

Screening for Acrylamide Levels in French Fries Using Portable Vibrational Spectrometers

THESIS

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By

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Abstract

In 2002, significant amounts of acrylamide were detected in some foods processed at high temperatures. Acrylamide is a known animal carcinogen and classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC). Current assays for acrylamide depend on expensive techniques, for example gas chromatography mass spectroscopy (GC-MS) and liquid chromatography tandem mass spectroscopy (LC-MS/MS) both of which require time consuming preparatory steps that produce hazardous waste. Generally, these methods require food producers to send samples out rather than perform in-house acrylamide testing. There is a need in the food industry to develop simpler, low-cost, and sensitive methods for routine monitoring of acrylamide in foods. Vibrational spectroscopy combined with chemometrics provides an alternative to chromatography that requires little sample preparation. Our objective was to develop a simple screening technique based on handheld and portable near-infrared (NIR) and mid-infrared (mid-IR) devices to detect acrylamide content in french fries. Frozen french fries (n=95) were kindly provided by an industry partner. They were manufactured by frying samples in ten second intervals that ranged from 0 minutes to 6 minutes. Acrylamide content of the french fry samples were determined using the QuEChERS method as a clean-up step and quantification using GC-MS. Frozen french fries were blended down into a powder and spectra were collected and analyzed by partial least squares regression (PLSR) to develop calibration models for predicting acrylamide

content in french fries. NIR and mid-IR showed good linear correlation between spectra and acrylamide levels ($r > 0.92$). NIR and mid-IR had standard error of validation (SEV) values of 200 $\mu\text{g/kg}$. The results suggest that handheld and portable spectrometers allow detection and quantification of acrylamide through spectral signature profiles enabling for real-time and field-based measurements for monitoring processing operations to limit acrylamide formation, addressing risk management and assessing product safety.

Dedication

To my loving family

Acknowledgments

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Chapter 1: Literature Review

1.1 Acrylamide background

Acrylamide ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$, prop-2-enamide, CAS Registry number 79-06-1), depicted in **Figure 1**, is a white crystalline solid with no odor. Its small molar mass (71.08 g/mol) along with its polarity allow it to be soluble in water, methanol, ethanol, acetone, acetonitrile, ethyl acetate, and chloroform in descending order (Habermann 1991). Acrylamide has a melting point of 84.5°C , a boiling point of 136°C at 3.3 kPa/25 mmHg and a vapor pressure of 0.007 mmHg at 25°C (Habermann 1991). Acrylamide contains an amide group and an electrophilic reactive double bond. Historically acrylamide has only been a concern for water quality purposes since it is used for many industrial processes. The main use of acrylamide for the manufacture of polymers that are used in oil drilling, paper making, and water treatment (Pelucchi and others 2011).

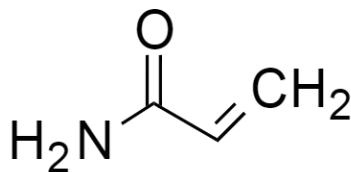


Figure 1: Chemical structure of acrylamide.

A report written by the IARC stated that acrylamide was only a major concern for industrial workers, since it was thought that acrylamide was a synthetic chemical that was contained in factories that worked with it (IARC 1994). In 2002 the Swedish National Food Agency along with researchers at Stockholm University announced that it had identified acrylamide in certain foods (Livsmedelsverket 2002). Specifically, foods that were high in starches that were baked or fried were found to have relatively significant levels of acrylamide. Acrylamide was found in moderate levels (5-50 $\mu\text{g/kg}$) and higher levels (150-4000 $\mu\text{g/kg}$) in protein-rich foods and carbohydrate-rich foods respectively, while no acrylamide was detected in unheated or boiled foods (Tareke and others 2002). The presence of acrylamide in foods has made it a compound of interest for the food industry.

1.1.1 Acrylamide formation

Acrylamide formation in foods is due to being exposed to thermal processing either at home or in an industrial setting. Even though the exact pathway for the formation of acrylamide has not been fully elucidated, there have been proposed pathways. One proposed pathway shows how acrylamide could be formed by heating decarboxylated asparagine (3-aminopropionamide) by deamination in the absence of reducing sugars (Granvogl and Schieberle 2006). Another proposed pathway has acrylamide as a product of the degradation of lipids with acrolein as the intermediate (Gertz and Klostermann 2002). Although there are other proposed pathways, it is generally accepted that the majority of acrylamide formed in foods is due to the Maillard reaction.

The high concentration of acrylamide in starchy foods that are baked or fried suggested that acrylamide may be a byproduct of the Maillard reaction. The Maillard reaction occurs when amino acids and reducing sugars interact in a heated environment. It results in favorable color and flavor development, but can lead to the formation of unwanted compounds such as acrylamide (Mottram and others 2002). In the Maillard reaction Strecker degradation leads to the decarboxylation and deamination of amino acids leading to the formation of aldehydes as seen in **Figure 2**. Mottram (2002) found that it is specifically the interaction between the amino acid asparagine and the reducing sugar glucose that leads to the formation of acrylamide. In the same study it was found that very high temperatures are not necessary for acrylamide formation.

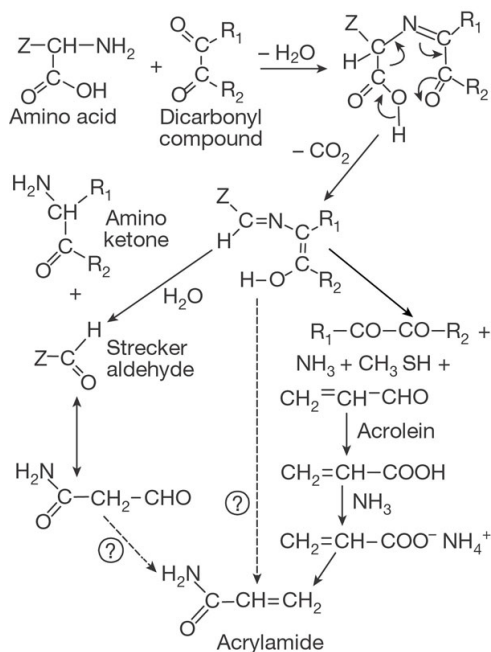


Figure 2: Proposed pathways for the acrylamide formation (Mottram and others 2002).

It was found that the amino acid asparagine was primarily responsible for the formation of acrylamide since contains an amide group attached to a chain of two carbon molecules (Mottram and others 2002). A study using ^{15}N -labeled asparagine and ^{13}C -labeled glucose has shown that the carbon atoms and the nitrogen atom of acrylamide was derived from asparagine. The acrylamide produced in this study did not contain any carbon atoms from the labeled glucose (Stadler and others 2004). The main mechanism for acrylamide formation appears to be when asparagine and a reducing sugar form the N-glycsoyl derivative of asparagine. Water is released at high temperatures and a Schiff base is formed which rearranges to form Amadori products. These products then undergo β -elimination to form acrylamide (Yaylayan and others 2003).

1.1.2 Metabolism

Upon ingestion of acrylamide, it is freely circulated in the body and is eventually excreted in the urine. In one human, study after consuming a meal containing 0.94 mg of acrylamide, sixty percent was recovered from the urine within a 75 hour window (Fuhr and others 2006). Once in the body acrylamide is metabolized into the epoxy derivative glycidamide. Although there are no metabolism studies on humans, in mice it was found that the formation of the epoxide glycidamide from acrylamide is facilitated by the cytochrome P450 enzyme CYP 2E1 in mice (Sumner and others 1999). Both acrylamide and glycidamide are then directly conjugated to with glutathione, as seen in **Figure 3**, before being excreted through the urine (Sumner and others 1999). Acrylamide and

glycidamide are both able to interact with nucleophilic sites of biological macromolecules. In particular, they interact with the thiol and amino groups found in proteins and nucleic acid nitrogens. Glycidamide can interact with the nitrogens in DNA, since they are nucleophilic, creating DNA adducts (Dybing and others 2005). The interaction between glycidamide and DNA is the main reason why it is believed to be the compound causing the carcinogenic and genotoxic symptoms associated with the consumption of acrylamide. Acrylamide is believed to be responsible for the neurotoxic properties since it is highly reactive with proteins (Dybing and others 2005).

