormancy of potatoes which induces sprouting, resulting in high economic loss [32]. White-fleshed potatoes have

been bred to withstand sprouting during reconditioning. However, color-fleshed potatoes have not, and are thus underutilized in potato processing. As a result, **there is a critical need to eliminate reconditioning before processing to reduce energy use and product loss due to sprouting.** I propose vacuum frying directly from cold storage as a potential process to eliminate the need for reconditioning and allowing for color-fleshed cultivars, i.e., Mountain Rose and Purple Majesty, to be more widely utilized in the chipping industry.

Traditionally, potato chips are manufactured by deep-fat frying at high temperatures (160-180 °C) which dehydrates the potato slice to produce a crisp texture. Deep-fat frying poses potential health risks due to oil uptake and the formation of potentially toxic lipid-oxidation products [57,58]. Additionally, frying at higher temperatures increases the rate of the Maillard reaction, which rapidly occurs at 120 °C and above [68]. Research on the byproducts of the Maillard reaction has suggested that some are potentially toxic. As mentioned previously, the increase in reducing sugar content due to cold storage increases the concentration of Maillard reaction products, as reducing sugars are a major reactant. However, the implementation of reconditioning alone does not fully eliminate the formation of toxic Maillard reaction products. Given this, **there is a need for alternative processing techniques to reduce the toxin formation during conventional, deep-fat frying of potato chips.** I propose vacuum frying as a potential technique to reduce the toxin formation by frying at a lower temperature for a longer time while still being perceived as favorable by consumers.

Commercially available potato chips have a characteristic crunch that is attributed to a moisture content of less than 2% [125]. Consumers also expect a light chip [94].

Therefore, **there is a need to maintain the same quality consumers expect when vacuum frying with or without reconditioning while reducing energy usage, product loss, as well as toxins.** Ultimately, any changes in storage or frying should produce a chip that consumers find comparable to the products they are accustomed to.

Current research on reducing potential toxin formation during deep-fat frying has only focused on the reduction of acrylamide. Mitigation strategies include chemical treatments which may have negative consumer perceptions. Other mitigation strategies have the potential to increase the formation of other potentially toxic Maillard reaction products. Additionally, previous studies have investigated single cultivars of potatoes at a time and have not fully studied color-fleshed cultivars. **Vacuum frying could potentially be used to eliminate reconditioning to create potato chips fried directly from cold storage which are similar to conventionally fried chips with reconditioning.** To achieve this, vacuum fried chips without reconditioning should be equal to in quality parameters and less than or equal to toxin concentrations of conventional fried chips with reconditioning. The implication of this work will be a reduction of (1) the costs and energy associated with reconditioning, (2) economic loss due to potato sprouting, and (3) toxin formation by high-temperature deep-fat frying. I believe that vacuum frying without reconditioning will produce a chip that is comparable in quality parameters to potato chips that are reconditioned and deep-fat fried.

I hypothesize that for all of the varieties studied:

(1) the quality attributes (i.e., texture, color, moisture) of vacuum fried chips without reconditioning will be similar to conventionally fried chips with

reconditioning as determined by commonly used instrumental methods (i.e., texture analyzer, colorimeter, moisture balance);

(2) the toxins acrylamide, advanced glycation end-products, and 5-hydroxymethylfurfural will be present at the highest concentrations in chips made using conventional frying without reconditioning followed by conventional frying with reconditioning and reduced in vacuum frying without reconditioning and lowest in vacuum frying with reconditioning;

(3) consumers will find vacuum fried chips without reconditioning comparable to conventional fried chips with reconditioning and preferred over conventional fried chips without reconditioning based on a blinded sensory evaluation (i.e., JAR, hedonic liking).

In order to test the hypotheses, the following specific aims are proposed:

1. Develop vacuum frying parameters that produce chips that are comparable to commercial chips.
2. Investigate the impact of vacuum frying and reconditioning on the quality attributes of chip from three cultivars of potatoes.
3. Investigate the impact of vacuum frying and reconditioning on the reduction of toxins in chips from three cultivars of potatoes.
4. Investigate the acceptability of vacuum fried chips without reconditioning using a consumer panel.

3 EFFECT OF VACUUM FRYING ON TOXIN FORMATION, RETENTION OF QUALITY ATTRIBUTES, AND CONSUMER ACCEPTABILITY AS A MEANS TO ELIMINATE RECONDITIONING AFTER LONG TERM COLD STORAGE IN WHITE-, RED-, AND PURPLE- FLESHED POTATO CHIPS

3.1 Introduction

Potatoes (*Solanum tuberosum L.*) are the largest fresh market vegetable crop in the United States with over 1 million acres harvested annually [1]. In 2016 Americans consumed on average 111.2 pounds/ person/ year [7]. Potatoes are sold for fresh market use or processed into a product such as potato chips and shoestrings. In 2016, the average annual consumption of potato chips and shoestrings per American was 17.8 pounds/person/year [7]. Color-fleshed potato chips are receiving more attention from consumers and producers as they are a rich source of phenolic compounds which have been shown to work as antioxidants, reduce the occurrence of certain cancers, and work as anti-inflammatory agents [50].

Potatoes can be stored from 2-12 months at low temperatures (2-10 °C) to keep a constant supply of potatoes by extending dormancy which will reduce sprouting and reduce waste due to rotting. During cold storage, the starch present in the potato is converted into sugar through a process called cold-induced sweetening. The potato industry has bred cultivars of potatoes that have extended periods of dormancy as well as resistance to cold-induced sweetening; however, color-fleshed potatoes are not resistant to cold-induced sweetening and are underutilized in the potato chip industry [12].

The accumulation of sugar in the tubers produces browning in fried products due to the Maillard reaction. This browning is undesirable to consumers due to taste and dark

brown color formation. The Maillard reaction produces toxins such as acrylamide, 5-hydroxymethylfurfural, and advanced glycation end-products [68].To reduce the effects of cold-induced sweetening, tubers which will be processed can be reconditioned by warming the tubers for 1-3 weeks at higher temperatures (15-20°C) [40]. During reconditioning, up to 80% of the reducing sugars are converted back to starch, and the remaining 20% is lost due to respiration [47]. Reconditioning breaks dormancy and causes potatoes to sprout. However, even after reconditioning, some Maillard toxins are formed during atmospheric frying due to the residual sugars.

Mitigation strategies for acrylamide include pretreatment methods of blanching, chemical additives, and natural plant extracts [96,97]. Mitigation strategies for 5-hydroxymethylfurfural have included altering the pH but have not had positive sensory attributes [123]. Reducing consumption of food products with advanced glycation end-products is the suggested mitigation strategy, with no work on reducing the occurance in the food products. Vacuum frying could limit the formation of Maillard reaction products. By lowering the pressure, the boiling point of the water present in the food is lowered [60]. The fried products can be fried at lower temperatures (110- 140 °C) [60–62] leading to fewer Maillard products.

In an effort to reduce the formation of Maillard reaction products and to reduce product loss due to sprouting, I propose vacuum frying directly from cold storage will produce a potato chip that is appealing to consumers and reduce toxins. Previous studies in our lab found vacuum frying retained health-benefitting anthocyanins and color compared to atmospheric fried chips. Vacuum frying reduced the levels of toxic glycoalkaloids to safe for consumption levels. Acrylamide levels were analyzed with

HPLC-PDA with no differences between storage treatments nor between vacuum or atmospheric frying [65].

I hypothesize that vacuum frying will:

(1) maintain the quality attributes (i.e., texture, color, moisture) of vacuum fried chips without reconditioning will be similar to conventional frying with reconditioning as determined by commonly used instrumental methods (i.e., texture analyzer, colorimeter, moisture balance);

(2) reduce the toxins acrylamide, advanced glycation end-products, and 5-hydroxymethylfurfural will the highest concentrations in conventional frying without reconditioning (C-CF) followed by conventional frying with reconditioning (R-CF) and reduced in vacuum frying without reconditioning (C-VF) and lowest in vacuum frying with reconditioning (R-VF);

(3) produce chips, without reconditioning, that consumers will find comparable to conventional fried chips with reconditioning and preferred over conventional fried chips without reconditioning based on a blinded sensory evaluation (i.e., JAR, hedonic liking).

3.2 Materials and methods

3.2.1 Potatoes

Three commercial cultivars of potatoes were grown at the San Luis Valley Research Center, Colorado State University, Colorado. One cultivar of each flesh color was used: white (Atlantic), red (Mountain Rose), and purple (Purple Majesty). Potatoes were planted in June 2017 and harvested in late August/ early September 2017. After harvest, the potatoes were stored as described below.

3.2.2 Storage

Potatoes were stored at the San Luis Valley Research Center, Colorado State University, Colorado at 3-4°C (37.4-39.2°F) for 75 days. The potatoes were shipped via UPS over two days. Potatoes were then stored in the dark at The Pennsylvania State University, University Park, Pennsylvania at 2-4 °C (35.6-39.2°F) for 52 days. Half of the potatoes remained in storage at 2-4 °C for an additional 21 days (without reconditioning/ cold storage potatoes) while the other half of potatoes were stored in a reconditioning room (reconditioned potatoes) maintained at 13 °C (55.4°F) for 21 days. Total time for potatoes at cold storage was 150 days.

3.2.3 Potato Raw and Chip Preparation

Potatoes were washed to remove excess dirt and sprouts from the skin. The potatoes were sliced to 1 mm thickness using a Cabela's heavy-duty deli slicer (Cabela's Inc., Sydney, Nebraska). The slices were placed in a strainer and washed under cool, running water for 1 minute while mixing. The slices were patted dry with paper towels to remove excess liquid. Raw slices were taken immediately to reducing sugar extraction

described below. Remaining potato slices were processed using vacuum frying or conventional frying, as described below. Approximately 80 g of raw slices were used for each trial of vacuum frying and conventional frying. Potatoes used for sensory evaluation were processed 24 hours prior to analysis.

3.2.4 Biological Replicates

Twenty trials were completed for Mountain Rose and Purple Majesty conventional fried with and without reconditioning and vacuum fried without reconditioning. Trials one through five were pooled as biological replicate one, trials six through ten as biological replicate two, eleven through fifteen as biological replicate three, and sixteen through twenty as biological replicate four. From biological replicates one, two, and three, 30 g of chips were taken from each for toxicant analysis, 5 g for texture, color, and moisture, and the remaining, as well as biological replicate four, went to sensory evaluation.

Three trials were completed for Atlantic conventional and vacuum frying with and without reconditioning and Mountain Rose and Purple Majesty vacuum fried with reconditioning. Each trial was a biological replicate. Chips (5 g) from each biological replicate were taken for texture, color, and moisture, and the remaining were taken for toxicant analysis.

All potato chips were stored at room temperature, in the dark, for 24 hours so that the chips would be at room temperature prior to the sensory evaluation. After 24 hours at room temperature, the potato chips were moved to -20 °C.

3.2.5 Frying Equipment and Conditions

3.2.5.1 Vacuum Frying

A vacuum fryer previous developed by our lab was utilized with modifications (Figure 1-1) [65,125]. The vacuum vessel was attached to a refrigerant condenser with ethanol. The condenser was attached to the in-house vacuum line.

The vessel was filled with 9 L of fresh, clear soybean frying oil (Bakers and Chefs Food Equipment PTE. LTD., Singapore). The oil was allowed to heat to 130 °C for 30 minutes, based on previous work in our lab [65]. Once the oil reached temperature, 80 g of potato slices were added into the basket in a single layer, and the basket was screwed into the basket rod. The lid was placed on the vessel, and the vessel was sealed. The vacuum was pulled to 25 in. Hg. (85 kPa). When vacuum was achieved, the potato slices were lowered into the oil and fried for the required time. The vacuum level and temperature were monitored every 30 sec. until completion of frying. At the completion of the fry time, the slices were raised out of the oil; the vacuum was released; once atmospheric pressure was achieved, the vessel was opened, the basket was removed, and the chips were placed on paper towels and blotted to remove excess oil. After the chips cooled to room temperature, the chips were stored in Ziploc bags.

3.2.5.2 Conventional Frying

Conventional frying was completed using an Avantco Single Countertop Fryer (Clark Associates Inc., Lancaster, Pennsylvania) filled with 5 L of fresh, clear soybean frying oil (Bakers and Chefs Food Equipment PTE. LTD., Singapore). The oil was heated to 170 °C, as it was experimentally convenient. Once the temperature was reached, 80 g of potato slices were laid flat in the frying basket. The slices were lowered into the oil

and stirred with a metal slotted spoon to prevent slices from sticking and to promote even frying. The slices were fried for the required time, and the temperature was monitored every 30 sec. The chips were removed from the oil and placed on paper towels and blotted to remove excess oil. After the chips cooled to room temperature, the chips were stored in Ziploc bags.

3.2.5.3 Oil Quality

The oil quality was measured after each frying trial for each frying treatment using a Testo 270 (Testo Inc., Sparta, New Jersey). The Testo 270 reports total polar materials as a percentage, which estimates the degradation of the oil. A percentage of total polar materials (%TPM) above 25% was considered the cut off for poor quality oil. The oil was changed after 10 trials in both the conventional fryer and vacuum fryer regardless if 25% TPM was not met.

3.2.6 Commercial Chips

Four commercially available brands of potato chips were purchased from a local grocery store (Wegmans, State College, Pennsylvania). Two varieties of each white- and purple-fleshed potato chips were purchased. Potato chips were defatted and stored as described below. Brands are referred to as White 1, White 2, Purple 1, and Purple 2.

3.2.7 Texture

Texture analysis was completed using a TA-XT Plus texture analyzer (Texture Technologies, Scarsdale, New York) equipped with a 0.635 cm spherical metal probe. One potato chip was placed on a 2 cm diameter cylinder sample holder. The probe was forced through the chip using a puncture test. The probe was set with a pre-test speed of 1.00 mm/sec until a trigger force of 0.049 N was reached. The probe traveled at 1.00 mm/sec for 3.00 mm followed by a post-test speed of 10.00 mm/ sec. Exponent (Stable Micro Systems, Godalming, United Kingdom) was used for data collection and analysis. Force (N) vs. distance (mm) curves were generated, and the maximum force was utilized to describe the hardness. A sample of two chips from each biological replicate was analyzed for each cultivar, storage treatment, and frying combination. A sample of 10 chips from each commercial sample was analyzed.

3.2.8 Color

Color analysis was done using the CIE Lab color scale. A digital colorimeter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) was used to measure the color of raw slices, conventional fried, and vacuum fried chips. The colorimeter was calibrated with a white standard plate, and the CIE Lab color scale was used: L^*- lightness, a^*- redness, and b^*-yellowness. Approximately 5 g of chips were crushed with a mortar and pestle and placed on a white background. Ten samples of commercial chips were analyzed. The color was analyzed at three different points for each biological replicate.

Color differences, ΔE, were calculated using the following equation:

$$\Delta E = \sqrt{(L^* - L_r^*)^2 + (a^* - a_r^*)^2 + (b^* - b_r^*)^2} \quad \text{(Eq. 3-1)}$$

where L^*, a^*, and b^* represent the Lab color scale reading of the fried chips and L_r^*, a_r^*, and b_r^* represent the color of the raw slices.

Digital photographs of the samples were taken using Canon EOS Rebel T3I 18 megapixel digital camera (Canon U.S.A. Inc., Melville, New York). Potato chip samples were placed on a white background.

3.2.9 Moisture Content

Moisture Content was done on an Ohaus MB45 Moisture Analyzer (Ohaus Corporation, Parsippany, New Jersey). Potato chips were crushed with a mortar and pestle, and 5 g was placed on a sample pan. The moisture analyzer was set to standard drying with a drying temperature of 135 °C with a timed stop of 5 minutes. Chips (5 g) from each of the 3 biological replicates of each cultivar, storage, and frying treatment combination were tested. Moisture content was done on 10 samples of each of the commercial chips.

3.2.10 Sensory Evaluation

3.2.10.1 Participants

Individuals (n=119, 32 males) with a median age of 41 years were recruited from the Pennsylvania State University campus and surrounding area (State College, Pennsylvania). Participants were selected off of the criteria of regularly consuming potato chips with 90.7% consuming potato chips within the past 6 months. Criteria for eligibility included not pregnant or breastfeeding, no known defects to smell or taste, no oral piercings, a nonsmoker in the last 30 days, no food allergies, sensitivities, or dietary restrictions, and no history of choking or difficulty swallowing. Participants provided

informed consent and were paid for their time. The procedures from this study were exempt from IRB review by the Penn State Office of Research Protections under the wholesome foods exemption in 45 CFR 46.101(b)(6) (Study Number 33164).

3.2.10.2 Samples

Each sample was assigned a three-digit random code and the order randomly assigned to each participant. Participants either received red- or purple-fleshed potato chips first and samples were randomized within cultivar. Two potato chips were presented to participants and served on a white plate. Participants received one treatment at a time. Participants had to wait 30 sec. between samples and 1 min. between cultivars. Participants were asked to rinse their mouth with water in-between samples. Participants evaluated Mountain Rose and Purple Majesty, vacuum fried without reconditioning and conventional fried with and without reconditioning.

3.2.10.3 Questionnaire

Before tasting the sample, participants were asked to rate how much they like or dislike the brightness of color using a 9-point hedonic scale (1=dislike extremely; 9= like extremely). Participants were then asked to rate the brightness of color using the Just-About-Right (JAR) scale with 3 being anchored as JAR, 1 as not at all, and 5 as much too much. The participants were instructed to taste the sample and answered JAR on crispiness, strength of flavor, and oiliness. They were then asked to indicate how much they liked or disliked the product overall using the 9-point hedonic scale. After tasting the 3 samples for one cultivar, they were asked to rank the samples in order of most preferred

to least preferred. After both cultivars were tested, participants were provided with the following statement:

> "Colored potatoes (red and purple) are a rich source of anthocyanins, which are antioxidants and potential anti-inflammatory agents. Knowing that colored potatoes may provide health benefits, how willing would you be to purchase colored potato chips over traditional white potato chips?"

Participants were asked to select their purchase intent (definitely would not purchase, probably would not purchase, might or might not purchase, probably would purchase, definitely would purchase). Participants were then given the following statement:

> "On average a single serving (1 oz. package) of traditional white potato chips (Lay's/ Herr's) costs $0.50. How much would you be willing to spend on similar size bag of colored potato chips?"

and were asked to select on a line scale the price they would be willing to spend. Participants were given a list of five commercially available products and asked to indicate the last time they purchased them. Commercial products were regular potato chips (Lay's/Herr's), Pringles, baked potato chips, colored potato chips (Terra Blues/ Jackson's Honest) and mixed vegetable chips/ straws.

3.2.11 Reducing Sugars

Reducing sugars were quantified by the Nelson-Somogyi method as described by Shao and Lin (2018) [126]. The copper reagent was prepared by dissolving 12 g of potassium sodium tartrate (Lot MKBJ6983V, Sigma Aldrich) and 24 g sodium carbonate (Lot G39585, Mallinckrodt Chemicals) in 250 mL deionized water. The solution was

stirred and 4 g of copper (II) sulfate pentahydrate (Lot MKCB1544V, Sigma Aldrich) and 16 g of sodium bicarbonate (Lot A0327947, Acros Organics) were added. Sodium sulfate (180 g) (Lot SX0760-3, EMD) was dissolved in 500 mL boiling deionized water. The two solutions were combined. Nelson reagent was prepared by dissolving 25 g ammonium molybdate tetrahydrate (Aldrich Chemicals) in 450 mL deionized water, after 21 mL of concentrated sulfuric acid (Lot SHBG8442V, Aldrich Chemistry) was added. Disodium arsenate (3 g) (Lot SLBT4798, Sigma) was dissolved in 25 mL of deionized water. Both solutions were mixed and stirred for 30 min at 55 °C.

Raw potato slices were homogenized with a coffee bean blade grinder (Mr. Coffee, Atlanta, Georgia) and 200 mg was put into a 25 mL glass screw cap test tube with 10 mL 80 v/v% 200 proof ethanol in water. The test tubes were placed into a water bath (Fisherbrand Isotemp Digital-Control Water Bath: Model 205, Fisher Scientific Company Waltham, Massachusetts) set at 95 °C for 30 min. The samples were removed from the water bath and cooled to room temperature. The supernatant (1 mL) was taken and put into a 10 mL glass screw cap test tube. Copper reagent (1 mL) was added, and the test tube was vortexed for 10 sec. The test tubes were placed into a water bath set to 100 °C for 10 minutes. The test tubes were removed and cooled to room temperature. Nelson reagent (1 mL) was added, and the tubes were degassed (VWR Ultrasonic Cleaners, Model 50D, VWR International, Radnor, Pennsylvania) for 1 min. Solutions (200 μL) were pipetted into a 96 well plate, and the absorbance was read at 520 nm (CLARIOstar plate reader, BMG Labtech, Ortenberg, Germany). Each cultivar and storage combination were run in triplicate.

