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Volatile organic contamination analysis in packaged foods

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by

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DEDICATION

I dedicate this work to Jesus Christ: The Lord God Almighty. He is my loving Creator, my source of inspiration, wisdom, and insight. To Him be all the glory. I also dedicate this work to my parents: Bill and Cindy Davis. Without their nurturing, protection, guidance and encouragement, I would not be writing these words. Finally, to my major professor, Dr. Keith Vorst, for his constant mentorship, friendship, and encouragement.

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ABSTRACT

Volatile Organic Compounds (VOCs) are unavoidable aspects of foods and their packaging. Some VOCs provide pleasant odors and contribute to flavor profiles, while others can cause negative health and environmental effects. VOCs are most commonly measured using headspace gas chromatography, and more recently, kinetic techniques such as solid phase microextraction (SPME). In microwave popcorn, diacetyl and possible other related substances (DAPORS) have recently become an emerging concern. Diacetyl was first discovered to cause bronchiolitis obliterans (BO) in microwave popcorn plant workers, but some claim levels of these compounds in microwave popcorn is of concern to consumers; particularly because of the high temperatures reached during cooking. Eight DAPORS were analyzed using SPME/GC-MS in both high-fat and low-fat varieties of microwave popcorn. Results found elevated levels of diacetyl and 2,3-pentanedione in low-fat varieties. Diacetyl was below the limit of detection in high-fat varieties. Because of the close proximity of these compounds to both plant workers and the consumers, solutions are now being developed to monitor VOC contamination in real time during manufacture of microwave popcorn. Additionally, real-time monitoring technologies can be applied to a wide variety of compounds and packaging substrates to monitor organic and inorganic contamination. A combination sensor array technology was developed in conjunction with a proprietary neural network. The array was successfully trained to detect and predict contamination in thermoplastics. This technology has application in detecting VOCs of interest both in total concentration, and speciation of certain chemical functional groups.

CHAPTER I

GENERAL INTRODUCTION

Nearly every food product sold in the market today is contained and protected by packaging. Consumption of food packaging has accelerated in recent years, with net revenues of over $39 billion globally in 2016 between paper and plastic materials (Hurley, 2017; Matsterson, 2016). Materials to make food packaging can be sourced from a variety of feedstocks but must comply with existing government regulations, such as those outlined by the US Food and Drug Administration, and the European Union. One such parameter of these regulations is the influence of volatile organic compounds on food safety and quality.

Volatile Organic Compounds (VOCs) are small molecules (i.e.- carboxylic acids, alkanes, ketones, alcohols, aldehydes, etc.) which have boiling points that make it possible for them to evaporate under atmospheric or elevated temperatures. A VOC is any organic compound having an initial boiling point less than or equal to 250° C measured at a standard atmospheric pressure of 101.3 kPa (EPA, Envrionmental Protection Agencey (EPA), 2016). VOCs can come from a variety of sources throughout manufacture, such as: material synthesis and pulping (Lin et al., 2011), extrusion and forming (Ezquerro et al., 2003; Feigenbaum et al., 2002), adhesive and printing inks (Aznar et al., 2015; Begley et al., 1991; Canellas et al., 2015), and the use of recycled feedstocks (Biedermann et al., 2013; FDA, 2006; Franz et al., 2004; Grob et al., 2009). In food packaging, VOCs are more likely to migrate through the many different layers of a laminated package into food because of higher diffusion rates and low molecular weights (Nerin et al., 2009). Current analytical techniques can successfully determine substances such as residual solvents or monomer, in low concentrations and complex matrices.

Detection of VOCs in Packaging

Detection of VOCs in both food and packaging has long been an important parameter to determine safety and quality of raw materials and finished goods. Analysis of VOCs can be used to determine a variety of important aspects of a food system, such as food or packaging composition, or contribution of packaging or ingredients to off flavors or odors. Historically, organic small molecules would be extracted via an appropriate solvent and then subsequently analyzed using liquid, and later in the 1950's, gas chromatographic separation techniques for volatile substances (Bartle et al., 2002). Headspace sampling techniques (where an aliquot of gas is sampled from a sample in a heated, sealed vial) were also developed in conjunction with gas chromatography (Poole, 2012).