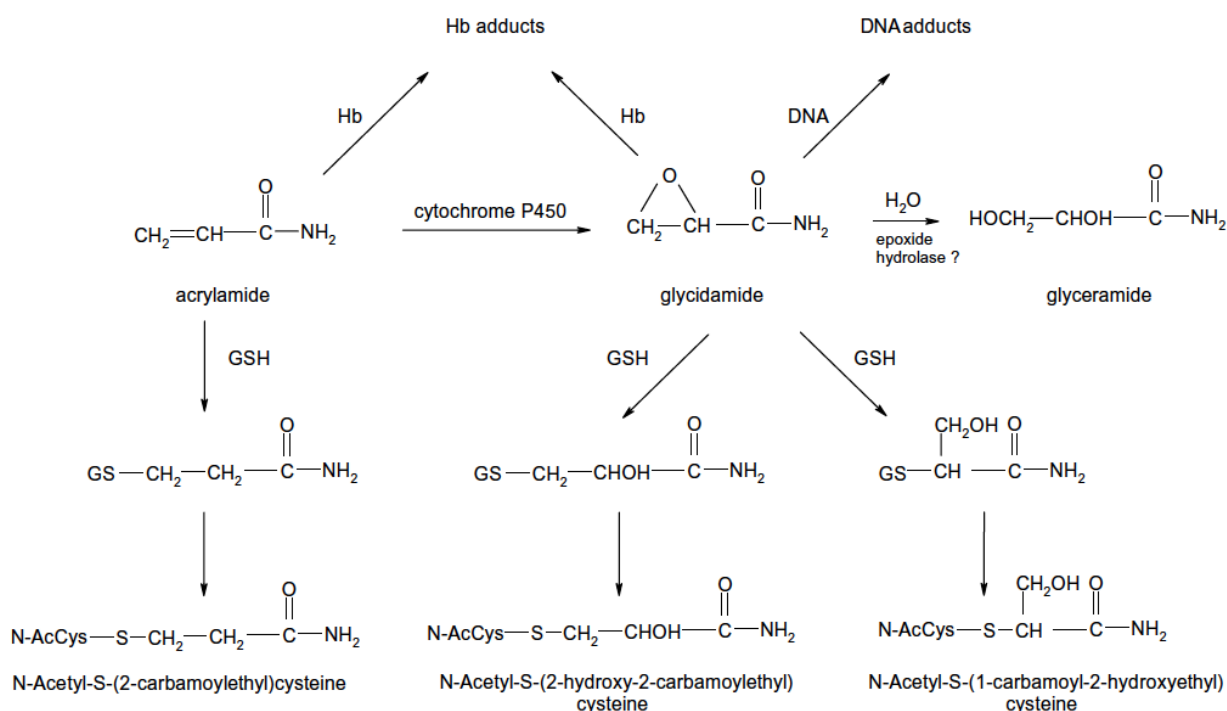


Figure 3: Major metabolic routes of acrylamide (Dybing and others 2005).

1.1.3 Genotoxicity

The genotoxicity of acrylamide refers to the chemical's ability to have an effect on DNA. Although acrylamide has relatively low reactivity with DNA under *in vitro* conditions, it is readily oxidized to form glycidamide. Glycidamide has been shown to produce a higher rate of DNA adducts than acrylamide (Gamboa da Costa and others 2003). DNA adducts are regions of DNA that have cancer-causing compounds bound to them. This study also found that glycidamide exposure to neonatal mice resulted in a 5-7 fold increase in DNA adduct formation which may be explained by the lower activity of oxidative enzymes in newborn mice (Gamboa da Costa and others 2003). In another genotoxicity study, male rats were injected with acrylamide at a dose of 5 x 50 mg/kg in 24 hour intervals. The offspring of these males were examined at 21 days of age for any mutant phenotypes that were associated with the marked loci. Upon exposure to acrylamide the rate of specific locus mutations significantly increased signifying that acrylamide may exhibit genotoxic characteristics (Russell and others 1991).

1.1.4 Carcinogenicity

The carcinogenicity of acrylamide specifically refers to the substance's ability to cause cancer. There have been several human studies that have tried to tie dietary exposure to the formation of cancer. Using prospective data, one study looked at 61,467 women in the Swedish Mammography Cohort in an attempt to find a link between acrylamide consumption and incidences of colorectal cancer. According to the study the mean intake of acrylamide through the diet was 24.6 µg/day with nearly 44% of acrylamide coming

from the consumption of coffee. Fried potato products, crisp bread, and other breads accounted for 16%, 15%, and 12% respectively. After adjusting for confounders there was no correlation between estimated acrylamide intake based on diets and incidence of cancer (Mucci and others 2006). Another study looked at data obtained from a network of Italian and Swiss hospital-based case-control studies to find the relation between dietary exposure to acrylamide and various types of cancer. In the analyzed data set there were no correlations between acrylamide intake and the development of cancer (Pelucchi and others 2006). No epidemiological studies have been able to show a good correlation between carcinogenicity and acrylamide exposure.

Although dietary studies in humans have not been able to strengthen evidence for the potential carcinogenicity of acrylamide, there have been animal studies that link acrylamide exposure to cancer development. Acrylamide was found to not only be a skin tumor initiator in rats, but also lead to increased formation of lung tumors in rats when acrylamide was introduced into their diets via drinking water (Bull and others 1984). The same study showed that acrylamide introduced systemically was more potent than topically applied acrylamide. One study spiked the drinking water of rats with 2 mg/kg bw/day of acrylamide for two years to study the chronic toxicity and carcinogenic effects of acrylamide. Rats that were exposed to this concentration had an increased incidence of several tumor types including the central nervous system, thyroid gland-follicular epithelium, and oral tissues (Johnson and others 1986). The development of cancer cells throughout the body is consistent with acrylamide circulating through the body once it is consumed.

1.1.5 Neurotoxicity

The neurotoxicity of acrylamide refers to damage to the nervous system due to exposure to the chemical. Acrylamide has been demonstrated to be a neurotoxin from accidental and chronic occupational exposure (Parzefall 2008). The main symptom of acrylamide exposure is peripheral neuropathy, which usually results in numbness or prickling. There have been other symptoms reported such as damage to the Purkinje cell and distal axon degradation of the central nervous system (Parzefall 2008). There have been several animal studies which have reproduced the observed neurotoxic effects of acrylamide. In the study conducted by Johnson and others (1986) rats that were exposed to acrylamide showed an increase in peripheral nerve damage, specifically degeneration of the tibial nerve (Johnson and others 1986). This same study determined a no-observed-adverse-effect-level (NOAEL) for nerve damage of 500 $\mu\text{g/kg bw/day}$ and a lowest-observed-adverse-effect-level (LOAEL) of 2000 $\mu\text{g/kg bw/day}$.

1.2 Acrylamide regulations

With a chemical that has as many toxic effects as acrylamide, one may expect there to be very tight regulations on the concentration limits that may be found in foods. However, due to the uncertainty of the mechanism of formation of acrylamide there have not been any maximum levels for acrylamide in foods set by regulators. Instead of enforcing a maximum level, the government has decided to take on mitigation strategies and monitoring procedures (Stadler and Lineback 2009). Currently the European Union is

deciding on when to vote to set maximum levels of acrylamide in certain food products.

If the law is passed, it will be the first law of its kind to set defined limits for acrylamide in foods (EU 2015).

In November 1986 the Safe Drinking Water and Toxic Enforcement Act, more commonly known as Proposition 65, was passed in the state of California. The Office of Environmental Health Hazard Assessment (OEHHA) is the main agency that is in charge of implementing Proposition 65. The law requires that businesses clearly provide warning to Californians about exposure to chemicals that may cause cancer, birth defects and other reproductive harm. Businesses must warn Californians whether the chemical is in the environment, buildings, or in the products that they purchase. There is a list of chemicals that are enforced by Proposition 65 and include over 900 chemicals that have been added since it was first published in 1987. Acrylamide was added to list of chemicals that are known to cause cancer, birth defect and other reproductive harm as defined by Proposition 65 in 1990. The maximum allowable dose level (MADL) for acrylamide is 140 µg/day, calculated based on a human male weighing 70 kg. Food companies are required to keep acrylamide levels below 275 parts per billion (ppb) in order to avoid having to place warning labels on the product (OEHHA 2015). For more information visit OEHHA's website at www.oehha.ca.gov.

In 1982 the Confederation of Food and Drink Industries (CIAA) of the EEC was founded. It consisted of food companies in Europe that helped promote the needs of the industry such as food safety and science, nutrition and health, consumer trust and choice, competitiveness, and environmental sustainability. In 2011 the CIAA became

FoodDrinkEurope with the same goals in mind. FoodDrinkEurope has published and updated an “Acrylamide Toolbox” that reviews scientific and technological developments for the mitigation of acrylamide formation in foods. The Acrylamide Toolbox takes fourteen parameters into consideration including reducing sugars, pH, asparaginase, and consumer guidance. These parameters are from different four different stages in the food chain: agronomy, recipe, processing, and final preparation. The guide covers all fourteen parameters as they pertain to certain high-risk acrylamide containing foods. More information can be found on FoodDrinkEurope’s website www.fooddrinkeurope.eu.

1.3 Extraction methods for acrylamide

Prior to the analysis through conventional techniques, acrylamide in samples must be extracted. There are several widely used techniques that have been developed for the efficient extraction of acrylamide. After the extraction process samples are ready to be analyzed via liquid chromatography or gas chromatography coupled with mass spectroscopy.