α-D-glucose standards (Aldrich Chemical Company, Milwaukee, Wisconsin) were prepared in deionized water at concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 μmol/mL. Standards (1 mL) were added to a 10 mL glass screw cap test tube, and the copper reagent and Nelson reagents were added as described above. Concentrations were run in triplicate, and average absorbance was used to fit a linear regression. Glucose concentrations were calculated from the linear regression of the calibration curve using the following equation:

$$y = 0.16274x + 0.0661, \mathrm{R}^2 = 0.96 \quad \text{(Eq. 3-2)}$$

3.2.12 Fat Extraction

Potato chips were defatted prior to all analyses. Fat extraction was done following the method described by Shamla and Nisha (2017) with modifications [127]. Potato chips were crushed using a mortar and pestle, and 3 g was added to 50 mL centrifuge tubes (VWR 89004-364, VWR International, Radnor, Pennsylvania). N-hexane (20 mL) was added (HPLC Grade, Alfa Aesar, Ward Hill, Massachusetts). Samples were vortexed for 1 minute at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts). Samples were centrifuged at 4,800xg for 25 min at 20 °C (Sorvall Legend XTR, Rotor TX-750, Fisher Scientific, Waltham, Massachusetts). Following centrifugation, the defatted chips were collected using a Buchner flask and funnel attached to a vacuum line. Samples were collected on filters (Whatman Filters, Grade 1, 70 mm pore, Cat. No. 1001070, GE Healthcare Life Sciences, Pittsburgh, Pennsylvania) and stored at -20 °C in Ziploc bags until analysis.

3.2.13 Methanol Extraction for Acrylamide

Methanol extraction of acrylamide was done following the method described by Pederson and Olsson (2003) with modifications [79]. Defatted potato chips (2 g) were placed in a 50 mL glass screw-top test tube. Methanol (40 mL) (HPLC grade, LabChem, Zellenople, Pennsylvania) was added. The initial mass of the chips and methanol was recorded. The test tubes were placed in a water bath (Precision Water Bath, model 2872, Thermo Fisher Scientific, Waltham, Massachusetts) set to 65 °C with a shaker set to 50 RPM. Every 24 hours, the mass was checked against the initial mass. If the weight was reduced by more than 1 g, methanol was added to get within 1 g of initial mass. After 7 days, the samples were removed, and weight was again checked, as described above. The samples were vortexed at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts) for 20 sec. The samples were filtered using a 45 μm nylon membrane filter (VWR syringe filter, Cat. No. 28145-489, VWR International, Radnor, Pennsylvania) on syringe (BD Disposable Syringe, Cat. No. 14-29-45, Fisher Scientific Company Waltham, Massachusetts) and stored –20°C until analysis in 50 mL centrifuge tubes (VWR Cat No. 89004-364, VWR International, Radnor, Pennsylvania).

To determine the accuracy of the extraction, 1 g of defatted Purple Majesty vacuum fried chips without reconditioning were weighed into glass screw cap test tubes, and a 2,000 ng/mL acrylamide (Lot NT055877, Ultra Scientific, Kingstown, Rhode Island) in methanol standard was added. The volume was adjusted to 20 mL with methanol. The mass was recorded, and the extraction protocol was followed as described above. Each concentration was performed in duplicate.

Matrix effect was determined by determining the percent recovery (%R) using the following equation:

$$\%R = \frac{(spiked\ sample\ result - unspiked\ sample\ result)}{Calculated\ concentration\ of\ standard\ added} * 100 \qquad \text{(Eq.3-3)}$$

3.2.14 Ethyl Acetate Extraction for 5-(hydroxymethyl)furfural

Ethyl acetate extraction for 5-(hydroxymethyl)furfural (HMF) was done following the method described by Marsol-Vall *et al.* (2016) with modifications [111]. Defatted potato chips (2 g) were placed into a 50 mL conical centrifuge tube (VWR Cat No. 89004-364, VWR International, Radnor, Pennsylvania). Ethyl acetate (10 mL) (HPLC grade, HiPerSolv Chromanorm, VWR International, Radnor, Pennsylvania) was added. The samples were vortexed at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts) for 20 sec. The samples were centrifuged at 4,700 x g at 4 °C for 10 minutes (Sorvall Legend XTR, Rotor TX-750, Fisher Scientific, Waltham, Massachusetts). The supernatant (5 mL) was filtered using a 45 µm nylon membrane filter (VWR syringe filter, Cat. No. 28145-489, VWR International, Radnor, Pennsylvania) on syringe (BD Disposable Syringe, Cat. No. 14-29-45, Fisher Scientific Company Waltham, Massachusetts) and stored –20°C until analysis in 15 mL centrifuge tubes (VWR Cat No. 21008-216, VWR International, Radnor, Pennsylvania).

To determine the accuracy of the extraction, 2 g of defatted Atlantic conventional fried chips without reconditioning were placed into a 50 mL conical centrifuge tube, a 1,000 ng/mL HMF standard (98%, Lot A0376643, Acros Organics, Thermo Fisher Scientific, New Jersey) was added and the samples were diluted to 10 mL the samples

were extracted as described above. Matrix effect was determined by determining the %R using Eq. 3, described above.

3.2.15 Phosphate Buffer Saline Extraction for Advanced Glycation End-products

Phosphate buffer saline was utilized for the extraction of N^{ε}-carboxymethyl)lysine in potato chips following the method described by Adisakwattana *et al.* (2014) with modifications [128]. Defatted potato chips (2 g) were weighed into 50 mL conical centrifuge tubes (VWR Cat No. 89004-364, VWR International, Radnor, Pennsylvania). Phosphate buffer saline solution (20 mL, 1x) (Cat. No. E404-200TABS, VWR International, Radnor, Pennsylvania) in deionized water was added, and the samples were vortexed at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts) for 30 sec. The samples were placed in a water bath (Precision Water Bath, model 2872, Thermo Fisher Scientific, Waltham, Massachusetts) for 30 min. at 90 °C. The samples were then centrifuged at 1,000 x g for 20 min. at 20 °C (Sorvall Legend XTR, Rotor TX-750, Fisher Scientific, Waltham, Massachusetts). The supernatant was collected and immediately analyzed as described below.

3.2.16 Acrylamide Quantification via GC-MS/MS

Samples were warmed to room temperature and vortexed at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts) for 30 sec. Extracted methanol (950 µL) and 50 µL of 200 ng/mL 2,3,3,d_3-acrylamide internal standard (Lot M110P20, CDN Isotopes, Pointe-Claire, Quebec, Canada) were

added to an amber GC vial (Amber vials with Bonded Red PTFE/White Silicone septa, Cat. No. 82030-974, VWR International, Radnor, Pennsylvania).

An Agilent 7890B gas chromatograph (GC) equipped with an Agilent 7693 autosampler and an Agilent 7000B triple quadrupole mass spectrometer (MS) was utilized. A WAXetr column (30 m x 0.25 mm x 0.25 µm, Agilent Technologies, Santa Clara, California) was used. The initial temperature of the GC oven was set to 70 °C and held for 1 min. The temperature was then raised to 240 °C at a rate of 25 °C/ min and held for 10.5 min. Helium (99.999% purity) was used as the carrier gas at a flow of 1 mL/min. The injector was run at pulsed splitless at 30 psi and an injection volume of 2 µL. The inlet liner was an ultra-inert, splitless, single taper linter with glass wool and autosampler fixed needle syringe has a 10 µL capacity with 23-26s/42/cone, PTFE-tip plunger (Agilent Technologies, Santa Clara, California). The injector and transfer line temperatures were held at 230 °C and 240 °C, respectively. The MS had a solvent delay of 5 min. The source temperature and quadrupole temperatures were held at 250 °C and 150 °C, respectively. Electron Impact (EI) Multiple Resonance Monitoring (MRM) acquisition mode was utilized with nitrogen (99.999% purity) as the collision gas. The MRM transitions monitored for acrylamide were the transition from 71 to 55 at 7 eV and 71 to 44 at 25 eV the transitions for 2,3,3,d_3-acrylamide were 74 to 58 at 7 eV and 74 to 30 at 20 eV. Ethyl acetate (HPLC grade, HiPerSolv Chromanorm, VWR International, Radnor, Pennsylvania) was used as the needle washing solution.

A full scan method was done to identify peaks using National Institute of Standards and Technology (NIST) software (Version 2.2, Gaithersburg, Maryland). EI scan mode was run monitoring ions between 30 and 300 amu with a scan time of 100 msec.

Standards were prepared in concentrations ranging from 2,000 ng/mL of acrylamide in methanol to 20,000 ng/mL acrylamide in methanol (HPLC grade, LabChem, Zellenople, Pennsylvania). Standards were prepared at higher concentrations so the addition of 50 µL of 200 ng/mL 2,3,3,d_3-acrylamide internal standard would yield a 20,000 ng/mL acrylamide concentration and a 10 ng/mL 2,3,3,d_3-acrylamide internal standard concentration. Acrylamide concentrations were calculated from the linear regression of the calibration curve using the following equation:

$$y = 2694x - 1535866\,, R^2 = 0.9962 \quad \text{(Eq. 3-4)}$$

Limit of detection (LOD) was calculated using the following equation:

$$LOD = \frac{3 * standard\ deviation\ of\ lowest\ concentration}{slope\ calibration\ curve} \quad \text{(Eq. 3-5)}$$

Limit of quantification (LOQ) was calculated using the following equation:

$$LOQ = \frac{10 * standard\ deviation\ of\ lowest\ concentraton}{slope\ of\ calibration\ curve} \quad \text{(Eq. 3-6)}$$

Percent difference was determined between commercial chip concentrations found in the present work and the Food and Drug Administration's (FDA) published survey data of acrylamide in food using the following equation:

$$\frac{|Concentration_1 - Concentration_2|}{\frac{Concentration_1 - Concentration_2}{2}} * 100 \quad \text{(Eq. 3-7)}$$

where concentration 1 was present work and concentration 2 was FDA data.

Data were acquired and analyzed using the MassHunter acquisition, qualitative, and quantitative platforms (Agilent Technologies, Santa Clara, California).

3.2.17 5-(Hydroxymethyl)furfural Quantification via GC-MS

Samples were warmed to room temperature and vortexed at 3,200 rpm (speed 10) (Fisherbrand Analog Cortex Mixer, Fisher Scientific Company Waltham, Massachusetts) for 30 sec. and 1,000 uL of sample was added to an amber GC vial (Amber vials with Bonded Red PTFE/White Silicone septa, Cat. No. 82030-974, VWR International, Radnor, Pennsylvania).

An Agilent 7890B GC equipped with an Agilent 7693 autosampler and an Agilent 7000B triple quadrupole MS was utilized. The GC was equipped with an HP 5 MS-UI column (30 m x 0.250 mm x 0.25 μm, Agilent Technologies, Santa Clara, California). The initial temperature of the GC oven was set to 70 °C and held for 1 min the temperature was then raised to 320 °C at a rate of 25 °C/ min and held for 2 min. Helium (99.999% purity) was used as the carrier gas at a flow of 1 mL/min. The inlet liner was an ultra-inert, splitless, single taper linter with glass wool and autosampler fixed needle syringe with 10 μL capacity and 23-26s/42/cone, PTFE-tip plunger (Agilent Technologies, Santa Clara, California). Injector was run at pulsed splitless at 30 psi and utilized a sandwich injection volume of 0.5 μL of sample, 0.2 μL air gap, 1 μL of derivatizing agent: N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) (GC derivatization grade >98.5%, Lot BCBR3924V, Sigma-Aldrich, Co., St. Louis, Missouri). The cap of the MSTFA vial was changed after 15 injections to prevent contamination. The injector and transfer line temperatures were held at 250 °C and 300 °C respectively. The MS had a solvent delay of 5 min. The source temperature and quadrupole temperatures were held at 250 °C and 150 °C, respectively. Electron Impact

(EI) with Selected Ion Monitoring (SIM) was utilized by monitoring for ions 183 m/z and 109 m/z.

A full scan method was done to identify peaks using NIST software (Version 2.2, Gaithersburg, Maryland). EI scan mode was run monitoring ions between 30 and 300 amu with a scan time of 100 msec.

Standards were prepared in concentrations ranging from 100 ng/mL to 20 ng/mL of HMF in ethyl acetate. HMF concentrations were calculated from the linear regression of the calibration curve using the following equation:

$$y = 29700x + 633144\,, R^2 = 0.9624 \quad \text{(Eq. 3-8)}$$

LOD and LOQ were calculated using Equations 3-5 and 3-6, respectively. Data were acquired and analyzed using the MassHunter acquisition, qualitative, and quantitative platforms (Agilent Technologies, Santa Clara, California).

3.2.18 Advanced Glycation End-products Quantification via N^{ε}-(carboxymethyl) lysine Enzyme-linked Immunosorbent Assay

Enzyme-linked Immunosorbent Assay for N^{ε}-(carboxymethyl) lysine (CML) (Biomatik, Wilmington, Delaware) was performed using the manufacturer's protocol. CML concentration was calculated from the linear regression of the calibration curve using the following equation:

$$y = -0.04862x + 0.217, R^2 = 0.9782 \quad \text{(Eq. 3-9)}$$

3.2.19 Statistical Analysis

Generalized Linear Model using Tukey adjustments and α=0.05 was utilized to determine the effects of cultivar, storage, and frying. Correlation of glucose, acrylamide,

HMF, and CML concertation was utilized. SAS Statistical Analysis System v 9.4 (SAS Institute Inc., Cary, North Carolina) was used for all analyses. Sensory analysis was done utilizing Copusense Cloud (Copusense, Guelph, Ontario, Canada).

3.3 Results and Discussion

3.3.1 Sugar Content

Reconditioning is utilized to counteract the effect of cold-induced sweetening. Conversion of the sugar back into starch reduces the excessive browning that is unappealing to consumers. By measuring the starting sugar concentrations of the raw slices, the correlation between sugar levels and toxins could be determined.

Glucose equivalence was measured in raw potato slices directly from cold storage (i.e., without reconditioning) and after reconditioning. Linear regression of solutions of glucose concentrations (Figure 3-1) allows for the use of Equation 3-2 to determine the glucose concentration of unknown samples. Previous work has studied the use of glucose for calibration curves of the Nelson-Somogyi method and found glucose had the highest reducing power and found that glucose calibration curves were acceptable for measuring fructose and glucose levels [126]. Kinetic modeling work of Maillard reaction products found that glucose concentrations were more accurate in predicting the amounts of acrylamide and 5-hydroxymethylfurfural than fructose and asparagine concentrations [121].

Statistical analysis showed significant differences between cultivars and between storage treatments. Atlantic slices had a 7.3% decrease in glucose equivalence when the potatoes were reconditioned while Mountain Rose and Purple Majesty had a 70.12% and

75.98% decrease, respectively. The data show that reconditioning effectively decreases the glucose concentration in raw potatoes. However, the red- and purple-fleshed potatoes that were reconditioned sprouted and many could not be used for frying due to shrinkage and soft texture of the raw tuber.

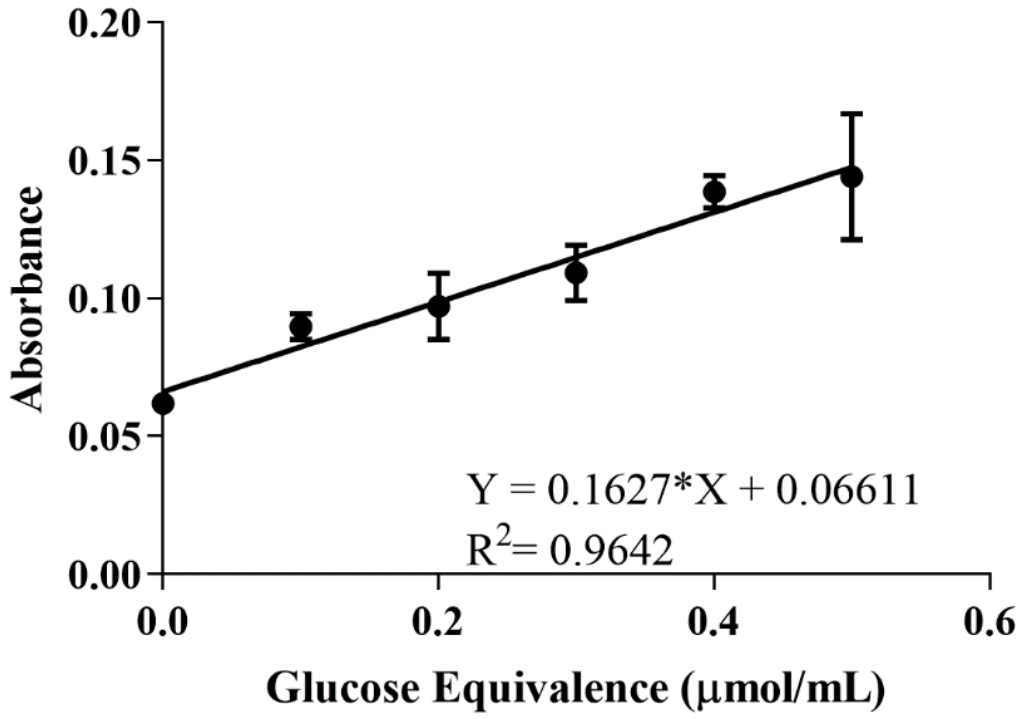

Figure 3-1 Calibration curve of absorbance as a function of the glucose equivalence. Error bars represent the standard deviation (n=3).

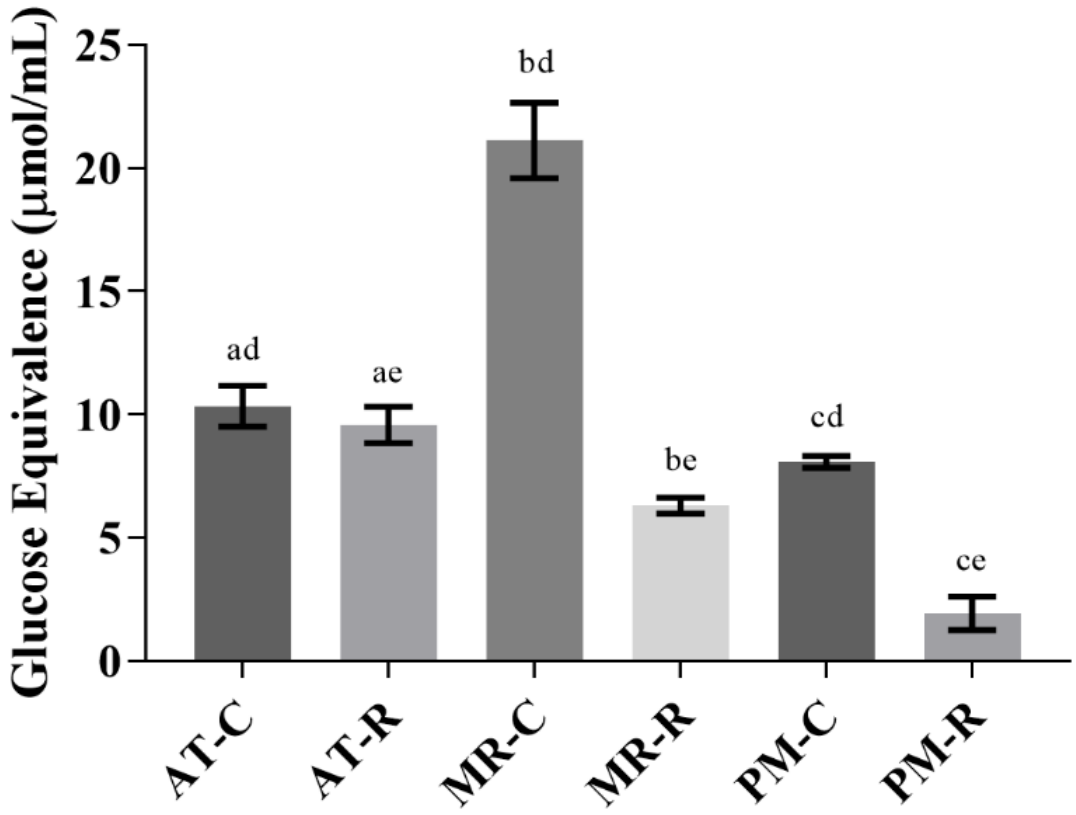

Figure 3-2 Glucose equivalence of raw potato slices for all cultivars: Atlantic (AT), Mountain Rose (MR) and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent the standard error of the mean (n=3). Different letters denote significant difference between cultivars (a, b, c) and storage (d, e).