The origins of headspace analysis using a sealed container are unclear, however, the technique has grown in popularity over the last 50 years with the advent of automated headspace gas chromatography in in the late 1960's (Poole, 2012). Later, solid-phase microextraction (SPME), a kinetic-based extraction technique, was developed by fixing polymer sorbents (i.e.- polydimethylsiloxane) fixed to various substrates (i.e.- titanium wire) for a predetermined amount of time. (Barnes et al., 2009). This technique has been successfully applied to analysis of a variety of samples, including flavor compounds and packaging materials (Ezquerro et al., 2002; Johnson et al., 2012; McCoy et al., 2017; Rosati, 2007).

Real-time Detection of VOCs in Packaging Manufacturing

Recently, real-time monitoring technologies have been developed to monitor VOCs in-situ. This allows for continuous monitoring with good sensitivity at ambient conditions (Dai et al., 2015; Hierlemann et al., 2000; Liao et al., 2013; McCoy et al., 2017). Additionally, if

packaging is formed from recycled materials, unwanted VOCs may be present in the finished article if clean-up parameters are not controlled for. Thus, this technology has multiple applications for monitoring safety and quality of food and packaging materials for both the plant-worker and the consumer.

The objective of this work was to characterize and quantify the level of DAPORS in high-fat and low-fat varieties of microwave popcorn, and to explore potential real-time solutions to monitor VOC levels in both food and packaging systems. It is expected that levels of diacetyl and other α-dicarbonyl substances will not be will be in quantifiable levels, but will not be in levels of inhalative concern.

Diacetyl and Possible Other Related Substances in Microwave Popcorn

Popcorn production in the United States is a $1.9 billion industry with $140 million in exports. 45.3% ($860.7 million) of this industry is comprised of un-popped popcorn most popularly sold in microwavable packaging (D'Costa, 2016). This is a contraction from the segments peak of $900 million in 2012, while the ready-to-eat (RTE) segment has seen tremendous growth of over 60% in the same period (Watson, 2015). While RTE sales have mostly expanded because of a renaissance of innovative branding and exotic flavors, some report potential health concerns of microwave popcorn to consumers, specifically flavorings and coatings (Egilman et al., 2012; Hari, 2013; Schafer, 2015).

BO is an inflammatory condition that affects the bronchioles of the lung, the smallest airways within the organ (Appendix I, Figure 1). Many different chemicals can cause lung injury of this kind, such as nitrogen oxides, welding fumes, and ammonia (King Jr, 2003). Symptoms of BO include a dry cough; shortness of breath; and/or fatigue and wheezing in the absence of a

cold or asthma (King Jr, 2003; Yousem et al., 1992). Repeated inhalative exposures to 2,3-pentanedione have been shown to cause fibrosis of intra-pulmonary airways in rats, demonstrating symptoms similar to BO in humans (Morgan et al., 2012).

Toxicology of DAPORS

Endogenous α-dicarbonyl compounds, such as those found in DAPORS (particularly diacetyl and 2,3-pentanedione) are reactive chemical species associated with the tendency to form cross-links with proteins (Miller et al., 2005). Furthermore, this same functional group has the capacity to form advanced glycation end products, or proteins (i.e.- arginine) that bind to sugar molecules (i.e.-glucose), specifically its electron-attracting carbonyl groups inherent to the structure (Figure 1.1) (Anders, 2017; Roberts et al., 1999).