1.3.1 Solid phase extraction

One popular method for the extraction of acrylamide from foods employs the use of solid phase extraction (SPE) cartridges in order to get rid of any materials that may interfere with analysis through chromatographic methods (Roach and others 2003). In this method developed by Roach, samples are mixed with water and shaken vigorously. The samples

are then centrifuged and an aliquot of the supernatant is further centrifuged to get rid of any solids. This supernatant is then filtered through an Oasis HLB SPE cartridge and subsequently filtered through an Accucat SPE in order to remove any residual compounds in the matrix (Roach and others 2003).

1.3.2 QuEChERS

In 2003, Steven Lehotay and Michelangelo Anastassiades at the USDA developed a quick method for the analysis of pesticide residues in foods (Anastassiades and others 2003). The method was coined QuEChERS which stands for “quick, easy, cheap, effective, rugged and safe.” The first step of the method is to weigh out a small amount of the sample into a Teflon centrifuge tube along with acetonitrile, magnesium sulfate and sodium chloride. This mixture is then shaken vigorously for one minute, then centrifuged. The sample will separate into an acetonitrile layer and a water layer on the bottom. An aliquot of the acetonitrile layer is then added into a microcentrifuge vial with primary-secondary amines (PSA) and more magnesium sulfate. This step acts as a dispersive SPE clean up that removes any residual carbohydrates or proteins that may interfere with the chromatography analysis. The vial is shaken, then centrifuged and the extract is transferred into an autosampler vial for analysis via GC-MS. Although this extraction technique was developed for pesticide analysis, it has since been modified for the analysis of other polar compounds in foods.

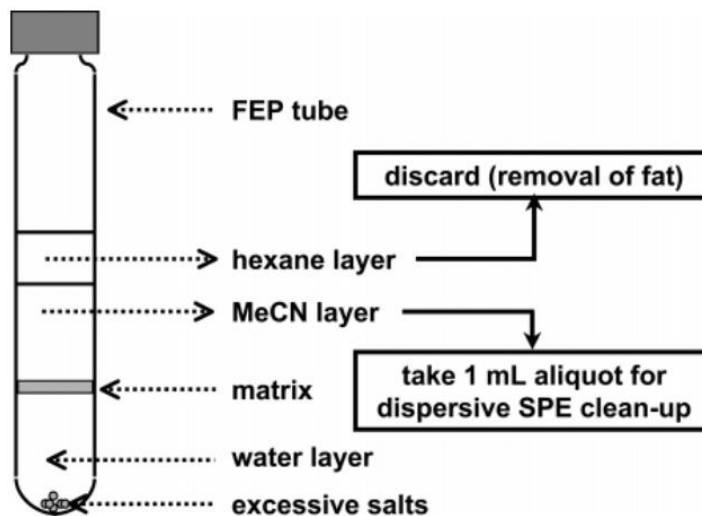


Figure 4: Schematic representation of the solvent layers after the first centrifugation step in the QuEChERS method (Mastovska and Lehotay 2006).

A modified QuEChERS method was developed for the extraction of acrylamide in foods (Mastovska and Lehotay 2006). This method was first tested out on potato chips, sweet potato chips, various crackers and snacks, peanut butter, chocolate and chocolate flavored syrup. The general procedure for this method was very similar to the original, except for a few changes. The samples are first defatted with hexane, then water is added along with acetonitrile in order to extract the acrylamide. After the centrifuging the top hexane layer, as seen in **Figure 4**, is discarded. The extract obtained from this method can be analyzed using both LC-MS/MS or GC-MS. An internal standard, d_3 -acrylamide, was used in this method in order to assess extraction efficiency. LC-MS/MS analysis showed a lower recovery rate opposed to GC-MS analysis which had a recovery rate that ranged from 88-113%. This extraction method for acrylamide provides a quick and relatively safe alternative for the direct determination of acrylamide using chromatography.

1.4 Detection methods

There are a variety of methods that have been applied to the detection and quantification of acrylamide in foods. There are two widely used methods for the quantification of acrylamide in foods, gas chromatography (GC) and liquid chromatography (LC). Both of these methods generally employ the use of a mass spectrometer (MS) as the detector. Most methods include the use of water in order to first extract the acrylamide from the food matrix.

1.4.1 Gas chromatography

Gas chromatography analysis of acrylamide has been used in a variety of products. In general GC methods are often coupled with a derivatization step that includes potassium bromate and potassium bromide in order to improve separation. In one study, researchers developed a method using GC with an electron capture detector (ECD) in order to measure the acrylamide content of a variety of fried foods including potato chips and chicken wings (Zhang and others 2006). Prior to GC-ECD analysis the samples were washed with a sodium chloride solution in order to extract the acrylamide from the food. The extract was then mixed with potassium bromate and potassium bromide in order to form 2,3-dibromopropionamide and 2-bromopropenamide. The use of ECD for the detection of acrylamide is a cheaper alternative to tandem MS methods. The limit of detection (LOD) for this method was estimated to be 0.1 µg/kg. Another study that applied a bromination technique with slightly different reagents in order to measure the

acrylamide levels in potato chips. This method included the use of potassium bromide and hydrogen bromide in order to derivatize the sample. In this case the acrylamide from the food samples were extracted using hot water. This extract was analyzed via GC-MS in selected ion monitoring (SIM) mode in order to identify the compounds by their m/z (Fernandes and Soares 2007). The limit of quantification (LOQ) and LOD were found to be 38.8 and 12.8 $\mu\text{g/kg}$ respectively.

Although derivatization is a popular technique used with gas chromatography methods, there have been studies that forego the use of such techniques. These studies usually employ expensive tandem MS detectors in order to detect acrylamide. One study measuring the acrylamide content in various heat-processed foods used a dispersive solid phase extraction technique, that included primary-secondary amine, followed by direct injection into a GC with a time-of-flight (TOF) mass analyzer. This technique resulted in a LOQ that ranged from 15 to 40 $\mu\text{g/kg}$ (Dunovska and others 2006). Another study used a triple-quadrupole mass spectrometer with a solid-phase microextraction (SPME) step in order to detect acrylamide with a LOD of 0.1 $\mu\text{g/L}$ (Lee and others 2007). Although most studies use some sort of tandem MS detector, there have been studies that have used direct GC-MS analysis in order to detect acrylamide in foods (Jezussek and Schieberle 2003).

1.4.2 High-pressure liquid chromatography

High-pressure liquid chromatography or high-performance liquid chromatography (HPLC) methods are the most widely used in laboratories for the analysis of acrylamide

in foods. Ever since the discovery of acrylamide in food there have been many LC methods developed using a variety of detectors in order to detect acrylamide. One laboratory was noted to have used HPLC coupled with an ultraviolet detector in order to measure acrylamide in a wide variety of food products. The samples were measured at 200 nm in order to quantify acrylamide and methacrylamide. This method had a LOD of 10 µg/L (Paleologos and Kontominas 2005). Another method was developing using HPLC coupled with a diode array detector (DAD) in order to determine acrylamide content of potato-based foods (Gökmen and others 2005). This method includes a methanol extraction of acrylamide following by clarification using Carrez I and II solutions. The sample is then evaporated and suspended with water before going through an Oasis HLB solid-phase extraction (SPE) cartridge. The DAD is set at 226 nm for analysis. This method has a LOQ that is estimated to be 4 µg/kg.

HPLC coupled with MS and tandem MS are some of the most popular methods for the determination of acrylamide in foods. LC-MS has been used in laboratories due to its relatively cheap cost when compared to its tandem MS counterparts. One study developed a method, that could be run both on single quadrupole LC-MS or GC-MS instruments, for the detection of acrylamide in complex matrices such as coffee, cocoa, and high-salt foods (Eberhart and others 2005). The method starts with an extraction with heated water followed by being mixed with ethylene dichloride. The sample is then extracted with ethyl acetate and concentrated before being analyzed by LC-MS. This method has a LOQ of 10 ppb and potato chip samples showed an relative standard deviation (RSD) of 5-8%. Several studies have developed methods for the determination

of acrylamide in foods using LC coupled with tandem MS. One study looking at acrylamide in cocoa and coffee products used a protein precipitation step along with SPE cartridges prior to LC-MS/MS analysis (Arisseto and others 2008). The method had a LOD and LOQ of 10 µg/kg and 20 µg/kg respectively.

1.5 Infrared Spectroscopy

When one is choosing an analytical method there are many factors to consider.

Typically, there are various methods that can be applied to any given analysis so it is important to weigh the advantages and disadvantages. For instance, there are very precise methods, GC-MS and LC-MS/MS, that can be used to measure the levels of acrylamide in foods but these methods are expensive, time-consuming, require trained operators and can produce hazardous waste. The disadvantages from these methods have created a need from the food industry to develop a quick and easy technique to ensure the quality and safety of foods.