3.3.2 Frying time optimization

In order to compare varieties and process conditions in potato chip manufacturing, it is necessary to standardize on either some measurement of chip quality (e.g., composition, flavor, texture) or a standard process time; this achieved specific aim 1. Previous work on potato chips has fried to final moisture content, marked by the stoppage of water bubbles in the oil, however; and a range of frying times was used [129,130]. Here, an attempt was made to find a standardize frying time that would achieve a similar

final moisture content and still produce acceptable potato chips for all samples. This approach has the advantage that the same time is allowed for chemical reactions but the conditions are not optimized for any particular potato.

The vacuum fryer was heated to 130 °C and vacuum was pulled to 25 in. Hg and slices were fried for 4 min. and 30 sec. based off of previous work (Figure 3-3) [65]. Using the conventional fryer heated to 170°C, a frying time of 2 minutes and 40 seconds (Figure 3-4) produced Atlantic, Mountain Rose, and Purple Majesty chips, without reconditioning, with a moisture content of < 2% moisture (Figure 3-5). Mountain Rose potatoes had a slightly higher moisture content of 2.7%. After 3 minutes of frying, the Mountain Rose still did not reach < 2% moisture. These data led to frying all conventional fried chips for 2 minutes and 40 seconds, regardless of final moisture content, so that they would be exposed to the high oil temperature for the same time.

While this work allows for comparisons of potato treatments, the processing of any individual potato could be further optimized by adjusting the process time and temperature.

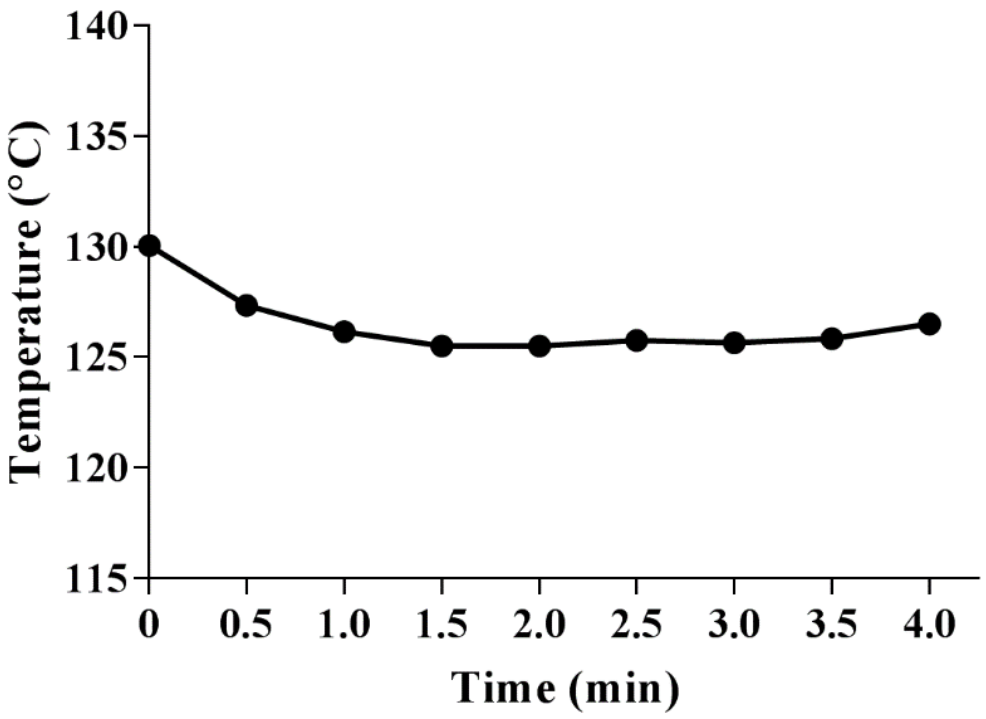

Figure 3-3 Temperature (°C) as a function of frying time of the vacuum fryer heated to 130 °C and fried with potato slices for 4 minutes. Data shown for Mountain Rose, without reconditioning, same trend was seen for all cultivars and storage treatments.

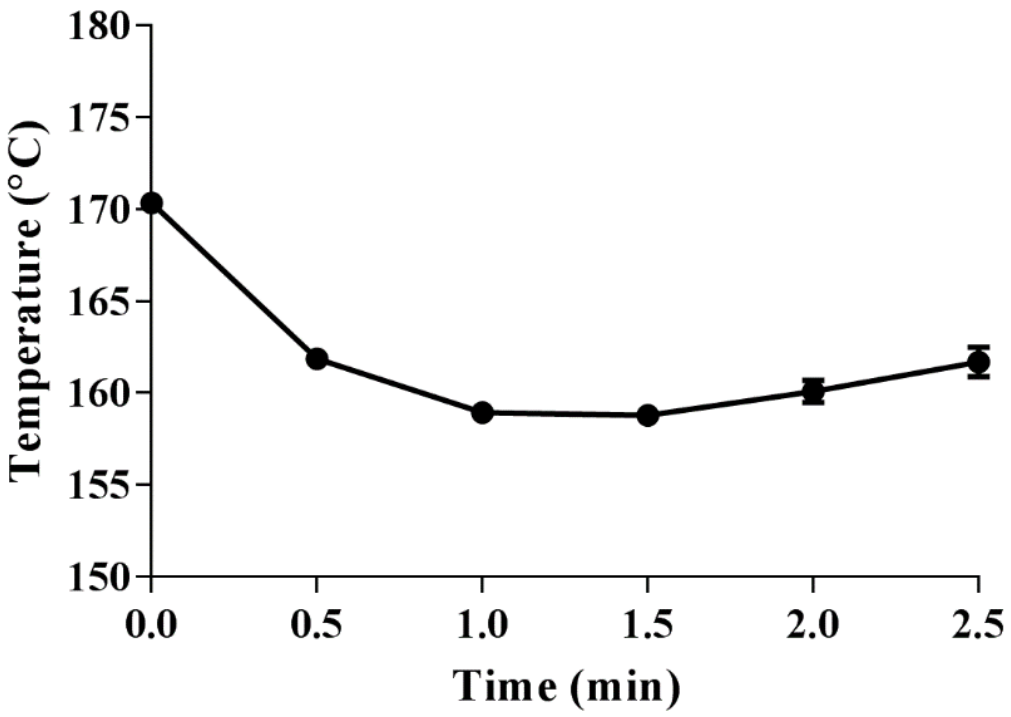

Figure 3-4 Temperature (°C) as a function of frying time of the conventional fryer heated to 170 °C and fried with potato slices for 2 minutes and 30 seconds. Data shown for Mountain Rose, without reconditioning, same trend was seen for all cultivars and storage treatments. Error bars represent standard error of the mean (n=20).

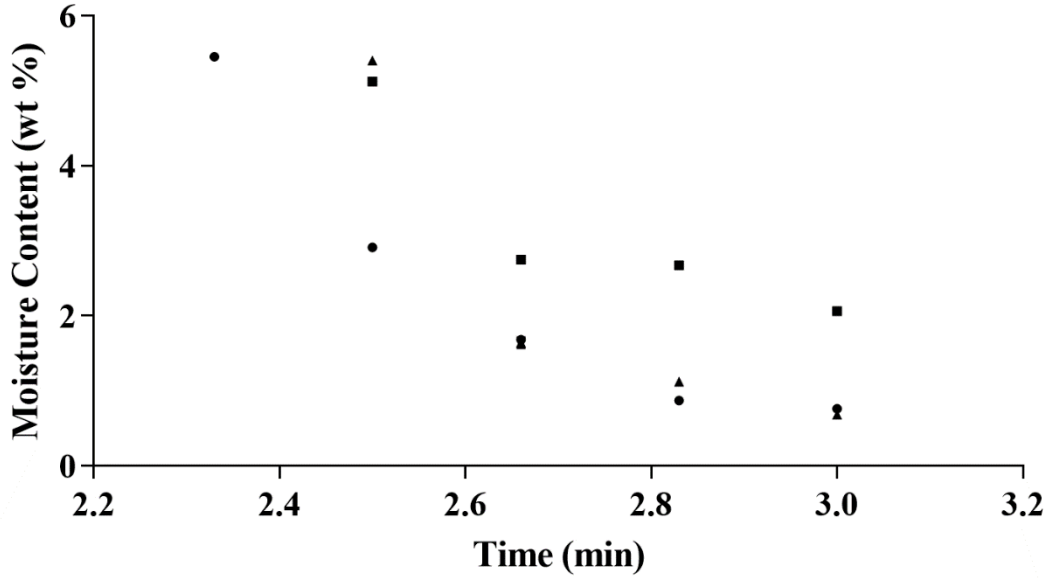

Figure 3-5 Moisture content, wet basis, as a function of frying time at 170 °C in the conventional fryer for each cultivar Atlantic (●), Mountain Rose (■), and Purple Majesty (▲), without reconditioning. Error bars at 2.66 min. represent the standard error of the mean (n=3). All other data points are single measurements.

3.3.3 Oil Quality

Vacuum frying proved to be an effective method for maintaining oil quality compared to conventional frying. Initial oil quality was at 7.5% TPM; in vacuum frying, this level was still maintained after 20 batches; comparatively, after 1 batch in the conventional fryer, oil quality degraded to 10% and continued to increase after 10 batches reaching 12.5% (Table 3-1). This allows for oil in a vacuum fryer to be utilized for longer periods of time before being replaced which decreases downtime of operation due to cooling and heating oil, costs of oil, and waste.

Table 3-1 Oil Quality represented as total polar molecules (%TPM) after vacuum frying (VF) or conventional frying (CF). Conventional fryer oil was changed after trial 10. Values taken for Mountain Rose cultivar without reconditioning. Similar trends were seen for all cultivars and storage treatments.

	% TPM	
Trial	CF	VF
Initial	7.5	7.5
2	10.0	7.5
3	10.0	7.5
4	10.0	7.5
5	10.5	7.5
6	10.5	7.5
7	11.0	7.5
8	11.5	7.5
9	12.0	7.5
10	12.5	7.5
11		7.5
12		7.5
13		7.5
14		7.5
15		7.5
16		7.5
17		7.5
18		7.5
19		7.5
20		7.5

3.3.4 Moisture Content

The characteristic crunch associated with potato chips is attributed to a chip with < 2% moisture. This produces a chip that is crunchy for consumers and also extends the shelf life of the product. In choosing to fry for a specific time, not to final moisture content, chips with different moisture contents were produced, this achieved specific aim 2.

Raw potatoes had 25-35% water (Table 3-2). There were significant differences found between cold storage and reconditioned potatoes indicating that some of the initial mass of the potato is lost due to respiration during the reconditioning process and subsequent sprouting. Frying reduced the amount of moisture in the chips (Table 3-2),

however, the different starting moistures of the raw slices produced chips with different final moisture contents when fried for the same time. Vacuum fried samples that were reconditioned had higher moisture contents than those fried without reconditioning.

No significant differences were found within the Atlantic cultivar between conventional fried with reconditioning and vacuum fried without reconditioning. Mountain Rose and Purple Majesty chips that were vacuum fried without reconditioning had significantly lower moisture contents than the chips that were conventional fried with reconditioning.

Data from moisture content indicated that the samples had a higher moisture content than the commercially available samples (Table 3-3). Purple Majesty chips that were vacuum fried without reconditioning produced a chip that similar to the commercial samples.

As stated above, the frying for a specific time allowed for comparisons between treatments and further optimizations are needed for each cultivar to produce a low moisture chip that is specific to that crop of potatoes.

Table 3-2 Moisture content, % wet basis, of raw, conventional fried (CF) and vacuum fried (VF) potato chips after cold storage (without reconditioning) or reconditioning. Values are expressed as mean ± standard deviation. Different letters denote significant difference between CF and VF samples (a, b) and raw slices (A, B, C).

Cultivar	Storage Treatment	Processing		
		Raw	CF	VF
Atlantic	Cold	35.34 ± 2.37^{A}	2.85 ± 1.92^{b}	5.05 ± 0.67^{ab}
	Reconditioned	26.82 ± 1.98^{BC}	6.16 ± 0.19^{ab}	6.34 ± 0.73^{ab}
Mountain Rose	Cold	36.10 ± 1.95^{A}	5.50 ± 1.35^{ab}	3.18 ± 1.04^{b}
	Reconditioned	32.17 ± 3.15^{ABC}	4.93 ± 1.22^{ab}	8.72 ± 0.66^{a}
Purple Majesty	Cold	33.82 ± 1.55^{BA}	7.42 ± 1.14^{a}	2.28 ± 0.42^{b}
	Reconditioned	25.54 ± 2.22^{C}	5.81 ± 2.06^{ab}	5.06 ± 0.56^{ab}

Table 3-3 Moisture content, % wet basis, of 4 commercially available potato chips. Values are expressed as mean ± standard deviation.

Brand	Moisture Content
White 1	1.71 ± 0.128
White 2	1.49 ± 0.113
Purple 1	2.52 ± 0.117
Purple 2	1.99 ± 0.104

3.3.5 Texture Analysis

The low moisture of a potato chip produces a crunch that could be further investigated utilizing texture analysis; this achieved specific aim 2. Since the frying time produced a higher moisture chip, it was necessary to study if this affected the texture of the final product.

Significant differences were found between the hardness of the chips of the Atlantic and Purple Majesty to Mountain Rose cultivars (Figure 3-6). No significant differences were found between storage and frying treatments for all cultivars. Mountain Rose chips that were vacuum fried after reconditioning had the highest moisture content and were the least hard. In all cultivars, samples that were vacuum fried without reconditioning were not statistically different to the more traditional conventional fried with reconditioning.

Atlantic samples were not significantly different in hardness to the two commercial samples. Purple Majesty samples were significantly different from one commercial sample which could be due to the thickness of the Purple 1 chips. The texture analysis signifies that vacuum fried chips directly from cold storage are comparable to traditionally processed chips and commercially available samples. The higher moisture

content in the chips would make them stale faster than commercial chips. However, the lack of textural differences indicates it could produce an acceptable chip.

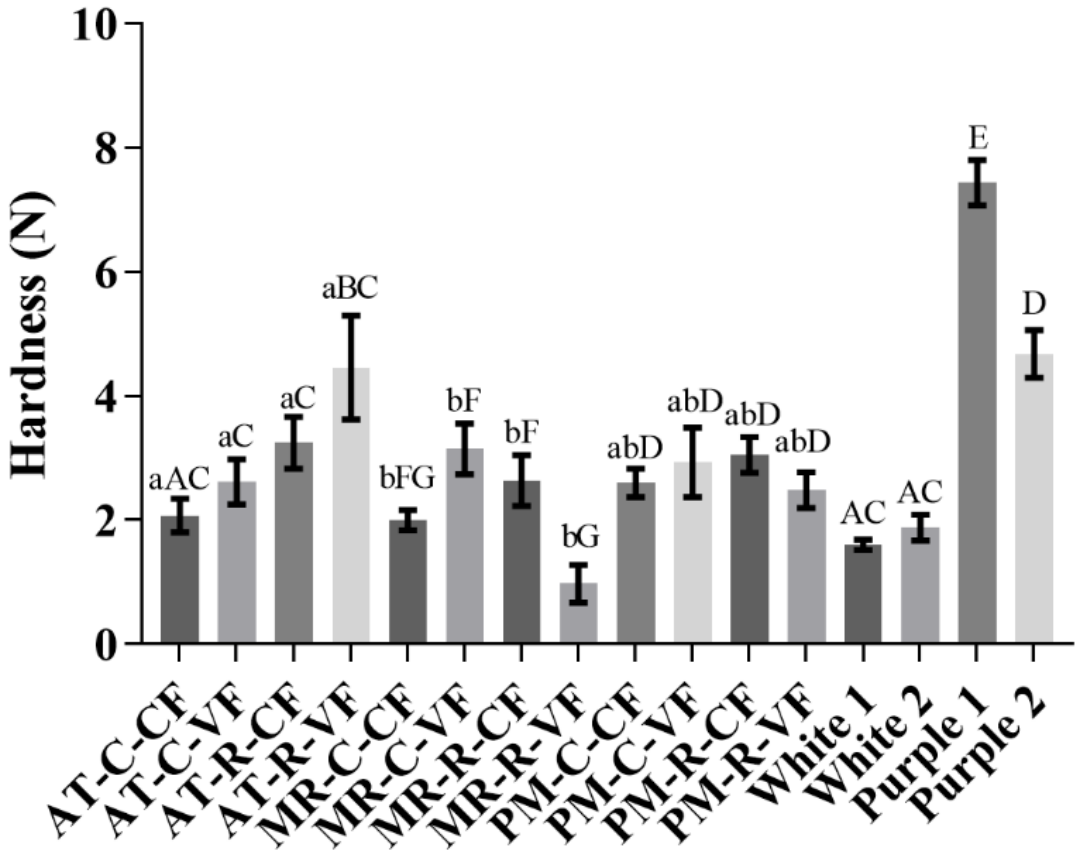

Figure 3-6 Hardness (N) of conventional fried (CF) and vacuum fried (VF) potato chips for all cultivars: Atlantic (AT), Mountain Rose (MR) and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R) and 4 commercial samples. Error bars represent standard error of the mean (n=3). Different letters denote significant difference between cultivars (a, b), no significant differences were found between storage or frying treatment. Different letters denote significant difference between commercial white chips to AT chips (A, B, C), between commercial purple chips to PM chips (D, E), and within MR chips (F,G).

3.3.6 Color Analysis

A major quality concern from the Maillard reaction in potato chip production is the excessive browning during frying which makes a chip unappealing to consumers. To avoid the excessive browning, potatoes are reconditioned before frying to reduce the amount of free reducing sugars. Investigation of the color of the potato chips, quantitative data could be utilized to achieve specific aim 2.

Variations in color are due to the uneven pigmentation of the color-fleshed potatoes (Figure 3-7). Purple- and red-fleshed raw potatoes have white centers and white rings around the outer layer causing more browning in those areas. In order to account for the variations in flesh pigmentation, the samples were ground prior to quantitative analysis. The ground potato chip samples were mixed in-between each measurement in order to get a more comprehensive value for the color of the chips.

Chips from the Atlantic cultivar had significantly different lightness (L*, Figure 3-8A) due to reconditioning and due to frying method. Chips from the Mountain Rose cultivar were not significantly different due to reconditioning but were due to frying method. Chips from the Purple Majesty cultivar had significant differences due to frying method but not due to reconditioning. Chips from the Atlantic cultivar (with and without reconditioning) were not significantly different from the commercial white samples if they were vacuum fried but they were different if they were conventional fried. Chips from the Purple Majesty cultivar (with and without reconditioning) that were vacuum fried and conventional fried with reconditioning were not significantly different from the commercial purple samples.

Chips from the Atlantic cultivar did not have significant red/greed differences (a^*, Figure 3-8B) due to reconditioning but were due to frying method. Chips from the Mountain Rose and Purple Majesty cultivars had significant differences due to reconditioning and due to frying method. Chips from the Atlantic cultivar (with and without reconditioning) were not significantly different from the commercial white samples if they were vacuum fried. Chips from the Purple Majesty cultivar (with and without reconditioning) that were vacuum fried were not significantly different from the commercial white samples. Chips from the Purple Majesty cultivar that were conventional fried were not significantly different from the commercial purple samples if they were reconditioned.

Chips from the Atlantic, Mountain Rose, and Purple Majesty cultivars had significant yellow/blue differences (b^*, Figure 3-8C) due to frying method but did not have significant differences due to reconditioning. Chips from the Atlantic cultivar (with and without reconditioning) did not have significant differences to the commercial white samples if they were vacuum fried. Chips from the Atlantic cultivar that were conventional fried did not have significant differences to the commercial white samples if they were fried without reconditioning. Chips from the Purple Majesty cultivar (with and without reconditioning) did not have significant differences to the commercial purple samples if they were vacuum fried; conventional fried (with and without reconditioning) had significant differences to the commercial purple samples.

Using Equation 3-1, ΔE values were calculated as the total change in color on frying (Figure 3-8D). Vacuum fried chips (with and without reconditioning) had lower ΔE than the conventionally fried samples, indicating a smaller change in color which

suggests that more of the natural pigments of the vacuum-fried samples are retained. The data suggest that vacuum frying retains more of the color of the raw slices and creates a chip closer in color to commercial samples than conventional frying, regardless of reconditioning or not.

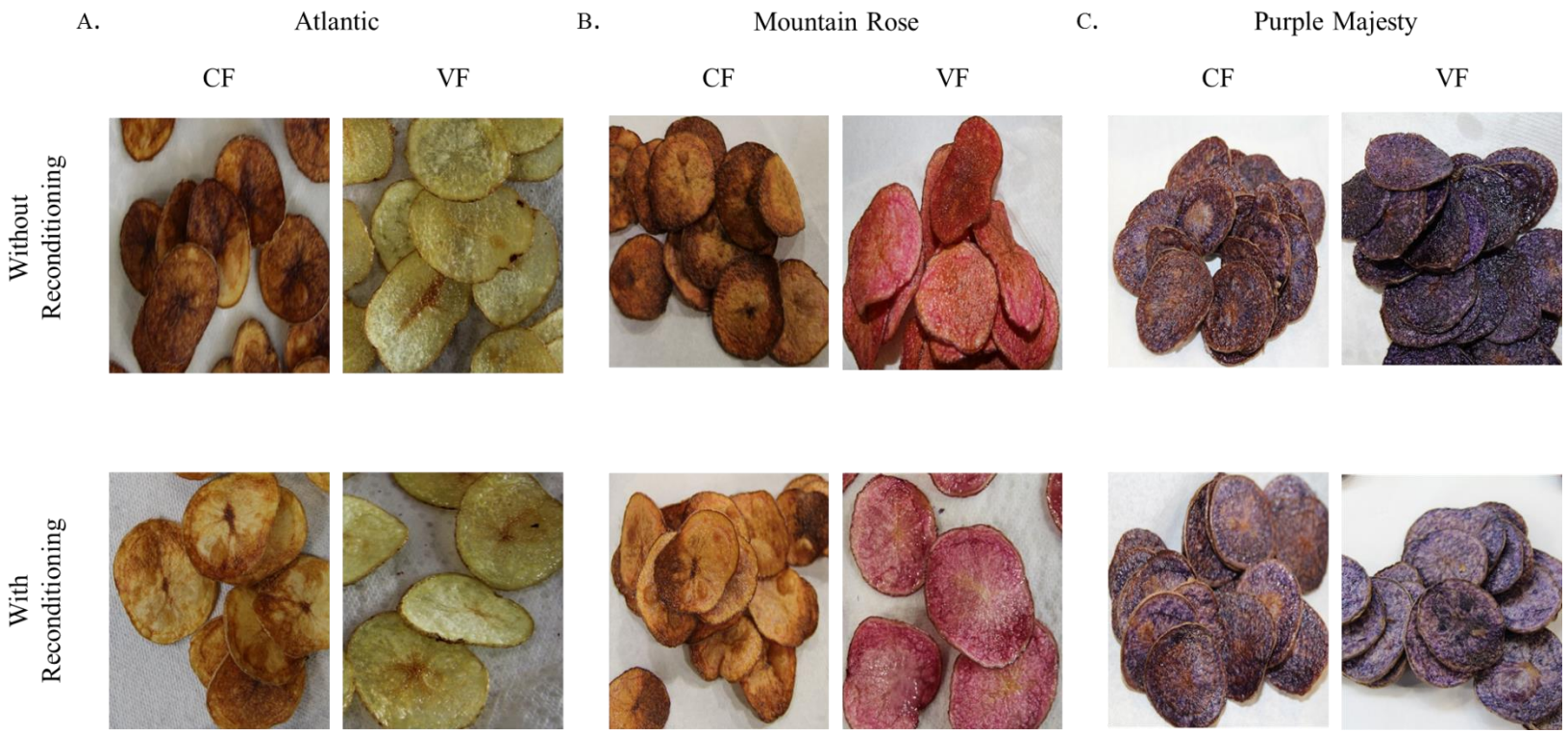

Figure 3-7 Photographs of three cultivars: (A) Atlantic, (B) Mountain Rose, and (C) Purple Majesty of conventional fried (CF) and vacuum fried (VF) chips with and without reconditioning.

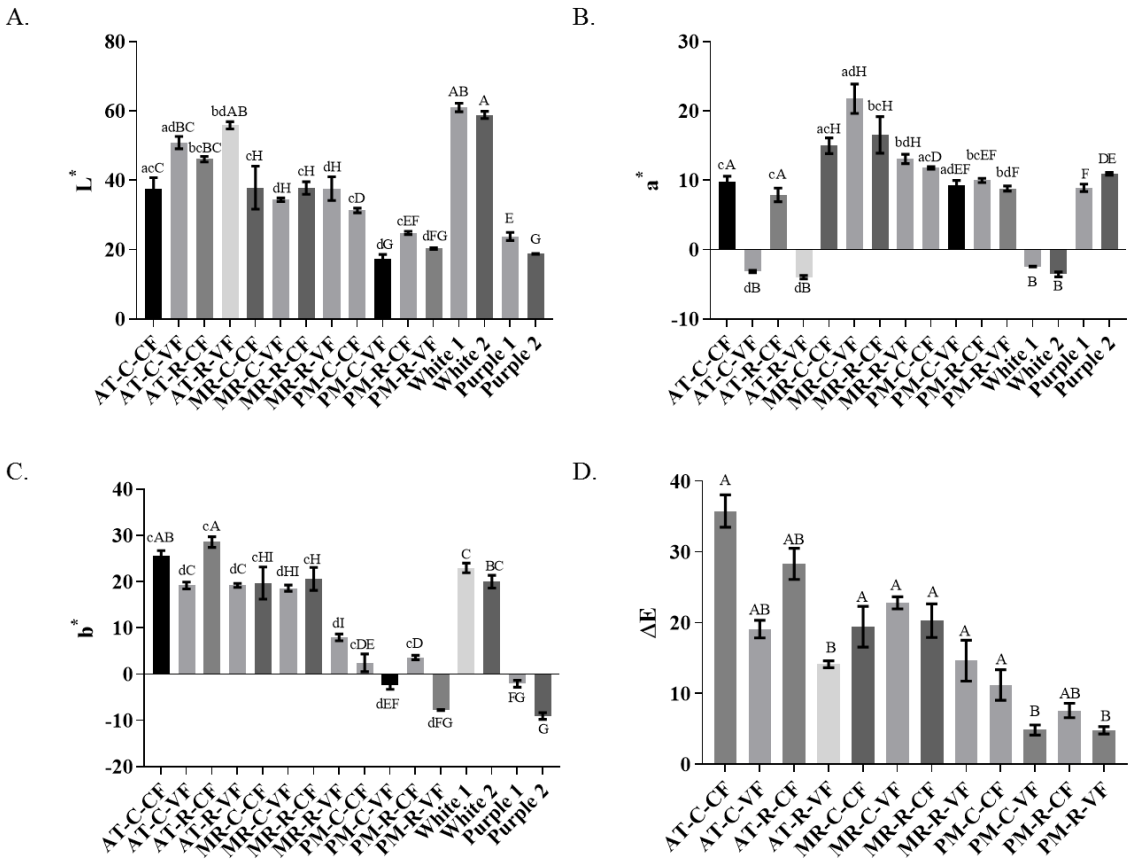

Figure 3-8 Color described using the International Commission on Illumination L^* a^* b^* color scale (A) L^* lightness, (B) a^* red/green, and (C) b^* yellow/ blue (D) ΔE color change, of conventional fried (CF) and vacuum fried (VF) potato chips for all cultivars: Atlantic (AT), Mountain Rose (MR), and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R) and 4 commercial samples. Error bars represent standard error of the mean (n=3). Different letters denote significant difference within the same cultivar between storage (a, b) and frying treatment (c, d). Different letters denote significant difference between commercial white chips and AT chips (A, B, C), commercial purple chips and PM chips (D, E, F, G), and within MR chips (H, I). Different letters denote significant differences in ΔE within the same cultivar (A, B).

3.3.7 Acrylamide

3.3.7.1 Standards and Calibration Curve

Reduction of acrylamide was studied to achieve specific aim 3. Utilization of GC-MS/MS allowed for the direct determination of acrylamide. The data indicates multiple reaction monitoring can effectively measure acrylamide at a retention time of 7.9 minutes (Figure 3-9). Multiple reaction monitoring allowed for the dual monitoring for acrylamide precursor ion 71 m/z and product ions of 55 and 44 m/z and the internal standard 2,3,3,d_3-acrylamide precursor ion 74 m/z and product ions 58 and 30 m/z (Figure 3-10). Quantification was done on product ions 55 and 58 m/z for acrylamide and d_3-acrylamide, respectively. Retention times for acrylamide and d_3-acrylamide were found to be 7.936 and 7.928 minutes, respectively. Through linear regression (Figure 3-11) of acrylamide standards, a correlation of 0.996 (Equation 3-4) indicates the calibration curve is useful in determining the concentration of unknown samples.

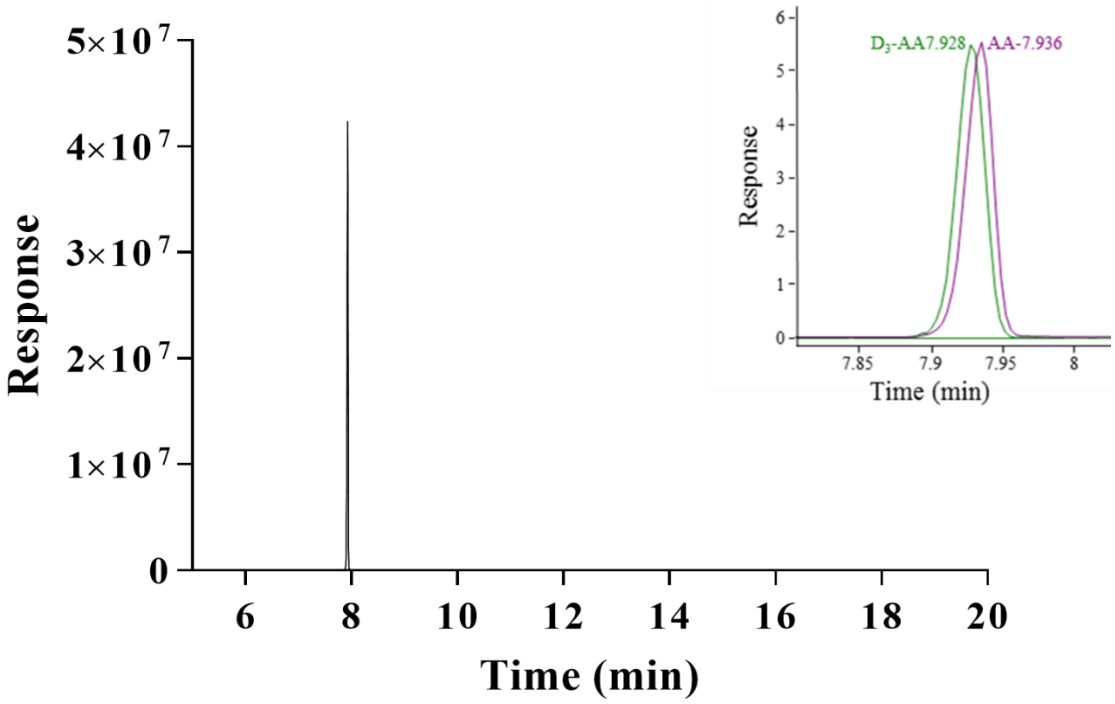

Figure 3-9 A typical GC MS/MS chromatogram of the peak of acrylamide in a standard of 20,000 ng/mL acrylamide in methanol with 10 ng/mL d_3-acrylamide internal standard. Peak corresponding to retention time 7.9 min was identified as acrylamide (AA) and d_3-acrylamide (D_3-AA) with precursor ions of m/z 71 and 74, respectively. Window insert is zoom for the 7.85-8.0 min region of the peaks of both transitions of D_3-AA with transition of 74 to 58 m/z and a retention time of 7.928 minutes and AA with transitions 71 to 55 m/z and a retention time of 7.936 minutes.

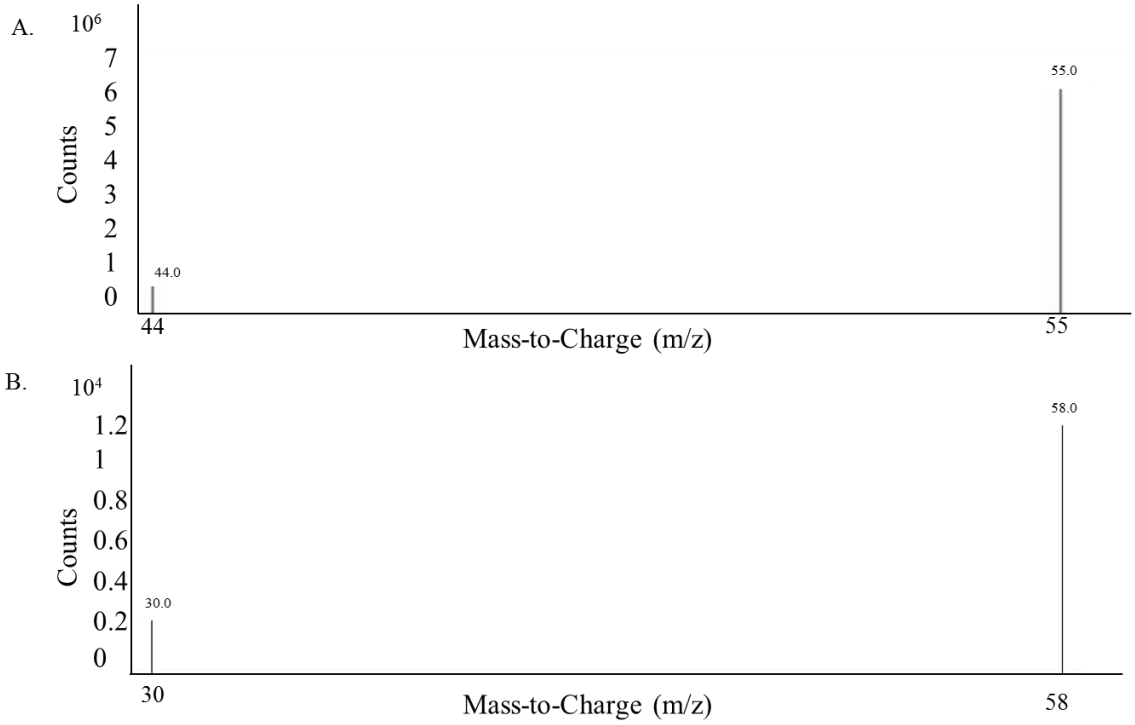

Figure 3-10 A typical GC MS/MS Total Ion Chromatogram (TIC) in Multiple Reaction Monitoring (MRM) of counts as a function of mass to charge ratio (m/z) of the fragmentation of acrylamide corresponding to the peak between 7.8 and 8 min. of a 20,000 ng/mL acrylamide with 10 ng/mL d_3-acrylamide internal standard. (A) Shows the precursor ion of acrylamide m/z 71 to product ions m/z 44 and 55 (B) shows the precursor ion of d_3-acrylamide m/z 74 to product ions m/z 30 and 58.

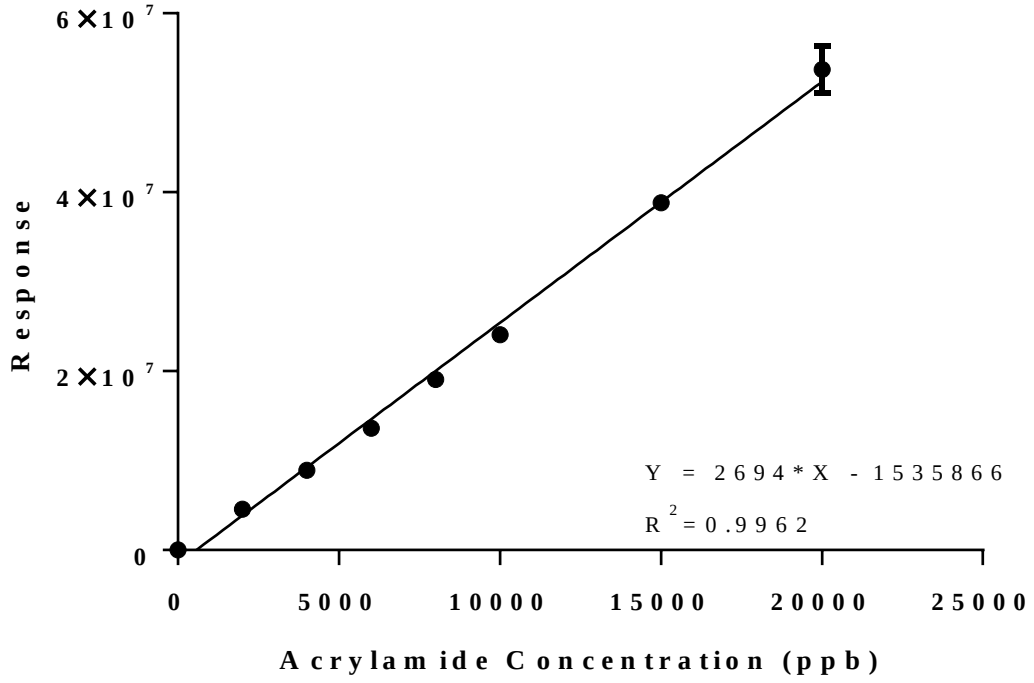

Figure 3-11 Calibration curve of GC-MS/MS response as a function of acrylamide concentration (ppb) in methanol. Error bars represent the standard deviation (n=3).

3.3.7.1.1 Limit of Detection and Quantification

Using Equation 3-5 the limit of detection (LOD) was determined to be 0.167 ppb. Using Equation 3-6 the limit of quantification (LOQ) was determined to be 0.560 ppb. All values found were outside of the LOD and LOQ. The LOD and LOQ further indicate that direct determination of acrylamide could be quantified reliably and accurately using GC-MS/MS.

3.3.7.2 Extraction Percent Recovery and Comparison to FDA

Using Equation 3-3, the percent recovery was found to be 101.54% showing that high recovery was seen through the methanol extraction method. Comparison of the

present extraction method and GC-MS/MS determination to the Food and Drug Administration's (FDA) published data on acrylamide values in food, using Equation 3-7, shows a 76.00%, 30.88%, and 34.26% difference between White 1, White 2, and Purple 1 samples (Table 3-4). These values indicate that the different extraction methods could influence the total acrylamide concentrations observed. The data could indicate the changes that have happened in the potato chip processing industry in the past 16 years since the initial push for acrylamide research was done by the FDA. Major potato chip companies have their own cultivars of potatoes which could change throughout the years [7]. There is little information about the specific cultivars that are owned by major companies, so there is potential that the present work was done on different samples with the same brand name.

Table 3-4 Acrylamide concentration (ppb) of acrylamide of present work (2017-2018) extracted in methanol and analyzed via GC-MS/MS compared to the Food and Drug Administration's (FDA) Survey data on acrylamide in food (2002-2006) using a different extraction method and analyzed via LC-MS. The same brand of commercial potato chips was analyzed for White 1, White 2, and Purple 1. Values are expressed as mean ± standard deviation.

Commercial Potato Chips	Acrylamide Concentration (ppb)	
	Present Work (2017-2018)	FDA (2002-2006)
White 1	780.26 ± 17.85	350.56 ± 83.11
White 2	771.33 ± 11.32	565 ± 97
Purple 1	1522.32 ± 78.93	1077
Purple 2	989.74 ± 18.08	NR

3.3.7.3 Sample Extraction

Utilizing one quadrupole of the triple quadrupole MS allowed for samples to be run and identified in a full scan mode (Figure 3-12). Through the NIST library search, the peak at 7.9 minutes was identified as acrylamide. Other compounds such as acetic acid, arginine, methyl palmate, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, methyl stearate, and 9,12-octadecadienoic acid (Z,Z)-, methyl ester have previously been identified as volatile found in plant products or in lipid-rich systems. The total ion chromatogram of the peak identified at 7.9 minutes (Figure 3-13) shows the typical ions of acrylamide of 71 m/z and 44 m/z.

Samples extracted in methanol for Atlantic (Figure 3-14), Mountain Rose (Figure 3-15), and Purple Majesty (Figure 3-16) indicate the same peak of acrylamide present in MRM mode at 7.9 minutes. The consistency in the starting and ending point of the acrylamide peak (Figure 3-17) further indicates the consistency of the methanol extraction and within the cultivar, storage, and processing treatments.