1.5.1 Infrared region of the electromagnetic spectrum

Infrared (IR) spectroscopy can be applied as an alternative method to screen quality parameters of foods in an industrial setting. The basis of infrared spectroscopy is studying the interaction between infrared light with matter (Smith 1999). Light waves are characterized by their wavelengths, frequency, hertz and energy. Infrared light is a certain region of light in the electromagnetic spectrum, as depicted in **Figure 5**. The infrared region is also divided into three distinct regions: near-IR (14,000 to 4,000 cm⁻¹),

mid-IR (4,000 to 400 cm^{-1}), and far-IR (400 to 50 cm^{-1}) listed in order of decreasing energy (Smith 1999).

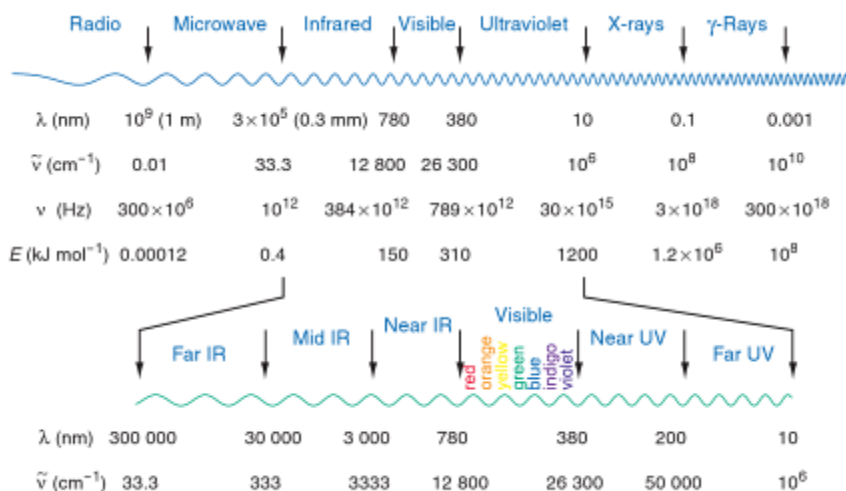


Figure 5: The electromagnetic spectrum and the wavelengths and wavenumbers associated with each region.

After samples are exposed to IR waves the components of the sample absorb some energy from the waves causing vibrations, bending, and twisting of the chemical bonds in the sample. Functional groups are sets of atoms that exhibit these movements when exposed to infrared light (Smith 1996). This phenomenon is particularly interesting because certain functional groups will absorb energy from the same spectral area, no matter what molecule they are a part of (Smith 1996). The absorption patterns that are shown in any given sample can give some insight on what type of functional groups are present. IR spectroscopy may be used to identify unknowns, confirm the identities of substances, and to quantify known components in a sample (Smith 1999). In theory this is an incredibly powerful instrument, but one major disadvantage of using IR

spectroscopy is that water in samples and other complex matrices can create a lot of noise in spectrograms making interpretation of the data difficult (Smith 1996).

1.5.2 Fourier-transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a rapidly growing technology that has recently been applied to the food industry for quality and safety purposes. An FTIR spectrometer consists of three major components that will be present in all instruments in one form or another, a light source, a beam splitter, and a detector. The main component that distinguishes a FT spectrometer from a regular spectrometer is a Michelson interferometer. A Michelson interferometer consists of three major components, a moving mirror, a fixed mirror and a beam splitter as shown in **Figure 6** (Bates 1976). The nature of the Michelson interferometer allows for the simultaneous data collection of all wavelengths compared to a grating spectrometer which only allows a single wavelength to be analyzed at a time.

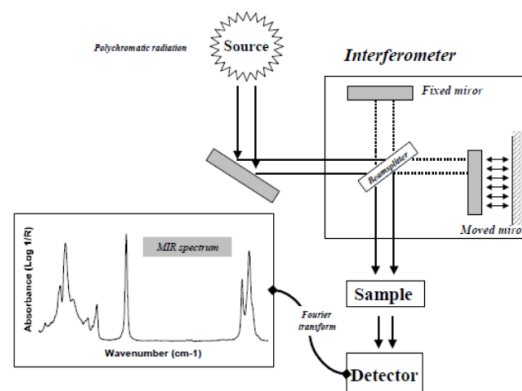


Figure 6: Schematic layout of an FTIR instrument including a Michelson interferometer (Baeton and Dardenne 2002).

There are a few advantages for using FTIR over a grating instrument (Griffiths and Haseth 2007). The first advantage, that was mentioned briefly, is called the Fellgett's advantage. The Fellgett's advantage is the higher signal to noise ratio that a FTIR instrument possesses due to measures the entire spectrum of a sample rather than scanning one wavelength at a time. The second is Jacquinot's advantage, which states that there is higher signal in FTIR instruments due to the higher amount of energy that reaches the detector simply because of how the instruments are constructed. The final is Conne's advantage which states that the collected data from an FTIR instrument is consistent between different analyses due to the use of a laser rather than a bulb as the light source. These advantages contribute to the compelling argument for using an FTIR instrument to create predictive models over grating instruments which may not give the sensitivity and reproducibility required.

1.6 Multivariate data analysis and chemometrics

Multivariate statistics is a branch of statistics that is used to understand data that has multiple outcome variables. The application of multivariate statistics is referred to as multivariate data analysis (MVA) and is useful for data sets that contain a large number of variables. MVA can be applied to a variety of fields including chemistry.

Chemometrics is described as the application of statistical methods, including MVA, to extract more information from chemical data (Workman and others 1997). Although chemometrics is not explicitly multivariable, many data sets that are obtained from chemical analyses are multivariable in nature and need to be analyzed as such. There are

many applications for chemometrics, but one of the most common methods used for multivariate calibration is partial-least squares regression (PLSR) (Wold and others 2001).

PLSR is a regression method that is used to analyze various X-variables that are correlated and noisy while also modelling several response variables or Y-variables. The models created using PLSR can be used for quantitative analysis of various components. Modern analytical instruments such as spectrometers provide data with a very large number of X-variables that tend to be highly correlated to each other (Wold and others 2001). The Y variable, dependent variable, is the response and can represent a variety of factors such as a property of chemical samples or the quality of a product. The robustness of models created using PLSR is increased by adding more relevant variables and observations. PLSR is capable of handling incomplete and noisy data with many variables in order to quantitatively model complicated relationships between X, predictors, and Y, responses(Wold and others 2001).

1.7 IR spectroscopy applied to detection of contaminants in food

IR spectroscopy has been applied to the food industry to monitor various quality and safety parameters. Generally, analytical tests on foods are time consuming, require trained individuals, and may produce toxic waste. Taking all of these factors into consideration, the food industry is constantly looking for alternative methods to ensure the quality and safety of their products. There have been numerous applications for IR spectroscopy for the detection of contaminants in foods.

NIR has been applied to the detection of mycotoxins in cereal grains. One study used NIR to detect mycotoxins created by several different species of fungi (Rasch and others 2010). In this study wheat was inoculated with several genera of fungi including *Fusarium* and *Alternaria* and the water activity was adjusted to facilitate mycotoxin production. Principal component analysis was used in order to group the different samples together. It was found that NIR has a lot of potential as a qualitative and a quantitative technique to identify different fungi and mycotoxins. Another study used NIR in order to predict the deoxynivalenol, a mycotoxin, concentration in wheat kernels (Pettersson and Aberg 2003). Unlike the previous study that used principal component analysis, this study used partial least squares regression in order to create a calibration model to predict the level of deoxynivalenol.

Vibrational spectroscopy has also been applied to the detection of melamine in different food products. Melamine has been a concern for food manufacturers, specifically infant formula manufacturers ever since Chinese companies were caught adulterating formulas with melamine in order to artificially increase the perceived protein content. One study applied NIR to the detection of melamine in soy bean meal (Haughey and others 2013). Like other studies seeking to develop a quantitative model, this study utilized PLSR in order to correlate spectral data with melamine concentration. The developed model showed good linear correlation (0.89-0.99) and a low square error of prediction (0.134-0.368%). Another study applied both NIR and mid-IR spectroscopy to predict the concentration of melamine in infant formula powder (Mauer and others 2009). The models created by this study showed high linear correlation (> 0.99) and were able to

detect 1 ppm of melamine in infant formula powder. Raman spectroscopy has also been applied to the detection of melamine in milk powder (Cheng and others 2010). Milk powders were spiked with melamine and subsequently analyzed using a Raman instrument. A detection limit of 0.13% was determined by the developed calibration model with good linear correlation (0.99).

Mid-IR and NIR have also been applied to the determination of toxicants in foods, specifically acrylamide in potato chips. One study looked at 64 commercial potato chips using both Mid-IR and NIR (Ayvaz and Rodriguez-Saona 2015). LC-MS/MS was used to determine the acrylamide concentrations, which ranged from 169-2453 µg/kg. Good linear correlation and standard error of predictions (SEP) lower than 100 µg/kg were found using these techniques. The promising results indicate that IR spectroscopy can be used as an alternative to acrylamide analysis in potato chips. Other studies have applied NIR to different fried potato products, such as french fries. One recent study developed a calibration method for the detection of acrylamide in french fries using NIR reflectance spectra coupled with GC-MS analysis (Adedipe and others 2016). The models developed by this study showed good linear correlation (0.98) and could detect levels of acrylamide as low as 50 ppb. The lowest observed standard error or prediction, without using any statistical treatments, was 209 ppb with 11 latent variables.

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Chapter 2: Screening for Acrylamide Levels in French Fries Using Portable Vibrational Spectrometers

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2.1 Abstract

In 2002, significant amounts of acrylamide were detected in some foods processed at high temperatures. Acrylamide is a known animal carcinogen and classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC). Current assays for acrylamide depend on expensive techniques, for example gas chromatography mass spectroscopy (GC-MS) and liquid chromatography tandem mass spectroscopy (LC-MS/MS) both of which require time consuming preparatory steps that produce hazardous waste. Generally, these methods require food producers to send samples out rather than perform in-house acrylamide testing. There is a need in the food industry to develop simpler, low-cost, and sensitive methods for routine monitoring of acrylamide in foods. Vibrational spectroscopy combined with chemometrics provides an alternative to chromatography that requires little sample preparation. Our objective was to develop a simple screening technique based on handheld and portable near-infrared (NIR) and mid-infrared (mid-IR) devices to detect acrylamide content in french fries. Frozen french fries (n=95) were kindly provided by an industry partner. They were manufactured by frying samples in ten second intervals that ranged from 0 minutes to 6 minutes. Acrylamide content of the french fry samples were determined using the QuEChERS method as a clean-up step and quantification using GC-MS. Frozen french fries were blended down into a powder and spectra were collected and analyzed by partial least squares regression (PLSR) to develop calibration models for predicting acrylamide content in french fries. NIR and mid-IR showed good linear correlation between spectra and acrylamide levels ($r > 0.92$). NIR and mid-IR had standard error of validation (SEV)

values of 200 µg/kg. The results suggest that handheld and portable spectrometers allow detection and quantification of acrylamide through spectral signature profiles enabling for real-time and field-based measurements for monitoring processing operations to limit acrylamide formation, addressing risk management and assessing product safety.

2.2 Introduction

Historically acrylamide has only been a concern for water quality purposes since it is used for industrial processes. The report written by the IARC stated that acrylamide was only a major concern for industrial workers, since it was thought that acrylamide was a synthetic chemical that was contained in factories that worked with it (IARC 1994). In 2002 the Swedish National Food Agency announced that it had identified acrylamide in certain foods (Livsmedelsverket 2002). Specifically, foods high in starches that were baked or fried, such as french fries, were found to have relatively significant levels of acrylamide. The occurrence of acrylamide in these foods suggests that acrylamide is a byproduct of the Maillard reaction. The Maillard reaction occurs when amino acids and reducing sugars interact in a heated environment. It results in favorable color and flavor development, but can lead to the formation of unwanted compounds such as acrylamide (Mottram and others 2002). Mottram found that it is specifically the interaction between the amino acid asparagine and the reducing sugar glucose that leads to the formation of acrylamide. In the same study it was found that very high temperatures are not necessary for acrylamide formation.

The toxicity of acrylamide in humans has not been very well studied, but there have been various studies conducted on laboratory animals concerning the toxicity and carcinogenicity of acrylamide. In one study, male rats were injected with acrylamide and the spermatogonial DNA was analyzed. Upon exposure to acrylamide the rate of specific locus mutations significantly increased indicating that acrylamide may exhibit genotoxic characteristics (Russell and others 1991). The mechanism of DNA damage caused by acrylamide is not well known, but an in vitro study showed that the oxidation product of acrylamide, glycidamide, which is formed as a metabolism byproduct in the body may be responsible for interacting with DNA (Gamboa da Costa and others 2003). In addition to the toxicity of acrylamide, the carcinogenicity of acrylamide has also been studied in vivo in laboratory animals. Acrylamide was found to not only be a skin tumor initiator in rats, but also lead to increased formation of lung tumors in rats when acrylamide was introduced into their diets via drinking water (Bull and others 1984). The same study showed that acrylamide introduced systemically was more potent than topically applied acrylamide. This finding is especially of concern when one considers that acrylamide is easily found in many foods that are regularly consumed.

The government has taken steps to protect the public from carcinogens in the food supply, for example proposition 65 in California requires that all products that contain potential carcinogens, including acrylamide, have a warning label. Companies are required to keep acrylamide levels below 275 ppb in order to avoid having to place a warning label on their product (OEHHA 2015). However, due to the uncertainty of the mechanism of formation of acrylamide there have not been any maximum levels for

acrylamide in foods set by regulators. Instead of enforcing a maximum level, the government has decided to take on mitigation strategies and monitoring procedures (Stadler and Lineback 2009). Currently the European Union is deciding on when to vote to set maximum levels of acrylamide in certain food products. If the law is passed, it will be the first law of its kind to set defined limits for acrylamide in foods (EU 2015).

There are several methods for the determination of acrylamide in food products. One of the most important steps to consider when analyzing any food product is sample preparation. With acrylamide there have been many methods used to prepare samples for liquid chromatography-tandem mass spectroscopy (LC-MS/MS) or gas chromatography-mass spectroscopy (GC-MS) analysis. One sample preparation technique, aptly named QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), used for the extraction and isolation of acrylamide from foods is the method developed by Mastovska and Lehotay. In this sample preparation method, the food product must be ground and then mixed with acetonitrile and water in order to efficiently extract acrylamide from the food matrix (Mastovska and Lehotay 2006). Although this method is much more efficient than older ones, it is still time consuming when the entire process is taken into consideration.

Although current analytical methods based on chromatography are able to accurately measure acrylamide content in foods there are several disadvantages to using these techniques. Some of these limitations include time-consuming preparation of samples, extensive training to operate analytical instruments, consumption and disposal of hazardous solvents, and low sample throughput. Infrared (IR) spectroscopy is a rapidly

improving technology that has been applied to the food industry for quality and safety screening purposes. It has many advantages such as a low upkeep cost, rapid time of analysis, high throughput, and little to no training required for the operator. Fourier transform infrared (FTIR) spectroscopy combined with chemometrics provides a robust and rugged technique that can be used to determine acrylamide content in french fries. In particular portable IR instruments can provide quality control technicians with a highly mobile method for screening french fries fresh off the line with minimal sample preparation.

The objective of this study was to develop a simple screening technique based on handheld near-infrared (NIR) and portable mid-infrared (mid-IR) instruments to detect acrylamide content in french fries with minimal sample preparation.

2.3 Materials and methods

2.3.1 French fry material

A total of 95 par-fried frozen french fry samples were received from McCain Foods (New Brunswick, Canada). French fry samples were fried for various amount of time ranging from zero minutes to six minutes in roughly ten second intervals. After frying, french fry samples were frozen, shipped, and received by the Department of Food Science and Technology at The Ohio State University.

2.3.2 Sample preparation

The frozen french fry samples were blended down with liquid nitrogen using a Waring blender (East Windsor, NJ, USA). The samples were blended until a fine powder was produced, liquid nitrogen was added as needed to ensure proper break down. Complete blending of the sample was necessary in order to ensure that the sample is homogenous and would be representative of each respective batch of french fries. The small particle size also helped with spectral collection and extraction for acrylamide analysis via GC-MS.

2.3.3 Acrylamide extraction

The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) was used to extract acrylamide from the frozen french fry powder. QuEChERS was originally developed for the analysis of pesticide residue in foods, but was modified by Mastovska and Lehotay for the extraction of acrylamide (Mastovska and Lehotay 2006). The method used for this study followed the experiment outlined by Mastovska and Lehotay. Extraction and dispersive QuEChERS kits were obtained from Agilent Technologies (Santa Clara, CA, USA) for the extraction and purification of french fries. The french fry powder (1 g) was placed into a 50 ml centrifuge tube. The sample was spiked with 0.5 ml of a 1 ppm solution of ^{13}C labeled acrylamide in acetonitrile. ^{13}C labeled acrylamide was acquired from Acros Organics (Fairlawn, NJ, USA). Hexane (5 ml) was added to the tube and the tube was vortexed for 30 seconds in order to defat the sample. After vortexing, 10 ml of water and 10 ml of acetonitrile were added to the centrifuge tube. Hexane, water, and acetonitrile were obtained from Fisher Scientific (Waltham, MA, USA). A salt mixture containing 4 g of MgSO_4 and 0.5 g NaCl was added to each

sample and the tubes were shaken vigorously for one minute. The tubes were then centrifuged for 6 minutes at 4500 rpm. The hexane layer was discarded and 1 ml of the acetonitrile layer was transferred into a 2 ml microcentrifuge vial containing 50 mg of primary secondary amine and 150 mg of MgSO_4 . The microcentrifuge vial was vortexed for 30 seconds and centrifuged for 1 minute at 5000 rpm. The supernatant was then transferred into an autosampler vial for GC-MS analysis.