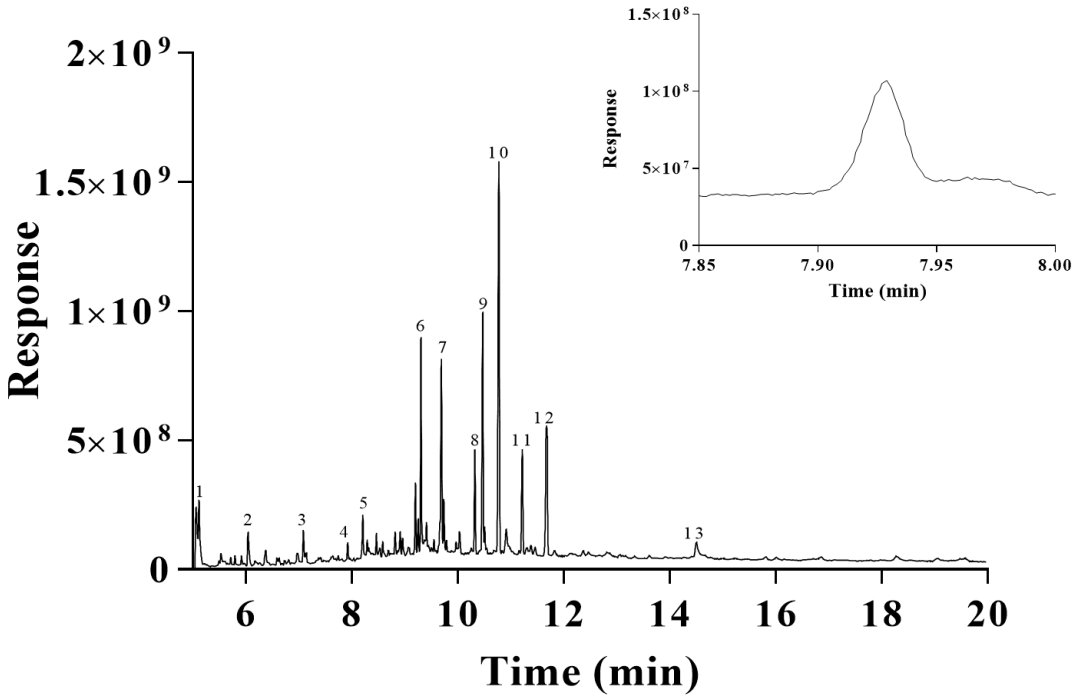

Figure 3-12 A typical GC-MS full scan chromatogram of acrylamide extracted in methanol of Atlantic chips that were conventional fried without reconditioning with 10 ng/mL d_3- acrylamide internal standard. Numbers indicate the following compounds: (1) acetic acid, (2) 4-cyclopentene-1,3-dione, (3) arginine, (4) acrylamide, (5) paromomycin, (6) methyl palmitate, (7) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, (8) methyl stearate, (9) 11-octadecenonic acid, methyl ester, (10) 9,12-octadecadienoic acid (Z,Z)-, methyl ester, (11) 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-, (12) DL-proline, 5-oxo-, methyl ester, and (13) Estra-1,2,5(10)-trien-17β-ol. Peaks 1, 3, 4, 6, 7, 8, 10 have been previously identified in plant and lipid-rich food products. Window insert is the peak corresponding to retention time 7.9 min (peak 4) and was identified as acrylamide and d_3-acrylamide with precursor ions of m/z 71 and 74, respectively.

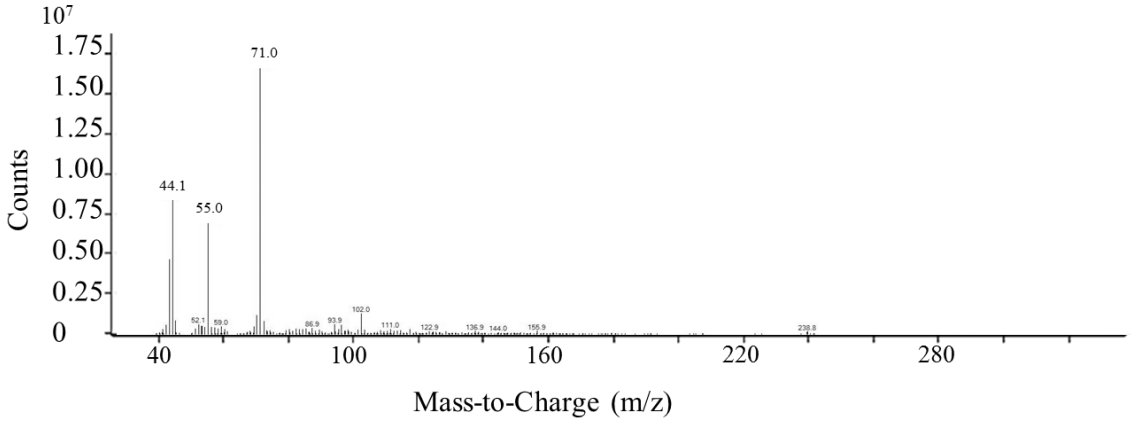

Figure 3-13 A typical GC-MS Total Ion Chromatogram (TIC) in full scan of counts as a function of mass to charge ratio (m/z) showing the ions present in the peak corresponding to 7.9 minutes of acrylamide extracted in methanol of Atlantic chips that were conventional fried without reconditioning.

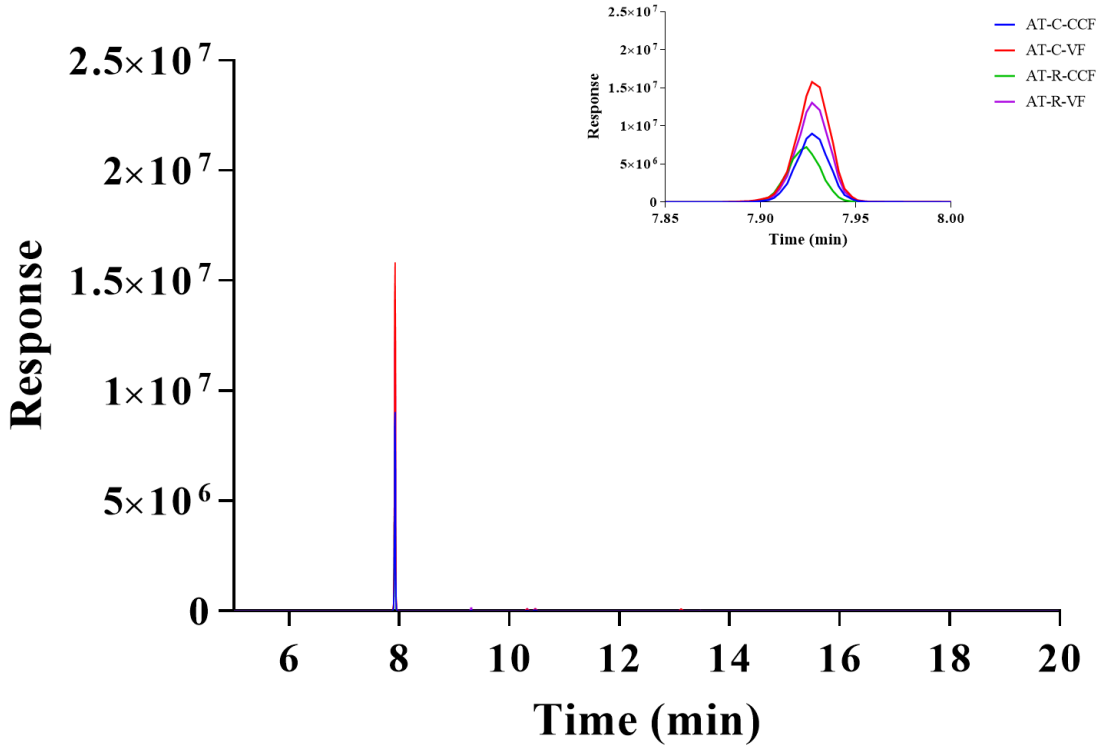

Figure 3-14 A typical GC MS/MS chromatogram of the peak of acrylamide in conventional fried (CF) and vacuum fried (VF) potato chips for the Atlantic (AT) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R). Window insert is zoom for the 7.85-8 min region and was identified as acrylamide and d_3-acrylamide with precursor ions of m/z 71 and 74, respectively.

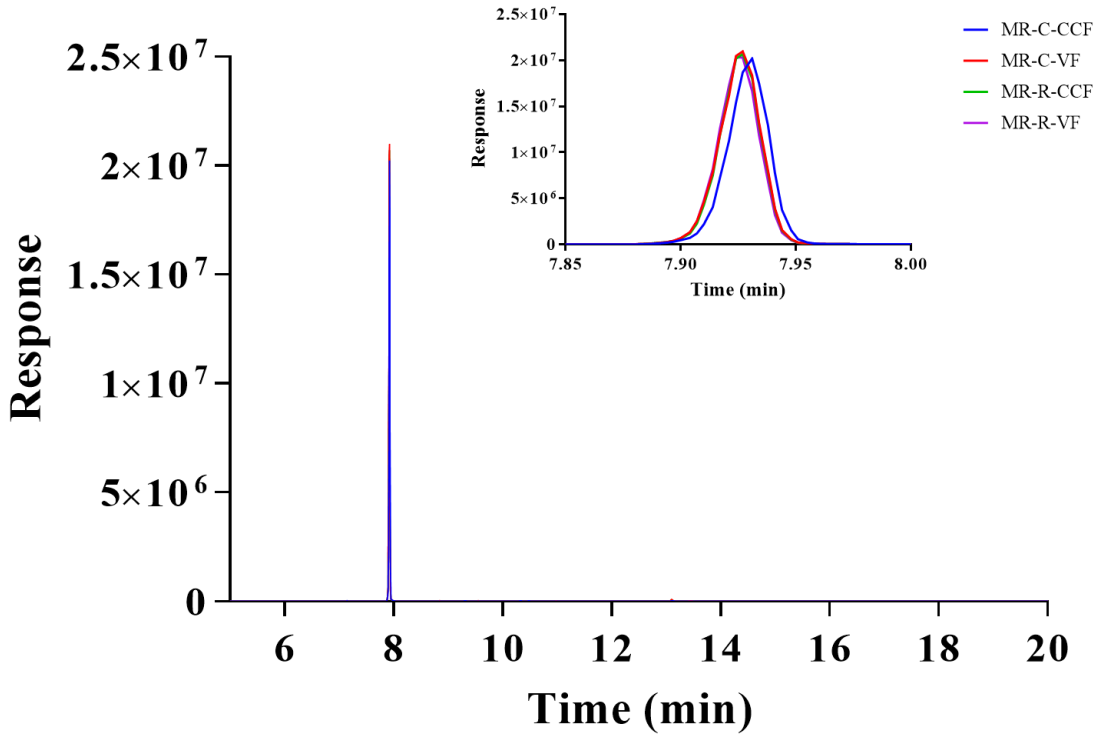

Figure 3-15 A typical GC MS/MS chromatogram of the peak of acrylamide in conventional fried (CF) and vacuum fried (VF) potato chips for the Mountain Rose (MR) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R). Window insert is zoom for the 7.85-8 min region and was identified as acrylamide and d_3-acrylamide with precursor ions of m/z 71 and 74, respectively.

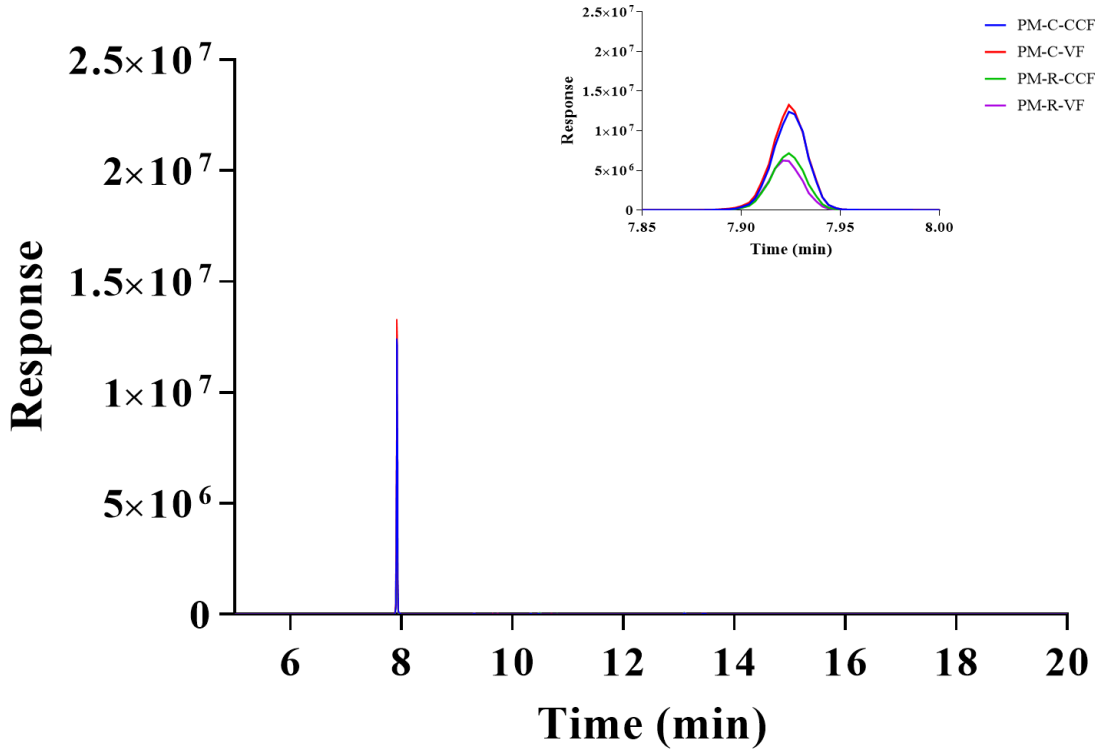

Figure 3-16 A typical GC MS/MS chromatogram of the peak of acrylamide in conventional fried (CF) and vacuum fried (VF) potato chips for the Purple Majesty (PM) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R). Window insert is zoom for the 7.85-8 min region and was identified as acrylamide and d_3-acrylamide with precursor ions of m/z 71 and 74, respectively.

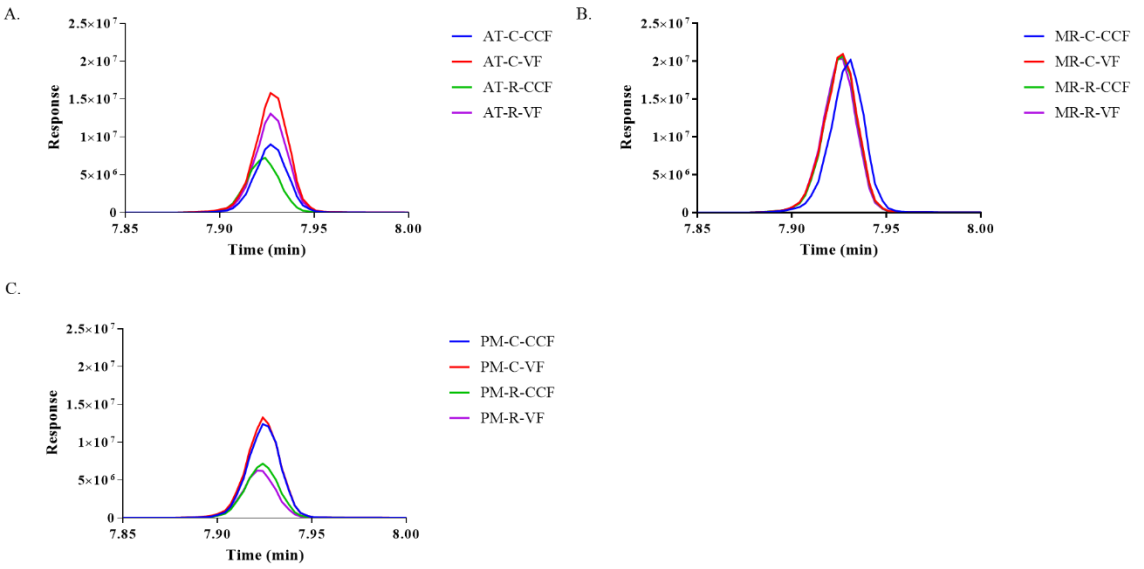

Figure 3-17 GC MS/MS chromatogram of the peak of acrylamide for the 7.85-8 min region in conventional fried (CF) and vacuum fried (VF) potato chips for (A) Atlantic (AT), (B) Mountain Rose (MR), and (C) Purple Majesty (PM) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R). Peaks were identified as acrylamide and d_3-acrylamide with precursor ions of m/z 71 and 74, respectively.

3.3.7.4 Analysis of Treatments

Concentrations of acrylamide were found in the range of 1,500-8,000 ppb in Atlantic, Mountain Rose, and Purple Majesty chips (Figure 3-18) and 700-1,500 ppb for commercial chips (Table 3-4). Chips from the Mountain Rose cultivar were significantly higher than the Atlantic and Purple Majesty cultivars. Reconditioning significantly decreased the amount of acrylamide compared to samples fried without reconditioning. There was no significant difference between conventional fried chips and vacuum fried chips within the same cultivar and with the same storage treatment. In the Atlantic and Mountain Rose potato chips fried without reconditioning, as well as in the Mountain Rose and Purple Majesty potato chips fried after reconditioning, the level of acrylamide was higher in the vacuum fried chips compared to the conventional fried chips.

It was hypothesized that vacuum frying at a lower temperature would reduce the formation of acrylamide. However, the vacuum fried chips were fried at 130 °C for 4.5 minutes. Acrylamide forms rapidly at 120 °C, this, combined with the longer time in the vacuum fryer, allowed for the formation of acrylamide. Lower levels of acrylamide in the conventional fried samples could be due to the high temperature for extended periods of time as previous studies have found acrylamide can degrade at high temperatures [100]. Reconditioning reduced the levels of acrylamide because of the change in glucose content, a significant correlation was found between the raw potato glucose and fried potato acrylamide concentrations. Variation in glucose concentration accounts for 61% of the acrylamide concentration indicating that reconditioning is still necessary to control for acrylamide levels.

Atlantic chips were all significantly higher than the commercial white samples. Purple Majesty chips that were conventional fried without reconditioning were significantly higher than the Purple 2 sample, all other Purple Majesty chips and purple commercial samples were not significantly different.

There are several possible reasons for differences in samples prepared for this study and the commercial samples. Pretreatments before and during frying could reduce the formation of acrylamide and browning in the commercial samples. Large potato chip companies have patents for their own cultivars of potatoes which could have lower levels of reducing sugars which would reduce the overall formation of acrylamide [7]. Commercial samples are fried in a continuous process where samples marketed as “kettle cooked” are batch fried and are a closer frying method to the conventional fried samples

[131]. Commercial samples are blanched prior to frying or are fried in oil with emulsifiers which create barriers around the potato chip [132,133].

The FDA does not regulate acrylamide in food, and it is suggested that Americans consume a healthy diet consistent with the 2015-2020 Dietary Guidelines for Americans, which is low in fried foods and snack foods, such as potato chips, to reduce exposure to acrylamide [135]. In 2017, the EFSA voted in favor of implementing mandatory measures to reduce the presence of acrylamide in foods, however, the final vote is expected to occur in spring 2018, where benchmark levels of concern will be set [136]. Research has suggested the tolerable daily intake for neurotoxicity and carcinogenesis to be 40 μg/kg-day and 2.6 μg/kg-day, respectively [86].

In 2016, the average annual consumption of potato chips and shoestrings per American was 17.8 pounds/person/year [7]. At the highest levels of acrylamide found in the data, Americans would consume 0.065 g of acrylamide annually due to potato chips.

The data indicate that reconditioning is still an effective method for reducing the levels of acrylamide in fried potato products and that vacuum frying without reconditioning produced similar levels of acrylamide to the more traditional conventional fried with reconditioning.

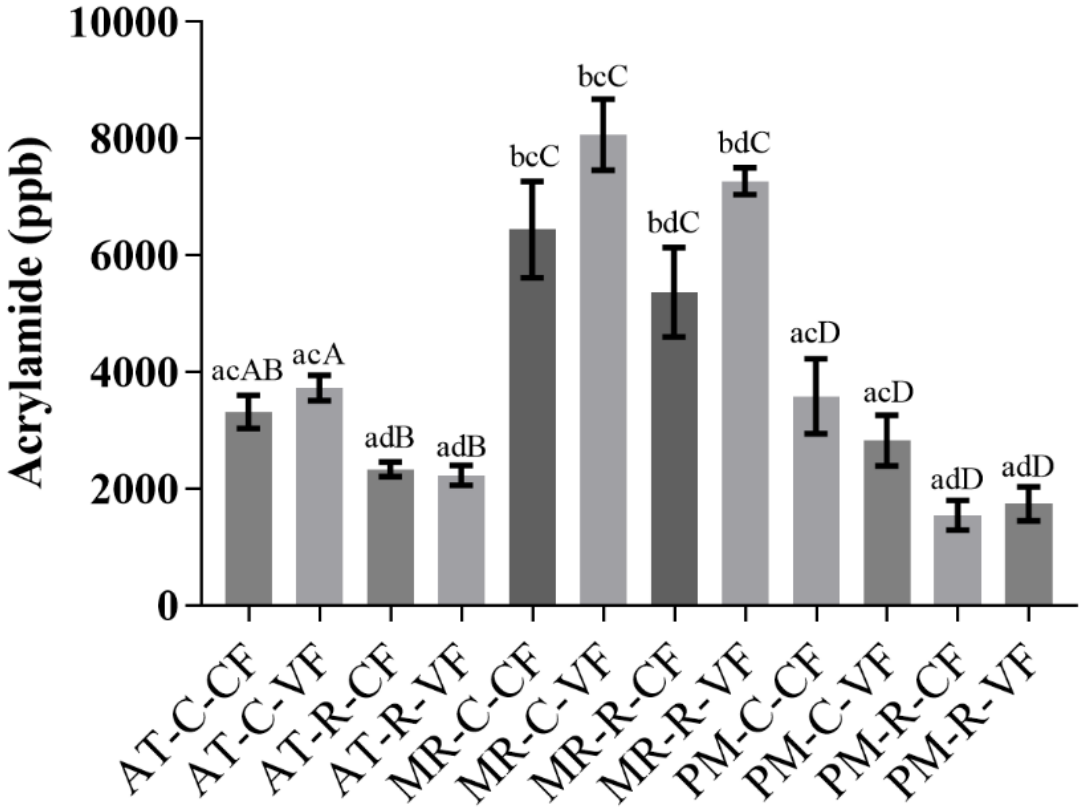

Figure 3-18 Acrylamide concentration (ppb) of conventional fried (CF) and vacuum fried (VF) potato chips for all cultivars: Atlantic (AT), Mountain Rose (MR) and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent standard error of the mean (n=3). Different letters denote significant difference between cultivars (a, b) and storage treatment (c, d), there was no significant difference between frying treatments. Different letters denote significant difference within AT chips (A, B), within MR chips (C) and within PM chips (D).