QuEChERS work by using both water and acetonitrile to extract the acrylamide from food samples. Since acrylamide is polar, it is readily soluble in both solvents. After the sample was mixed with the solvents, a salt mixture consisting of MgSO_4 and NaCl was added to fully saturate the water in the solution with salt. This step forces acrylamide along with a few other compounds into the acetonitrile phase. The dispersive solid phase extraction (SPE) step using the primary secondary amine is crucial in order to remove any residual carbohydrates or proteins that may interfere with the chromatography analysis.

2.3.4 Gas chromatography-mass spectrometry analysis

A standard curve using acrylamide (99%+) and ^{13}C labeled acrylamide, obtained from Acros (Fairlawn, NJ, USA), was created in order to quantify the concentration of acrylamide in extracts via mass spectroscopy. The concentration of ^{13}C labeled acrylamide was kept constant throughout the standard curve in order to account for any pipetting errors. An initial concentration of 1000 ppb was created and a serial dilution was used in order to obtain a range of 7.8 ppb to 1000 ppb.

An Agilent Technologies 7820A GC was used with a 5877B MSD (Santa Clara, CA, USA). This instrument is a single quadrupole mass spectrometry machine that uses electrospray ionization to ionize the sample. An HP-5MS column produced by Agilent Technologies (Santa Clara, CA, USA) was used for analysis. The column is 50m in length with a width of 0.250mm and an inner diameter of 0.25um.

The initial oven temperature was set at 50°C and the temperature was increased at a rate of 20°C/min with a final temperature of 300°C and 3 minutes of hold time. The injection volume for the analysis was 1 µL. The injection method used was a pulsed splitless method with an injection port temperature of 250°C. The pressure of the injection port was set at 9.785 psi. The flowrate of helium through the column for this analysis was set at a constant 1.2ml/min.

The mass spectrometer source temperature was set at 230°C and the quadrupole temperature set at 150°C. The electron energy of the MS was set at 70 eV and the mass spectrometer was set to single ion monitoring mode. The two major ions that were measured and used for quantification in this study were 71.1 and 74.1 m/z.

2.3.5 Infrared analysis

2.3.5.1 Near-infrared spectral analysis

A ThermoFisher microPHAZIR AG handheld unit was used to collect near-IR spectra (Waltham, MA, USA). This instrument is equipped with a MicroElectroMechanical System (MEMS) in order to disperse light and uses a single indium gallium arsenide (InGaAs) detector. The spectral range of the instrument is 1600-2400 nm with an optical resolution of 11 nm. French fry powders were placed into 20 ml beakers and the beakers

were placed bottom down on the detector. Spectral data were collected from 1600 to 2400 nm or 6250 to 4166 cm^{-1} . The collection of spectral data took less than 10 seconds per french fry powder sample. Background collection was taken automatically by the microPHAZIR. Spectra were collected in duplicate for each sample.

2.3.5.2 Mid-infrared spectral analysis

Samples were treated with hexane prior to analysis using the Agilent Cary 630. Roughly 1 g of sample was placed into 2 ml centrifuge tubes, 1 ml of hexane was added to the tube and samples were vortexed. After being vortexed samples were centrifuged for 10 minutes at 10,000 rpm. The hexane layer was discarded and this process of washing the sample with hexane was repeated twice. After washing the sample with hexane, the sample was then dried using a vacuum centrifuge in order to get rid of any residual hexane.

An Agilent Technologies Cary 630 FTIR Spectrometer (Santa Clara, CA, USA) was used with the single-bounce diamond ATR accessory along with a deuterated triglycine sulfate (DGTS) detector. The instrument uses a Michaelson interferometer in order to disperse light. A uniform pressure was applied to the sample using the provided apparatus for sampling solids. Spectra were collected from the frequency range 4000 cm^{-1} to 700 cm^{-1} , using 64 background scans and 64 sample scans in order to improve the signal-to-noise ratio. The spectral resolution was set to 4 cm^{-1} and background was collected after each sample. The samples were collected using Agilent Microlab PC software (Agilent Technologies, CA, USA). Each french fry powder sample was measured in duplicate in

order to collect independent measurements. Acetone was used in between each sample in order to clean the crystal.

2.3.6 Chemometric analysis

Chemometrics is the extraction of information from chemical data by statistical, usually multivariate, means. The spectral data were analyzed using a multivariate technique known as partial least squares regression (PLSR). PLSR is a regression technique that is frequently used to detect patterns in large data sets and to correlate these patterns to known reference values. PLSR works by creating latent values from a larger set of data, in this case spectra, and using these variables to explain variance (Wold and others 2001). Thus, the spectral data was correlated with acrylamide concentrations obtained through GC-MS analysis.

The software used for all statistical analyses was Pirouette version 4.0 (Infometrix, WA, USA). Separate PLSR models were created from the spectral data obtained from the microPHAZIR and the Cary 630. For the NIR model using the microPHAZIR, data transformation included normalizing and Savitsky-Golay smoothing function (35 pt gap). For the mid-IR model using the Cary 630, data transformation included normalizing, Savitsky-Golay smoothing function (35 pt gap) and the 2nd derivative. Outlier Diagnostic using leverage and residuals were used in order to identify spectra that did not fit the regression model and that introduced noise.

2.4 Results and discussion

2.4.1 Reference values for acrylamide

The retention time for native acrylamide and the ^{13}C labeled acrylamide, the internal standard, was 3.2 minutes **Figure 7**. The m/z 's used for the quantification of acrylamide and the internal standard were 71.1 and 74.1 respectively. The acrylamide concentration of the french fries tested ranged from 0 to 2992 ppb, which is consistent with values reported in literature (Tareke and others 2002; Becalski and others 2003). The frying times for 95 of the samples were provided so a comparison between the frying time and acrylamide concentration could be made for those samples. As seen in **Figure 8A**, the concentration of acrylamide increased as the frying time increased. This trend was expected since acrylamide formation is associated with the Maillard reaction (Mottram and others 2002). The longer the fries are exposed to the heated oil, the more time the Maillard reaction has to develop flavor and color compounds as well as acrylamide.

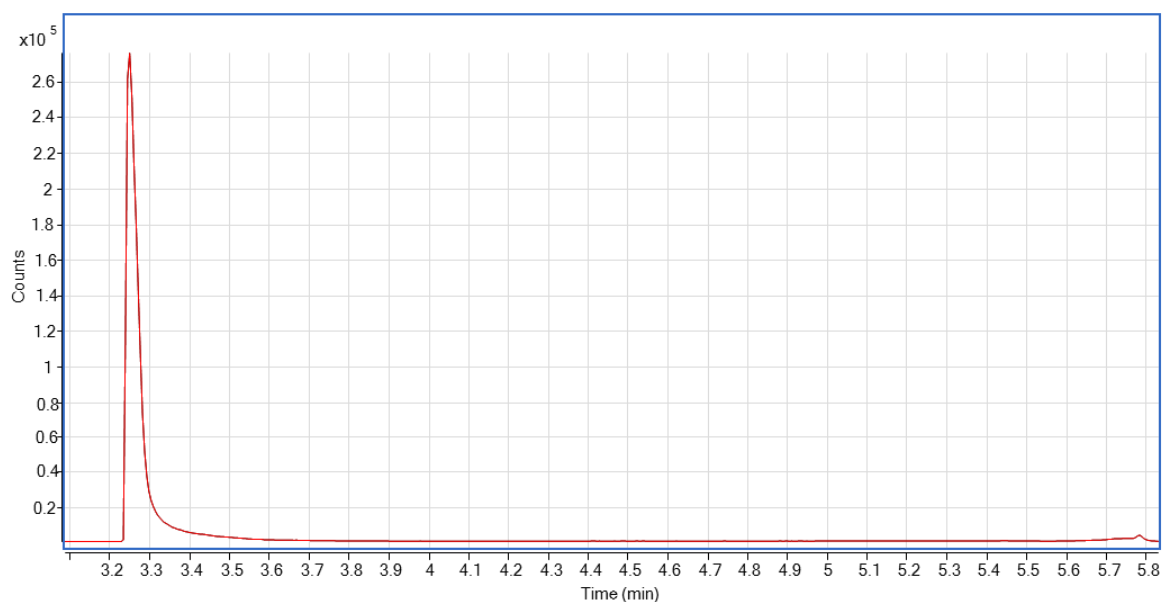


Figure 7: Sample chromatogram used to quantify acrylamide obtained from gas chromatography-mass spectroscopy analysis.

The log of the acrylamide concentration was plotted against the frying time as seen in **Figure 8B**. From the plot, the kinetics of acrylamide formation appear to follow a first order reaction rate. One interesting finding from this graph is that the plot does not intersect at 0 indicating that there may be a non-zero minimum level of acrylamide formation the instant that potatoes come in contact with hot oil.

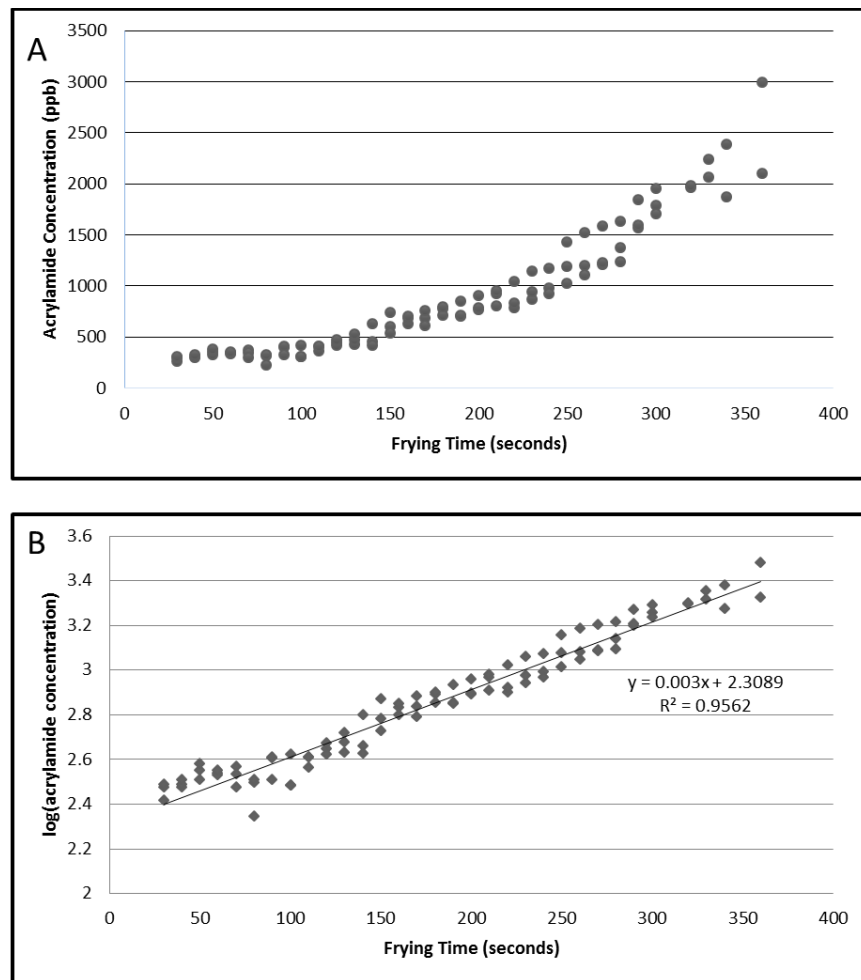


Figure 8: Plot of the A) average acrylamide concentration measure by GC-MS against the frying time and B) the log of the acrylamide concentration plotted against the frying time

A standard curve as seen in **Figure 9** was used in order to quantify the level of acrylamide in samples. The individual samples were analyzed in triplicate and the average was taken in order to ensure that the calculated concentrations were precise. The relative standard deviation (RSD) was calculated for each sample and the values were all lower than 10% except for two samples which had RSD values of 10.06 and 11.54. Mastovska and Lehotay (2006) reported z-scores of -0.42 to -0.02 when testing out different food matrices with the QuEChERS method in conjunction with LC-MS/MS analysis. These low values indicate that the QuEChERS extraction and subsequent GC-MS analysis have a high level of reproducibility. Compared to other methods that were tested in preliminary studies, the QuEChERS method proved to not only be more reliable but also required much less time.

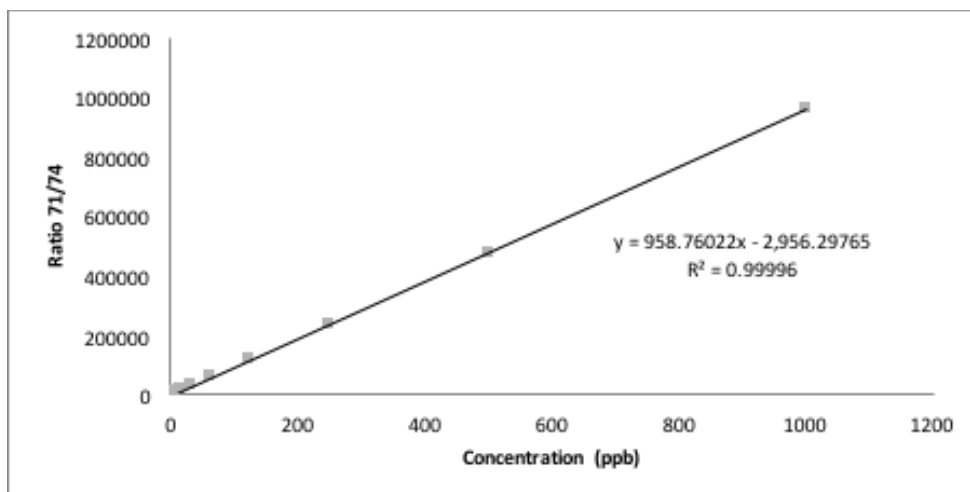


Figure 9: Standard curve created using acrylamide and ^{13}C labeled acrylamide in acetonitrile.

2.4.2 Calibration models for acrylamide concentration in french fries using mid-infrared and near-infrared instruments

Spectral collection using the Cary 630 was done on the defatted french fry powder. The powder was applied onto the ATR accessory and pressure was applied using the provided clamp. The mid-infrared spectra for the french fry powder (**Figure 10A**) was collected from 4000-700 cm^{-1} . The mid-infrared region provided the most resolution in terms of showing distinct and discernable peaks. There were several distinct bands that were visible in the spectra that are associated with certain functional groups. The broad bands that appear around 3600-2900 cm^{-1} and 1700-1500 cm^{-1} are associated with water (Scibisz and others 2011). The bands in the 3100-2800 cm^{-1} range represent the =C-H and C-H stretching vibrational modes and the 1746-1720 cm^{-1} represents the C=O functional group, both of which are associated with lipids. The bands associated with peptide linkage can be seen at around 1690-1600 cm^{-1} for amide I, 1575-1480 cm^{-1} for amide II, and 1301-1229 for amide III (Kong and Yu 2007). Finally the bands located around 1200-900 cm^{-1} are linked to the C-O and C-C stretching as well as the C-O-H and C-O-C deformation of carbohydrates (Shiroma and Rodriguez-Saona 2009).

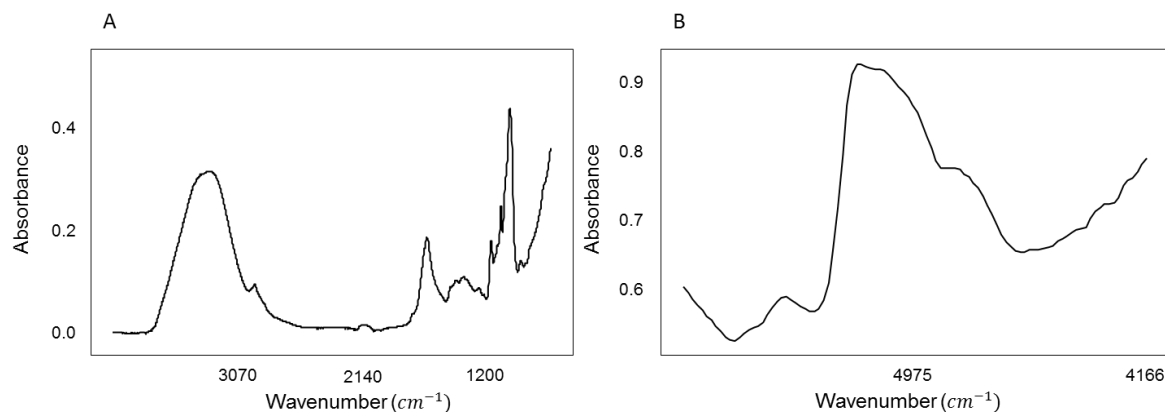


Figure 10: Representative mid-infrared and NIR spectra of french fry powder collected using A) an Agilent Cary 630 and B) a Thermo Fisher microPHAZIR AG respectively.