3.3.8 5-Hydroxymethylfurfural

3.3.8.1 Standards and Calibration curve

Reduction of HMF was studied to achieve specific aim 3. Utilization of GC-MS and derivatization of HMF with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) in the GC injection port allowed for quantification of HMF in potato chips. The data indicate that derivatization in the injection port can reduce sample preparation time and can measure derivatized HMF at a retention time of 5.7 minutes (Figure 3-19). Single ion monitoring (SIM) allowed for monitoring of the ions 183 and 109 m/z which are typical of HMF with a trimethylsilyl (TMS) group (Figure 3-20). Through linear regression (Figure 3-21) of HMF standards, a correlation of 0.96 (Equation 3-8) indicates the calibration curve is useful in determining the concentration of unknown samples.

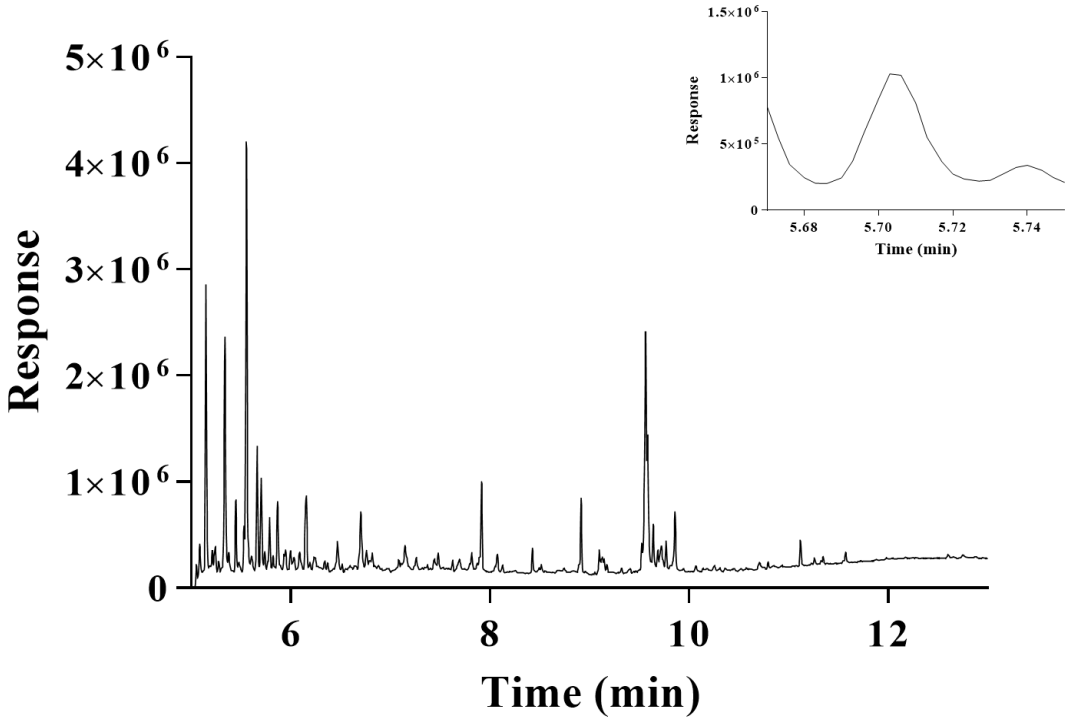

Figure 3-19 A typical GC MS Selected Ion Monitoring (SIM) chromatogram of the peak of 5-(Hydroxymethyl)furfural (HMF) derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) in a standard of 100 ng/mL HMF in ethyl acetate. Peak corresponding to retention time 5.7 min was identified as the derivatized sample. Window insert is zoom for the 5.67-5.75 min region

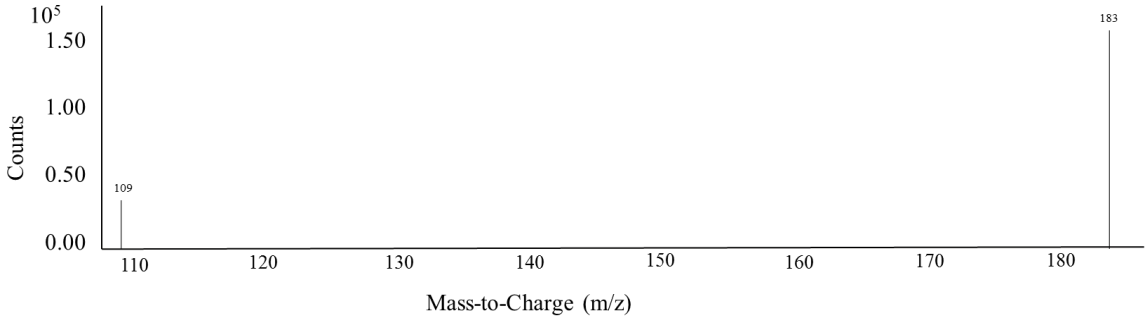

Figure 3-20 A typical GC MS Total Ion Chromatogram (TIC) in Selected Ion Monitoring (SIM) of counts as a function of mass to charge ratio (m/z) of the ions of 5-(Hydroxymethyl)furfural (HMF) derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) in the peak between 5.67 and 5.75 min. of a standard of 100 ng/mL HMF.

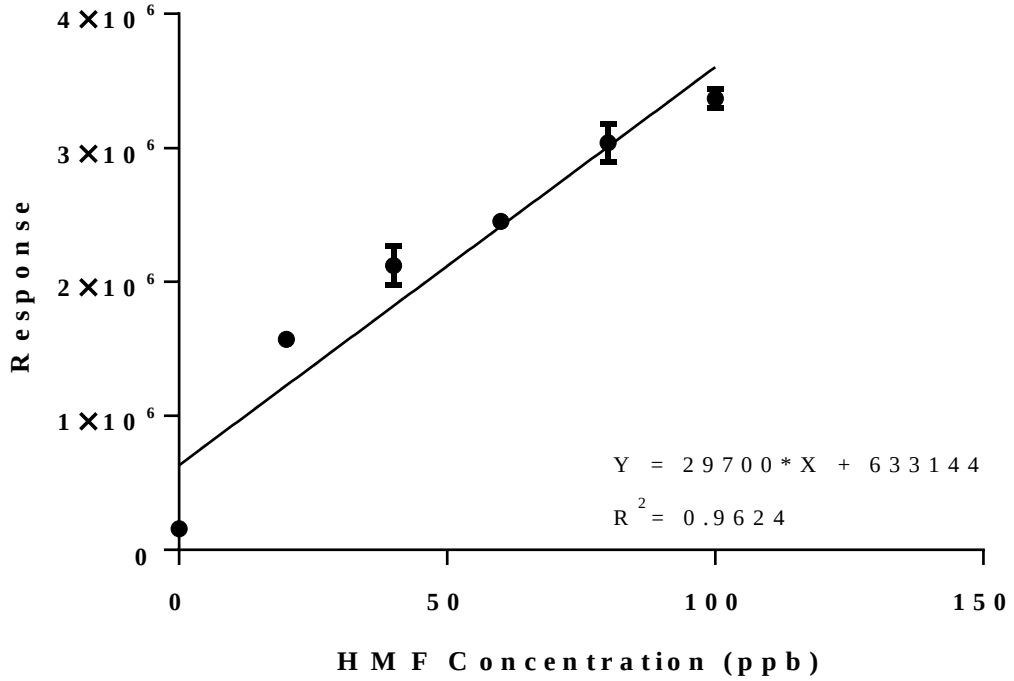

Figure 3-21 Calibration curve of GC-MS response as a function of 5-Hydroxymethylfurfural concentration (ppb) in ethyl acetate. Error bars represent the standard deviation (n=3).

3.3.8.1.1 Limit of Detection and Quantification

Using Equation 3-5, the LOD was determined to be 0.0067 ppb. Using Equation 3-6, the LOQ was determined to be 0.022 ppb. All values found were outside the LOD and LOQ. Determination of HMF via derivatization with MSTFA allowed for quantification using GC-MS.

3.3.8.2 Extraction Percent Recovery

Using Equation 3-3, the recovery was found to be 83.3% which shows there is a good recovery of HMF through ethyl acetate extraction.

3.3.8.3 Sample Extraction

By utilizing one quadrupole of the triple quadrupole MS, samples could be run and identified in full scan mode (Figure 3-22). Through the NIST library search, the peak at 5.7 minutes was identified as 5-trimethylsiloxymethylfurfural. Other predominant peaks were identified as TMS derivatives due to excess MSTFA. The total ion chromatogram of the peak identified at 5.7 minutes (Figure 3-23) shows the ions of HMF of 183 and 109 m/z. The strong peak of other TMS derivatives shows the need for optimizing the amount of derivatizing agents or others to be studied for their potential to derivatize HMF more strongly.

Samples extracted in ethyl acetate for Atlantic (Figure 3-242), Mountain Rose (Figure 3-25), and Purple Majesty (Figure 3-26) cultivars indicate the same peak for HMF present in SIM mode at 5.7 minutes. The consistency in peaks between cultivars (Figure 3-27) indicates the consistency in the ethyl acetate extraction between cultivar, storage, and frying treatments.

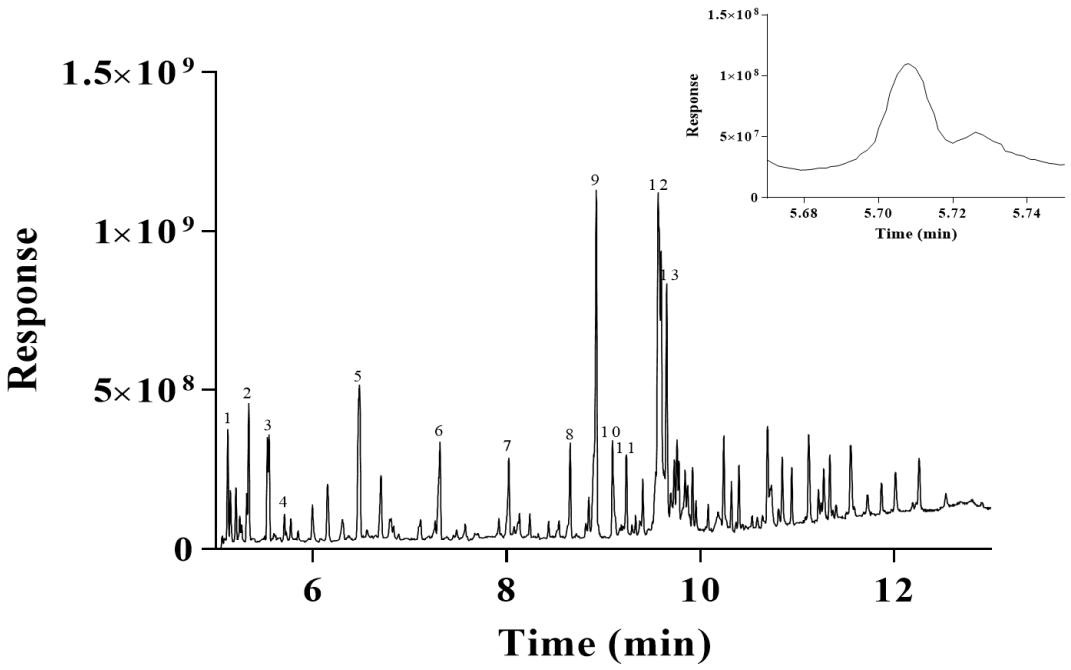

Figure 3-22 A typical GC-MS full scan chromatogram of 5-(Hydroxymethyl)furfural (HMF) extracted in ethyl acetate and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) in chips from the Atlantic cultivar that were conventional fried without reconditioning. Numbers indicate the following compounds: (1) Ethanolamine, 3TMS derivative, (2) Octanoic acid, TMS derivative, (3) glycerol, 3TMS derivative, (4) 5-trimethylsiloxymethylfurfural, (5) pentasiloxane, dodecamethyl- (6) 5-octadecenal (7) myristic acid, TMS derivative (8) heptasiloxane, hexadecamethyl- (9)Palmitic acid, TMS derivative (10) 9,12,14 octadecatrienoic acid, 1,3-dihydroxypropyl ester (Z,Z,Z)-(11) 9,12-octadecadienoic acid (Z,Z)- TMS derivative (12) Oleic Acid, (Z,), TMS derivative (13) Petroselinic acid, TMS derivative. Window insert is the peak corresponding to retention time 5.7 min (peak 4) and was identified as derivatized HMF, 5-trimethylsiloxymethylfurfural, with the ions m/z 183 and 109.

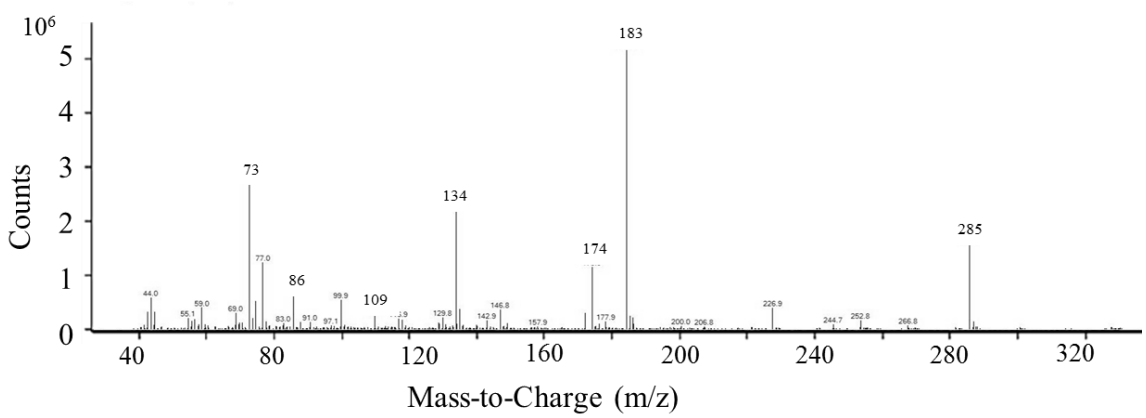

Figure 3-23 A typical GC-MS Total Ion Chromatogram (TIC) in full scan of counts as a function of mass to charge ratio (m/z) showing the ions present in the peak corresponding to 5.7 min. of 5-(Hydroxymethyl)furfural (HMF) extracted in ethyl acetate of chips from the Atlantic cultivar that were conventional fried without reconditioning and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA).

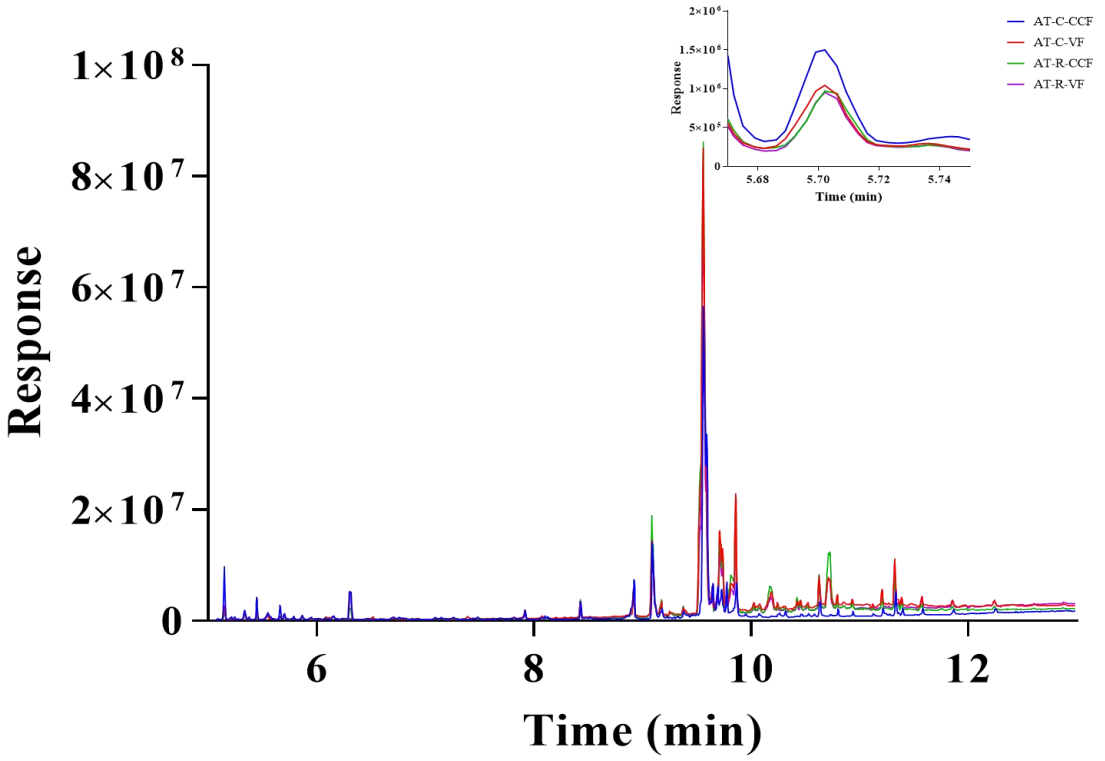

Figure 3-24 A typical GC MS chromatogram of the peak of 5-(Hydroxymethyl)furfural (HMF) extracted in ethyl acetate in conventional fried (CF) and vacuum fried (VF) potato chips for the Atlantic (AT) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R) and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). Window insert is zoom for the 5.67-5.75 min region.

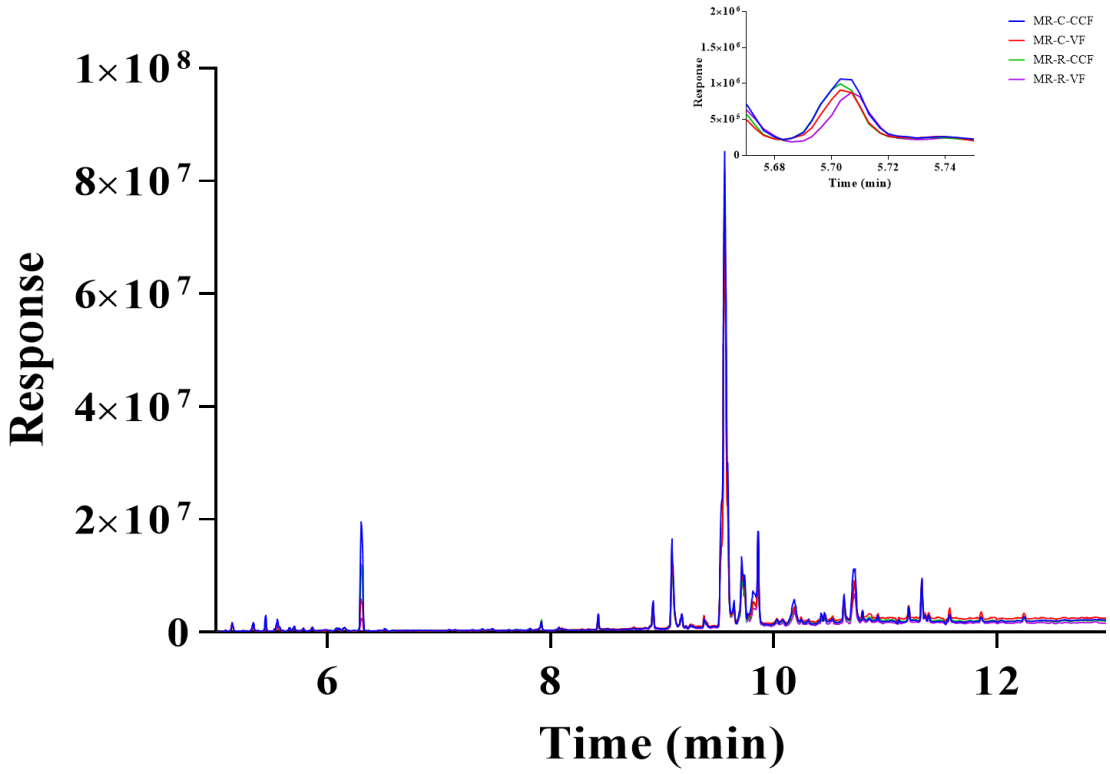

Figure 3-25: A typical GC MS chromatogram of the peak of 5-(Hydroxymethyl)furfural (HMF) extracted in ethyl acetate in conventional fried (CF) and vacuum fried (VF) potato chips for the Mountain Rose (MR) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R) and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). Window insert is zoom for the 5.67-5.75 min region.