The NIR spectra were collected from 6250-4166 cm^{-1} as seen in **Figure 10B**. The NIR region that the instrument captured did not provide very much resolution, nonetheless it captured a few regions that are associated with certain functional groups. The region around 4800 cm^{-1} is associated with the $-\text{CH}=\text{CH}-$ functional groups that are associated with fatty acids (Hourant and others 2000). There are a couple of regions in the spectra that are associated with different functional groups found in proteins. The region around 4900-4500 cm^{-1} is associated with the amide band combinations and N-H stretching overtones (Wang and others 1994). The region from 4300-4000 cm^{-1} are correlated with the C-H stretching combinations (Wang and others 1994). The bands around 5320-5180 cm^{-1} are associated with the O-H stretch and H-O-H deformation mode that is associated with the water in the sample (Hourant and others 2000). Due to the design of the portable NIR instrument that was used in this study, Thermo Fisher microPHAZIR, the range of the spectra were limited. This limitation is a result of the microPHAZIR being a MEMS-based device which limits the scanned region.

As seen in **Figure 11** there is good linear correlation ($R_{\text{val}} > 0.92$) between the predicted acrylamide concentration values and the reference acrylamide concentration values obtained through GC-MS analysis. The calibration models developing using mid-infrared and near-infrared devices showed good linear correlation. Outliers were determined by using X residuals and the leverage of samples. Samples with large residuals (> 2) or odd residual patterns were excluded from the calibration curve since including these samples may introduce noise into the model. High leverage samples could have a profound influence on a model, often leading to overestimating or underestimating the predicted values (Hawkins 2004).

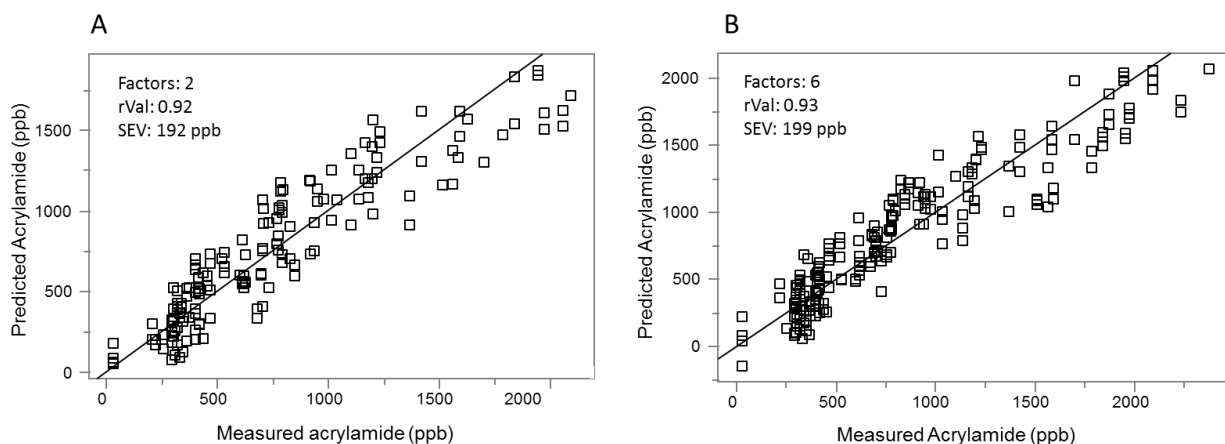


Figure 11: PLSR correlation plots for estimating acrylamide concentration using A) an Agilent Cary 630 unit equipped with an attenuated total reflectance (ATR) accessory and B) a ThermoFisher microPHAZIR AG handheld unit. The instruments measure mid-IR and NIR respectively.

The number of PLS factors used in the model created with the Agilent Cary 630 unit was 2 whereas the number of PLS factors for the microPHAZIR unit was 6. The number of

PLS factors used in a model describes how many components are necessary in order to explain the variance in the values. It is important to be mindful of factor selection when developing a model since using too many factors can lead to overfitting of models (Hawkins 2004). Overfitting overcomplicates the model and can lead to the modeling of noise rather than signal (Hawkins 2004). The standard errors of validation (SEV) for the model created using mid-IR and NIR were 192 ppb and 199 ppb respectively. Overall both models performed similarly with consistent correlation coefficients and SEV.

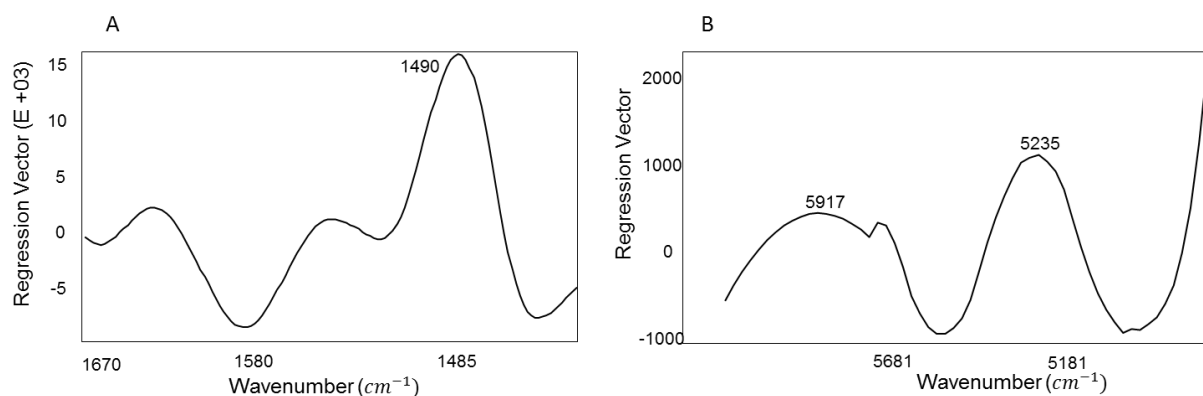


Figure 12: PLSR regression vector plot of the models created using A) mid-IR and B) NIR. The regression vectors for mid-IR and NIR are using 2 and 6 factors respectively.

The regression vectors were analyzed in order to determine which mid-IR and NIR region were most influential towards developing each respective model. As seen in **Figure 12A**, the range that explained the most variance in the mid-IR model was between 1670 to 1430 cm⁻¹. This region is associated with the amide I (1600 -1690 cm⁻¹) and amide II (1480 - 1575 cm⁻¹) bands (Kong and Yu 2007). This region is also associated with the C-H bending vibrations of -CH₂ and -CH₃ (Shiroma and Rodriguez-Saona

2009). As seen in **Figure 12B**, the range that explained the most variance in the NIR model was between 6270 to 4820 cm^{-1} . The resolution of the NIR spectra is not as clear as that of the mid-IR, so it is difficult to determine which peaks are most influencing the model. It appears as though the most influential region for the NIR calibration model is the region associated with O-H stretching (Hourant and others 2000).

Table 1: Summary of PLSR models developing using mid-IR and NIR instruments.

Instrument	Samples used for model	Range of concentrations predicted (ppb)	Range of spectra used (cm^{-1})
Agilent Cary 630 (mid-IR)	86	80 - 1872	1670 - 1430
Thermo Fisher microPHAZIR AG (NIR)	90	40 - 2070	6270 - 4820

The results from this study are comparable to results obtained from similar studies done on potato chips. One study applied handheld NIR and mid-IR spectrometers to the detection of acrylamide in french fries (Ayvaz and Rodriguez-Saona 2015). This study used LC-MS/MS as a reference method in order to determine the concentration of acrylamide in commercial potato chip samples. The models developed by Ayvas and Rodriguez-Saona showed good linear correlation and a standard error of prediction lower than 100 ppb were reported by this study. Another study processed a single variety of potato tuber using different frying times and temperatures in order to create a range of acrylamide concentrations. Acrylamide contents were modeled using NIR spectra with

reference data collected by liquid chromatography high-resolution mass spectroscopy and PLSR in order to correlate the two. In this study a SEV of 285 ppb using 4 PLS factors without the use of any statistical pretreatments (Segtnan and others 2006). Another study reported standard errors of prediction (SEP) as low as 209 ppb without any statistical pretreatments with their NIR spectroscopy models. This model was created using reference values obtained through GC-MS analysis (Adedipe and others 2016).

2.5 Conclusion

The data suggests that portable infrared spectroscopy methods can be developed to reliably screen for acrylamide levels in french fries. PLSR model performance statistics using mid-IR ($r_{\text{Val}} = 0.92$, SEV = 192 ppb) and NIR ($r_{\text{Val}} = 0.93$, SEV = 199 ppb) demonstrate that these portable instruments can accurately predict acrylamide levels. Both instruments showed consistent and strong correlation of acrylamide levels. Future work could be done to increase the sample size of the model in order to increase its robustness. Portable IR spectroscopy has several advantages over the traditional techniques including having a lower upkeep cost, a higher sample throughput, and the potential for in-field applications. The Cary 630 and microPHAZIR provide the industry with rapid and sensitive alternatives to the conventional LC-MS/MS and GC-MS based methods that are currently in use.

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