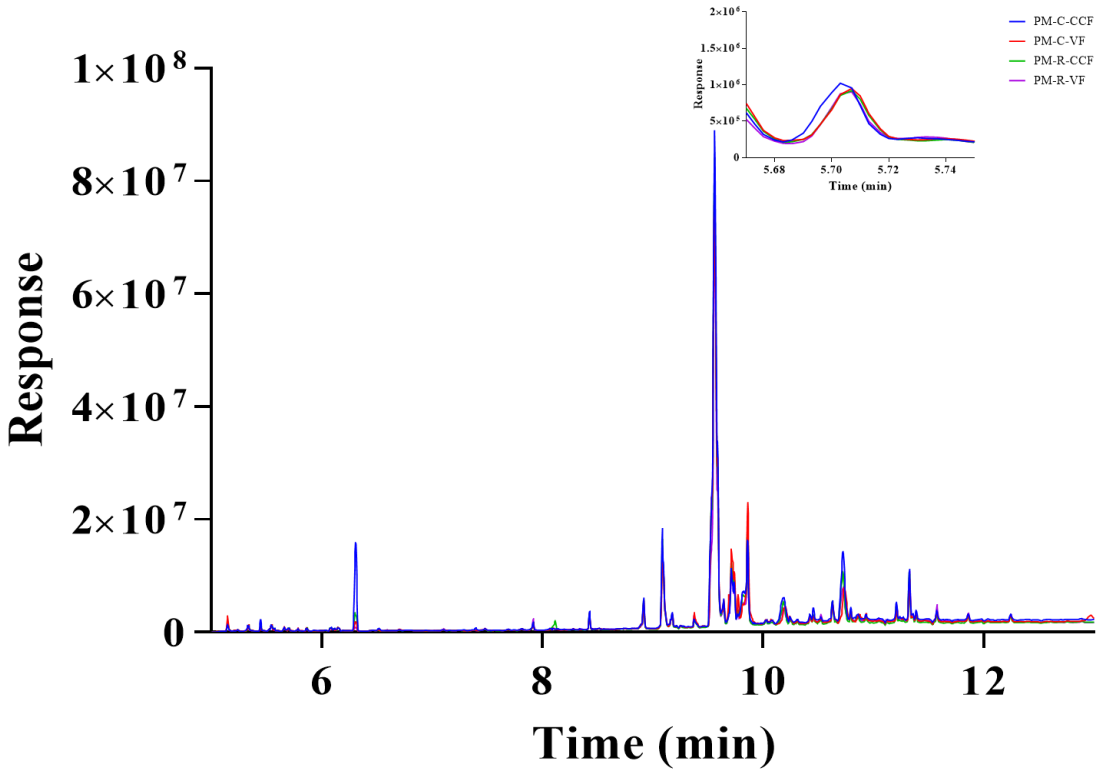

Figure 3-26 A typical GC MS chromatogram of the peak of 5-(Hydroxymethyl)furfural (HMF) extracted in ethyl acetate in conventional fried (CF) and vacuum fried (VF) potato chips for the Mountain Rose (MR) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R) and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). Window insert is zoom for the 5.67-5.75 min region.

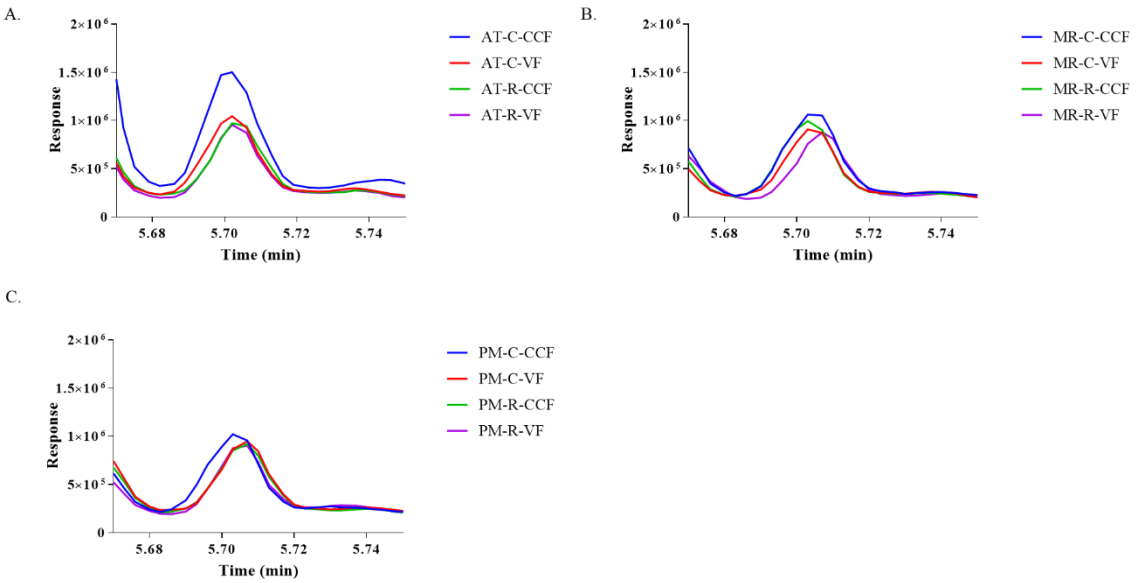

Figure 3-27 GC MS chromatogram of the peak of 5-(Hydroxymethyl)furfural for the 5.67-5.75 min region in conventional fried (CF) and vacuum fried (VF) potato chips for (A) Atlantic (AT),(B) Mountain Rose (MR), and (C) Purple Majesty (PM) cultivar directly from cold storage, without reconditioning, (C) and after reconditioning (R) and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA).

3.3.8.4 Analysis of Treatments

HMF can be formed from fructose or glucose via the Maillard reaction and forms rapidly at temperatures above 150 °C [119]. HMF formation is highly dependent on the ratio of amino acids to reducing sugars which would result in different cultivars having different amounts [119].

Concentrations of HMF were found in the range of 20-125 ppb in processed samples (Figure 3-28) and 21-24 ppb in commercial samples (Table 3-5). Chips from the Mountain Rose cultivar had significantly higher levels of HMF than Atlantic and Purple Majesty. Significant differences due to storage and frying treatment were found in all cultivars. Atlantic, Mountain Rose, and Purple Majesty cultivars conventional fried without reconditioning had the highest amount of HMF within the cultivar. Atlantic,

Mountain Rose, and Purple Majesty cultivars conventional fried after reconditioning had the next highest amount of HMF within the cultivar. The data shows that the conventional frying at 170 °C allows for the formation of HMF in potato chips both with and without reconditioning. Chips from the Mountain Rose cultivar were significantly higher in HMF suggesting that the cultivars studied had different ratios of amino acids to reducing sugars. A significant correlation was found between the raw potato glucose and fried potato HMF concentrations. Variation in glucose concentration accounts for 50% of the HMF concentration. Raw potatoes without reconditioning from the Mountain Rose cultivar had the highest levels of glucose as well as the highest levels of HMF, suggesting that reconditioning is useful for controlling levels of HMF regardless of amino acid composition.

Table 3-5 Concentrations of 5-hydroxymethylfurfural (HMF) in 4 commercially available potato chips. Values are expressed as mean ± standard error of the mean.

Brand	HMF (ppb)
White 1	21.14 ± 0.299
White 2	21.63 ± 1.475
Purple 1	22.85 ± 0.601
Purple 2	23.92 ± 1.540

Atlantic chips that were conventional fried were significantly different from the commercial white samples if they were not reconditioned but were not significantly different after reconditioning. Atlantic chips (with and without reconditioning) had no significant differences to the commercial white samples if they were vacuum fried. Purple Majesty chips (with and without reconditioning) were not significantly different from the commercial purple samples regardless of frying method. Ultimately, the amount

of HMF was not statistically different between vacuum fried samples without reconditioning and conventional fried samples with reconditioning.

The EFSA reports the level of concern for HMF in food as 540 μg/ person/ day [114]. In 2016, the average annual consumption of potato chips and shoestrings per American was 17.8 pounds/person/year [7]. At the highest levels of HMF found in the data, Americans would have consumed 0.1009 mg of HMF annually due to potato chips, i.e., 5 times less than the daily level of concern. While there were significant differences between treatments, the concentrations of HMF found in potato chips were below 125 ppb. Other studies have found concentrations of HMF in cereal and honey to be 46,800 and 38,300 ppb, respectively [120]. The average American consumes 14.2 and 1.3 pounds/person/year of cereal and honey, respectively [137,138]. The total intake of cereal is similar to that of potato chips, and with higher levels of HMF, reduction in cereal products should be prioritized.

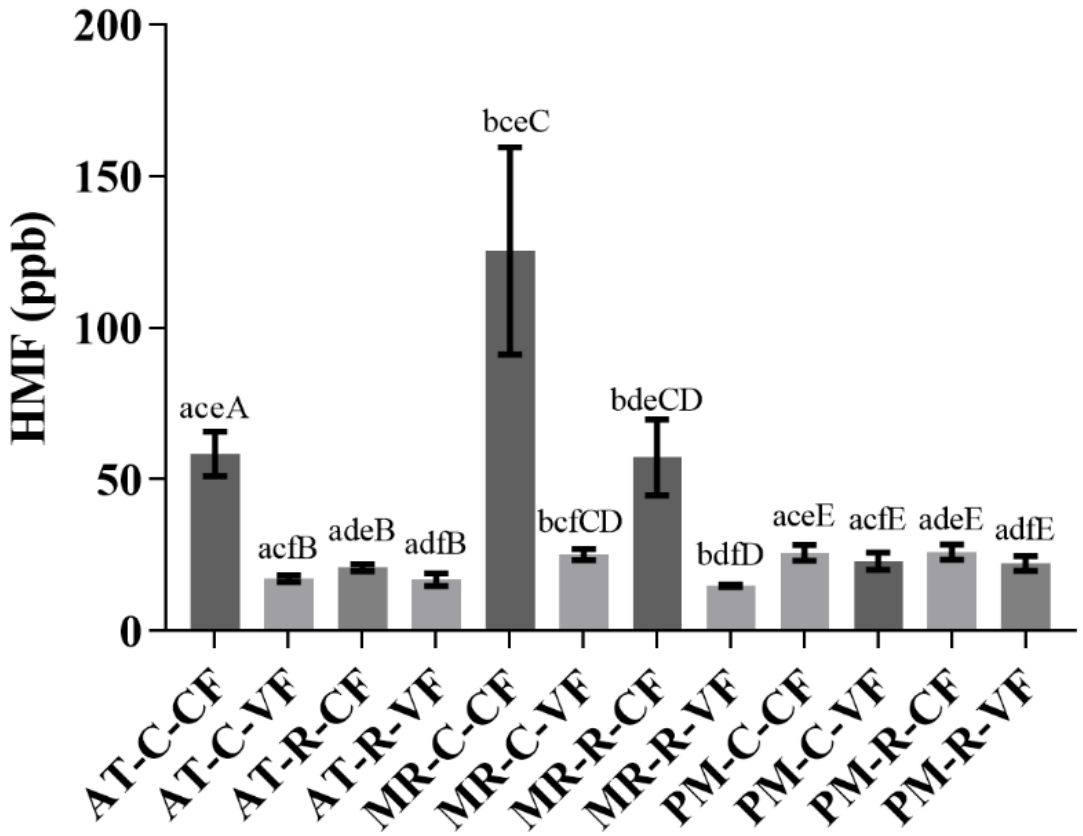

Figure 3-28 5-(Hydroxymethyl)furfural (HMF) concentrations (ppb) of conventional fried (CF) and vacuum fried (VF) potato chips for all cultivars: Atlantic (AT), Mountain Rose (MR) and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent standard error of the mean (n=3). Different letters denote significant difference between cultivars (a, b), storage (c, d), and frying treatment (e, f). Different letters denote significant difference within AT chips (A, B), within MR chips (C, D), and within PM chips (E).

3.3.9 Advanced Glycation End-products

3.3.9.1 Calibration Curve

The concentrations of CML in potato chips were determined using a CML ELISA kit. The test response was linearly related (R^2=0.978) to the logarithm of the concentration of CML (Equation 3-9) allowing their determination in unknown samples (Figure 3-29).

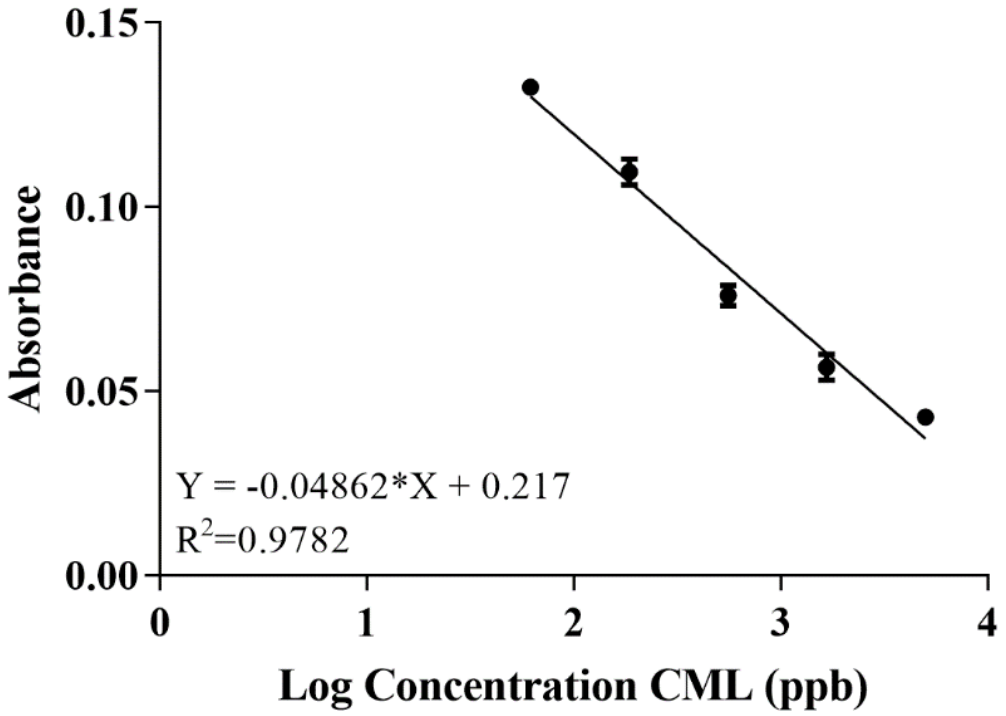

Figure 3-29 Calibration curve of absorbance as a function of the log concentration of N^{ε}-carboxymethyl lysine (CML). Error bars represent the standard deviation (n=3).

3.3.9.2 Analysis of Treatments

Reduction of CML was studied to achieve specific aim 3. CML is the most widely studied AGE in food products due to its stability and ability to be measured analytically [103]. Concentrations of CML are typically reported as arbitrary units of AGEs per gram of food [102,104,106,134]. In this work, I expressed CML concentrations as their logarithms to reduce skewedness and achieve normality in the data.

Concentrations of CML were found between 20-30 ppb in all samples (Figure 3-30, Table 3-6). Significant differences were found between the Atlantic and Mountain Rose cultivars to Purple Majesty. Significant differences were found due to storage and frying treatments. There were no significant differences found between Atlantic samples and commercial white samples. A significant difference was seen between Purple Majesty Vacuum fried after reconditioning and all other prepared and commercial samples. Purple Majesty vacuum fried after reconditioning had the highest concentration of CML with all other treatments having similar levels. No significant differences were found within the chips of the same cultivar for levels of CML.

Table 3-6 Concentrations of N^{ε}-carboxymethyl lysine (CML) in 4 commercially available potato chips. Values are expressed as mean ± standard error of the mean.

Brand	CML (ppb)
White 1	23.22 ± 0.013
White 2	23.16 ± 0.007
Purple 1	22.28 ± 0.001
Purple 2	22.77 ± 0.007

One brand of commercial chips (White 1) was analyzed by Goldberg *et al.* (2004) and found 28,000 U/g of AGE; as stated previously, the only known similarity is the

brand name [104]. The same study found an increase of AGEs in potato products after being baked at higher temperatures [104].

The cultivar of potato influences the starting concentration of lysine, which would influence the formation of CML; also, processing of the same cultivar delivers different final concentrations of lysine. White-fleshed potatoes have 0.09 g lysine/ 100 g potato when raw and 0.11 g lysine/ 100 g potato when baked [140]. Red-flesh potatoes have 0.10 g lysine/ 100 g potato when raw and 0.012 g lysine/ 100 g potato when baked [140]. Potatoes that were processed into French fries or shoestrings were found to have 0.13-0.19 g lysine/ 100g potato depending on their initial processing and reheating method. Controlling for the level of lysine in cultivars could reduce the occurrence of CML. Soaking raw potato slices in a lysine solution has been shown to reduce acrylamide formation in potato, therefore, there is a need to study how the reduction of one Maillard reaction product influences the formation of others [136].

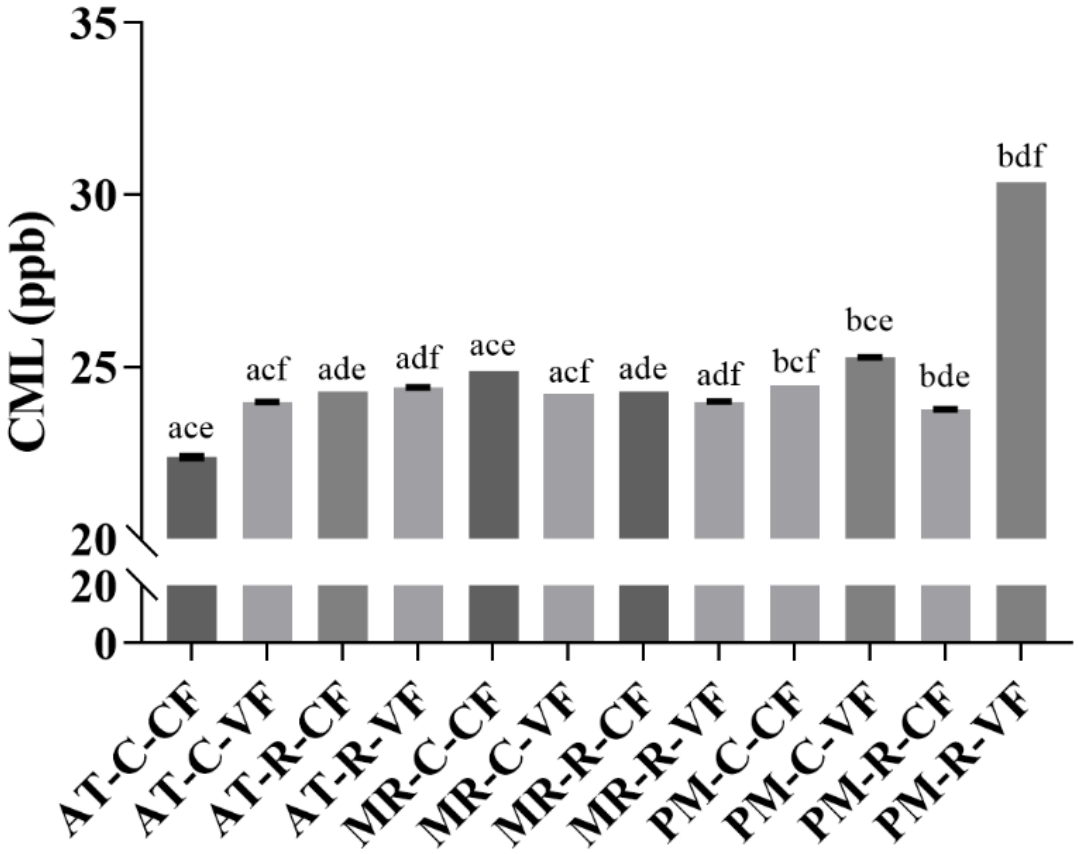

Figure 3-30 Concentration of N^{ε}-carboxymethyl lysine (CML) of conventional fried (CF) and vacuum fried (VF) potato chips for all cultivars: Atlantic (AT), Mountain Rose (MR), and Purple Majesty (PM), directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent standard error of the mean (n=3). Different letters denote significant difference between cultivars (a, b), storage (c, d), and frying treatment (e, f). No significant differences were found within the cultivars.

3.3.10 Sensory Analysis

One of the aims of this study, specific aim 4, was to determine if vacuum frying at lower temperatures could be used to avoid the need for reconditioning in the manufacturing of good-quality potato chips. To test this consumer perception of vacuum fried chips produced directly after removing the potatoes from cold storage, without

reconditioning, was compared to the more standard conventional fried chips after reconditioning and to another alternative of conventional frying without reconditioning.

Participants ranked the color (Figure 3-31A) of vacuum fried chips prepared from non-reconditioned potatoes as just-about-right significantly more than the conventional fried samples from non-reconditioned potatoes. Participants ranked the crispiness (Figure 3-31B) of vacuum fried chips prepared from non-reconditioned potatoes as just-about-right significantly more than the conventional fried samples with and without reconditioning. Participants ranked the flavor (Figure 3-31C) of the samples as significantly different for the Mountain Rose vacuum fried without reconditioning, and conventional fried with reconditioning as the conventional fried with reconditioning did not have a strong enough flavor. All other rankings of flavor were not significant. However, they tended to be rated with not enough flavor. Participants ranked the oiliness (Figure 3-31D) as significantly too much oil in the Purple Majesty vacuum fried without reconditioning compared to the conventional fried chips with and without reconditioning. Chips from the Mountain Rose cultivar had no significant differences but were all ranked highly with too much oil.

Consumer liking of color, in Purple Majesty and Mountain Rose, was highest in the vacuum fried without reconditioning and lowest in conventional fried without reconditioning (Figure 3-32A). Overall liking of the potato chips was significantly the highest in the vacuum fried without reconditioning (Figure 3-32B). Participants ranked their most preferred and least preferred potato chip sample within the cultivar giving a total rank sum where a lower score is the more preferred sample (Figure 3-33). In both cultivars, the vacuum fried chip without reconditioning was ranked significantly higher

more often, and conventional fried without reconditioning was ranked significantly lower more often.

Vacuum frying tended to retain more of the natural color of the potato chips and produced a chip closer to the commercially available samples (Figure 3-5). Participants ranking the color as just-about-right confirms the possibility of vacuum frying without reconditioning to produce a commercially similar chip. The liking of color on the 9-point hedonic scale further exemplifies that vacuum frying produced a chip that was more visually appealing. The challenges of frying to a specific moisture content are further proven as the chips that were not reconditioned were not crispy enough. Frying times and temperatures must be optimized for each batch of tubers to account for the internal characteristics of the raw tuber that changes throughout storage. The chips being ranked not quite strong enough in flavor could be due to the fact that the chips were served unsalted which is not typically the way consumers eat potato chips so the consumers do not have a frame of reference of what an unsalted potato chips should be in flavor. Industrial potato chips processing uses air to remove some of the oil from the chips. Therefore, the samples being ranked as slightly too much oil could be attributed to utilizing paper towels to blot off the excess oil.

Overall, participants were not accustomed to colored potato chips, 80% of participants had purchased white potato chips in the last month where only 20% had purchased colored potato chips. Color-fleshed potato chips retain the phenolic compounds found in the raw tuber, which have been shown to act as antioxidants with anti-carcinogenic and anti-inflammatory properties [51]. After informing participants of the potential health benefits of colored potato chips, 73.95% of participants were likely to

purchase colored chips over traditional white chips and were willing to spend $1.10 for a single serving of colored potato chips, a 120% increase over a single serving of white potato chips.

The vacuum fried chips were liked more than the conventional fried samples showing it is an attractive alternative to this type of batch frying. However, participants' preference ranking shows that conventional frying without reconditioning is not enough to fix the problems associated with reconditioning. Participants were willing to purchase colored chips over white chips, and pay more for them, showing that there is a market for this type of health-benefitting snack food.

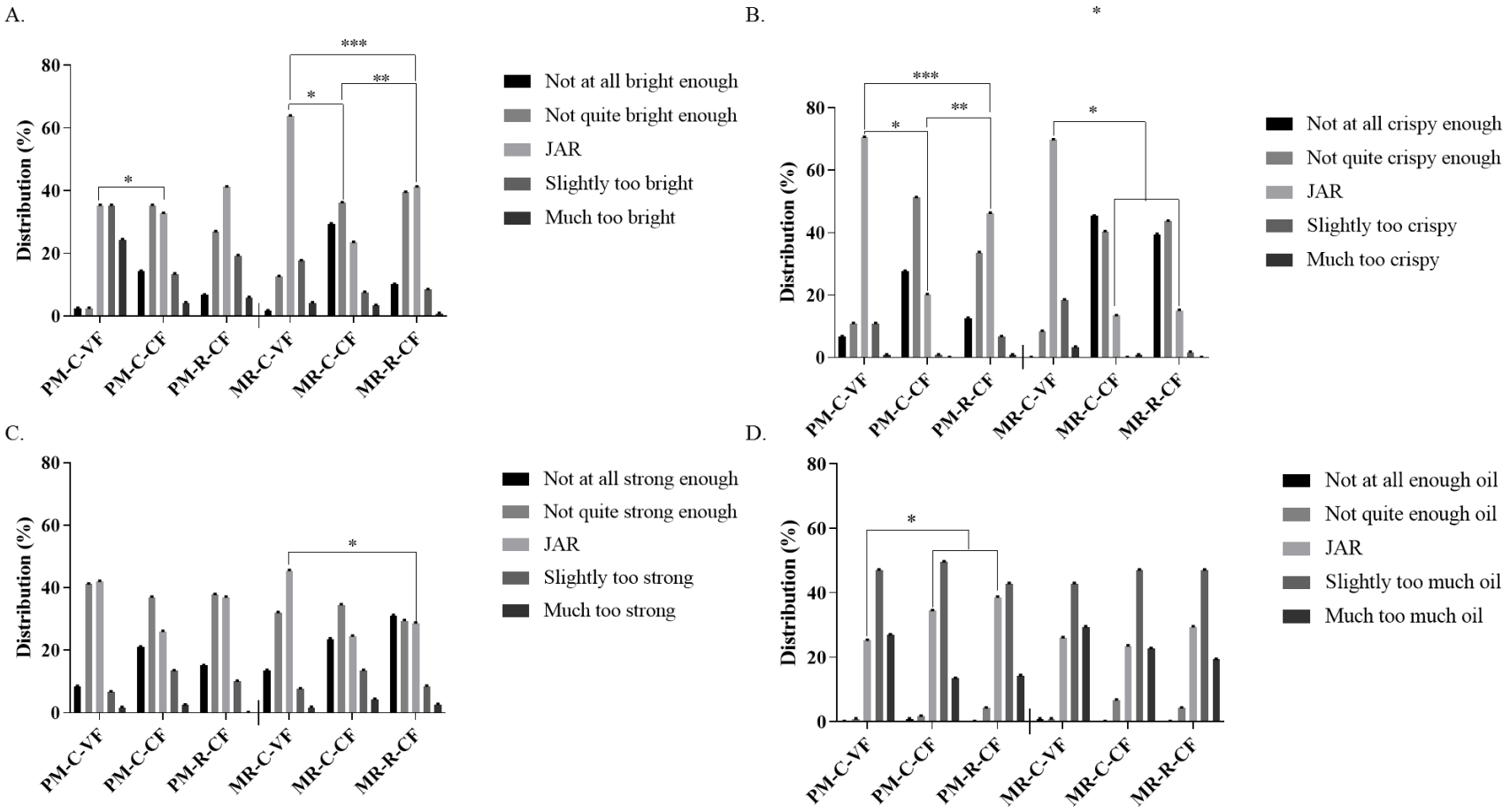

Figure 3-31 Distribution of participants just about right (JAR) rating for potato chips for (A) color (B) crispiness, (C) flavor, and (D) oiliness of conventional fried (CF) and vacuum fried (VF) potato chips for Mountain Rose (MR) and Purple Majesty (PM) cultivars, directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent Tukey's honest significant difference (n=119). Asterisks (*) denotes significant differences within Purple Majesty and Mountain Rose samples.

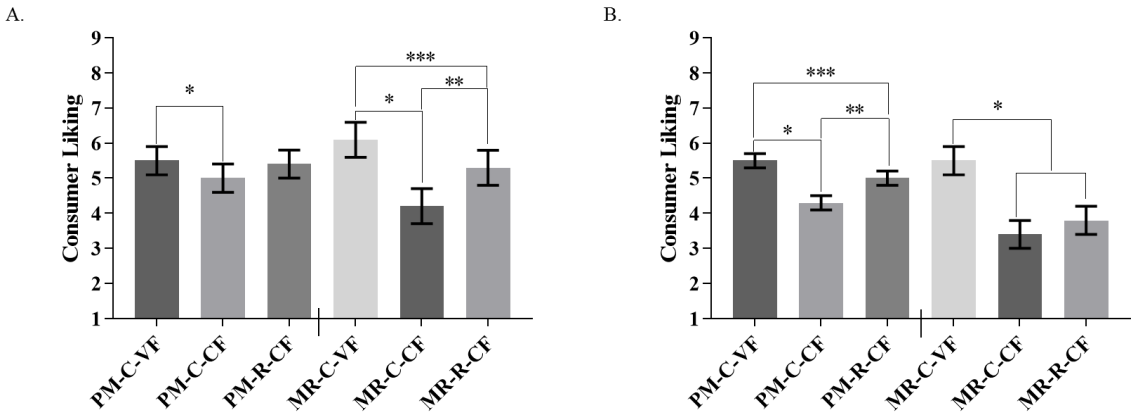

Figure 3-32 Participants rated potato chips samples on a 9-point scale where 1 is dislike extremely and 9 is like extremely of (A) color and (B) overall liking of conventional fried (CF) and vacuum fried (VF) potato chips for Mountain Rose (MR) and Purple Majesty (PM) cultivars, directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent the Tukey's honest significant difference (n=119). Asterisks (*) denotes significant differences within Purple Majesty and Mountain Rose samples.

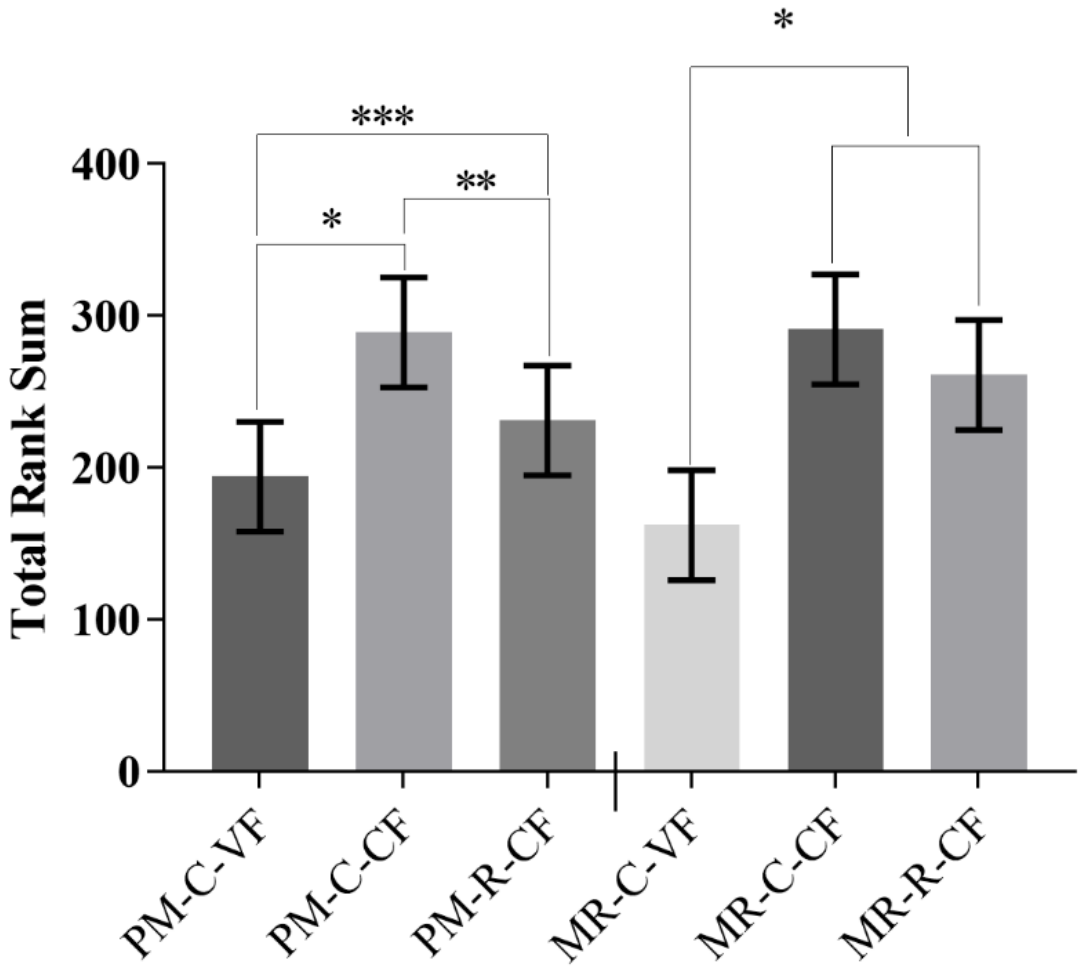

Figure 3-33 Total rank sum of participants' ranking of most preferred to least preferred of conventional fried (CF) and vacuum fried (VF) potato chips for Mountain Rose (MR) and Purple Majesty (PM) cultivars, directly from cold storage, without reconditioning, (C) and after reconditioning (R). Error bars represent the Tukey's honest significant difference (n=119). Asterisks (*) denotes significant differences within Purple Majesty and Mountain Rose samples.

3.3.11 Conclusions

Vacuum frying with and without reconditioning was investigated as an alternative to reduce the cost of reconditioning, reduce Maillard reaction products, and maintain quality parameters to produce an acceptable chip.

In the present work, reconditioning effectively decreased the glucose concentration (Figure 3-2). However, the Mountain Rose and Purple Majesty potatoes sprouted during reconditioning, and many tubers could not be utilized for frying due to the soft texture and shrinkage. Sprouting combined with the cost of a secondary reconditioning room was an incentive to investigate a way to fry directly from cold storage without excessive browning and formation of toxic Maillard reaction products due to the increased levels of free reducing sugars.

Vacuum and conventional frying parameters were developed to produce a chip comparable to commercial chips in texture, moisture, and color. This work allows for comparisons of potato treatments. However it has shown that the processing of individual potatoes should be further optimized by adjusting the process times and temperatures for a specific cultivar of potatoes prior to processing.

The quality attributes of moisture, texture, and color were investigated to determine if the vacuum frying parameters produced vacuum fried chips without reconditioning that were similar to conventional frying with reconditioning. Vacuum frying without reconditioning produced a lower moisture chip (Table 3-2) that was more similar to commercially available samples (Table 3-3). Vacuum fried chips without reconditioning produced a higher moisture chip than conventional fried with reconditioning, which further illustrates the need to develop frying methods for specific

storage conditions. The hardness of the vacuum fried chips without reconditioning was not statistically different to the conventional fried chips with reconditioning and to commercially available samples (Figure 3-6). Vacuum frying retained more of the natural pigments of the potatoes, regardless if it was reconditioned or not (Figure 3-8). Vacuum fried chips, with and without reconditioning, produced chips that had a more similar color profile to commercial samples than conventional fried with and without reconditioning. Vacuum fried chips without reconditioning produced chips that were comparable in quality parameters to conventional fried with reconditioning as well as chips that were more similar to commercial samples.

Levels of three Maillard reaction products, which are potential toxins, were investigated to determine if vacuum frying without reconditioning could maintain comparable levels to chips that were conventional fried with reconditioning. Acrylamide levels in the three cultivars were statistically lower when the potatoes were reconditioned within the same frying treatment (Figure 3-18). In comparing the frying treatments within the same storage conditions, the acrylamide levels were not statistically different. 5-hydroxymethylfurfural concentrations were significantly higher in conventional fried samples without reconditioning and not statistically different in other treatments (Figure 3-28). Concentrations of the advanced glycation end-product N^{ε}-carboxymethyl lysine were not significantly different (Figure 3-30). Chips that were vacuum fried without reconditioning had levels of these three Maillard reaction products that were not significantly different to the conventional fried with reconditioning showing the potential to vacuum fry without reconditioning and still maintain acceptable levels of potential toxins in the potato chip.

Maintaining quality parameters and reducing toxins via vacuum frying is useful but not no avail if consumers do not find vacuum fried chips without reconditioning comparable to conventional fried chips with reconditioning and preferred over conventional fried chips without reconditioning. Consumers preferred the chips that were vacuum fried without reconditioning over conventional fried samples, with and without reconditioning (Figure 3-33). Consumers were willing to purchase colored chips over white chips when they were told that there was a potential health benefit associated with the colored chips. Future studies should investigate increasing the flavor and reducing the oiliness of the vacuum fried samples, as these were rated poorly by the consumer panel (Figure 3-31).

Vacuum frying without reconditioning maintained instrumental quality parameters and showed potential in a consumer panel. The levels of potential toxins found were within the safe ranges for these in food products. Additionally, vacuum frying proved to maintain oil quality longer than conventional frying in this study. This demonstrates an added benefit to vacuum frying beyond the reduction of toxins as it allows producers to keep the oil for longer periods of time which would result in lower oil costs and reduced down time.

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4 OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Potatoes are an important vegetable crop worldwide which are stored and processed prior to consumption. During low-temperature storage, some of the starch in potatoes converts to reducing sugar in a process known as cold-induced sweetening. If potatoes were fried directly from cold storage with conventional, atmospheric frying, the consumers. In addition, some products of the Maillard reaction (e.g., acrylamide, 5-hydroxymethylfurfural, and advanced glycation end-products) have been found to be toxic. Reduction of these products as well as maintaining chip quality makes reconditioning potatoes at higher temperatures prior to processing essential. During reconditioning, the tubers break dormancy, white-fleshed potatoes have been bred to withstand reconditioning, but color-fleshed cultivars such as red- and purple-fleshed potatoes have not and thus sprout during the reconditioning process. In this work, I examined whether vacuum-frying (i.e., lower temperature) potatoes that had not been reconditioned would allow the formation of similar-quality chips with lower toxins than from potatoes conventionally fried (i.e., higher temperature) after reconditioning.

Results presented here show that colored potato chips vacuum fried without reconditioning produced a chip that was acceptable to consumers as well as maintained color and texture properties in comparison to commercially available samples. Under these current vacuum frying parameters for Atlantic, Mountain Rose, and Purple Majesty cultivars, reconditioning is still necessary to reduce the glucose and acrylamide concentrations. 5-Hydroxymethylfurfural was highest in atmospheric fried chips without reconditioning further illustrating the need for reconditioning. Advanced glycation end-

product levels were not affected which could indicate that frying in any capacity increases these values. This study demonstrates the possibility of eliminating reconditioning if toxins are reduced with a secondary mitigation strategy.

Analyzing acrylamide directly with GC-MS/MS allowed for the direct determination of acrylamide without timely bromination. Methanol extraction proved to be effective. Other extraction methods should be studied to extract acrylamide from food matrices. The data suggest that acrylamide could be degraded at higher temperatures of atmospheric frying which creates the need for further studies of acrylamide and other toxin degradation. 5-hydroxymethylfurfural derivatization in the injection port allowed for quantification using GC-MS. The use of N-Methyl-N-(trimethylsilyl)-trifluoroacetamide derivatized 5-hydroxymethylfurfural, however many other trimethylsilyl compounds were formed creating the need for further investigation of these compounds. Analysis of N^{ε}-carboxymethyl lysine, a common food advanced glycation end-product was effectively determined using an ELISA. Further work needs to be completed on new cultivars of potatoes as well as the nutritional aspect of these tubers prior to processing in order to fully understand the concentration of advanced glycation end-products and changes due to storage and different frying techniques.

Vacuum frying maintained a higher quality of oil than conventional frying. This would allow for more use out of a batch of oil with less waste and less downtime from cooling and heating the oil throughout the day. Future work should be done on other oil quality parameters, such as the release of polycyclic aromatic hydrocarbons that are typically released during atmospheric frying. Vacuum frying has the potential to be a more environmentally friendly alternative than conventional frying.

Sensory analysis showed comparable results of vacuum fried without reconditioning and atmospheric fried after reconditioning (a typical chip) found that the vacuum fried was more desired or similar on quality attributes. This suggests that vacuum frying could eliminate reconditioning with the need for additional studies on reduction of oil uptake and an increase of flavor.

Overall, vacuum frying without reconditioning has potential to eliminate reconditioning while still producing a chip that is similar in quality to commercial samples and acceptable to consumers. This study highlighted the need to fit frying parameters to a specific cultivar of potato prior to frying. This study found that vacuum frying produced similar levels of toxins to conventionally fried samples. The levels found in the studied potato chips are lower than the levels of these toxins found in bakery products and coffee. Given this, the levels of toxins found in potato chips would be of less concern than these other products which are consumed more frequently and in higher quantity.

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