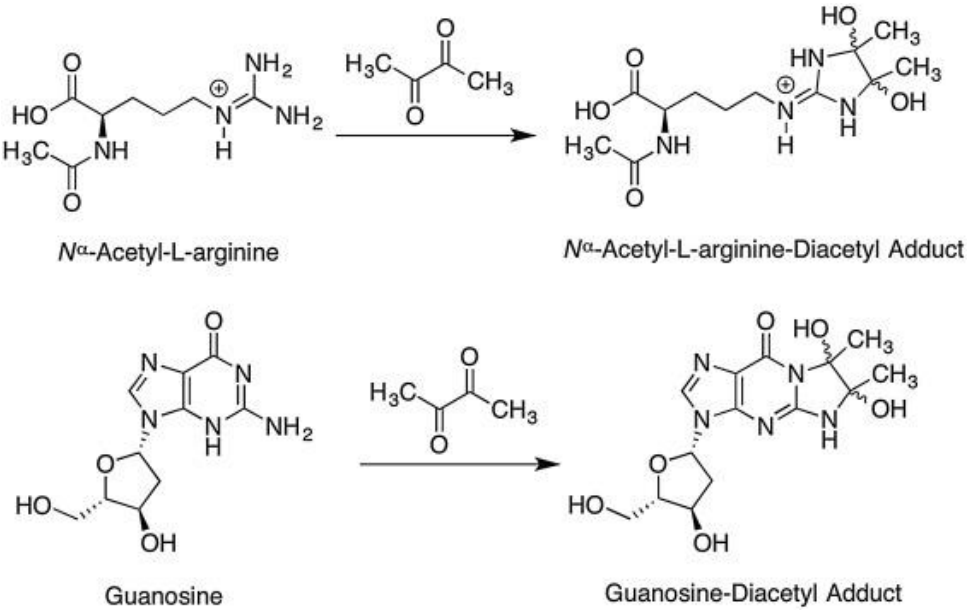

Figure 1.1. Products of the reaction of diacetyl with Nα-acetylarginine and with 2-deoxyguanosine (Anders, 2017).

2,3-pentanedione has been reported to be even more reactive than diacetyl (Epperly et al., 1989; Flake et al., 2016; Hubbs et al., 2012). Acetoin (3-hydroxy-2-butanone) is a structurally similar compound to diacetyl, and is often included in may butter flavor formulations. However, an α-hydroxyketone in acetoin replaces the reactive α-diketone associated with the toxicity of diacetyl and 2,3-pentanedione (Figure 1.2) (Hubbs et al., 2012). To confirm this phenomenon, the US National Toxicology program (NTP) conducted a 90-day study on inhalative exposure to diacetyl and acetoin in Sprague-Dawley rats. Significantly higher levels respiratory tract lesions from exposures as low as 25ppm of diacetyl were found in both rats and mice when compared to acetoin (National Institute for Occupational Safety and Health (NIOSH), 2016).

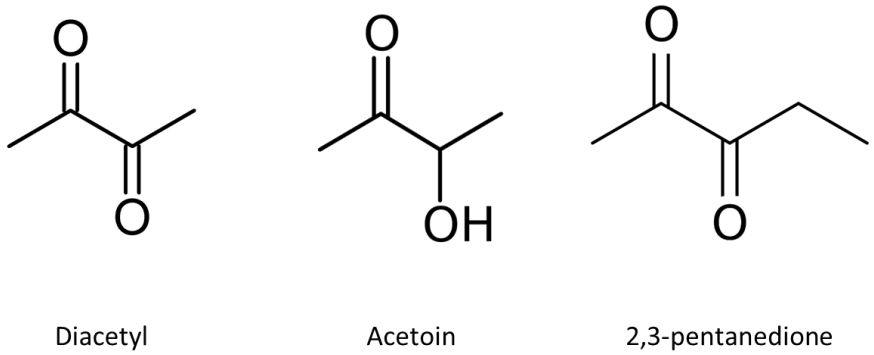

Figure 1.2. Structural identification of diacetyl, acetoin, and 2,3-pentanedione

Numerous studies have linked BO to inhalative exposure to ketone-type flavoring compounds such as diacetyl (Boylstein et al., 2006; Fedan et al., 2006; Flake and Morgan, 2016; Hubbs et al., 2008; Lockey et al., 2009; Rigler et al., 2010; Starek-Swiechowicz et al., 2014; van Rooy et al., 2007). Other studies claim microwave popcorn could pose a significant health risk to consumers (Egilman et al., 2011). These claims have been used to justify several substantial lawsuits in both plant workers in microwave popcorn facilities and consumers.

Litigation Involving BO and Diacetyl

In May of 2000, workers from a microwave popcorn plant in Missouri reported a fixed airway obstruction of the lungs; a disease known as bronchiolitis obliterans (BO) (Akpinar-Elci et al., 2005; Hubbs et al., 2008; Kreiss et al., 2002). Workers in manufacturing facilities of popcorn oil had developed similar severe symptoms of bronchiolitis obliterans, who filed a lawsuit against their employers in Missouri, Montana, Illinois, Nebraska, and Iowa. Plaintiff verdicts in Missouri ranged from $2.7 million to $20 million from 2004 to 2008. $7 million and $30.4 million verdicts were awarded to former Iowa and Illinois popcorn butter flavor mixer workers, respectively (Finley, 2014; Lehr, 2010). Manufacturers of microwave popcorn and other popcorn oils with various butter flavorings have since employed rigorous safety precautions to protect workers from such severe exposure in future. Additionally, the US Occupational Safety and Health Administration (OSHA) set Time Weighted Averages (TWAs) for exposure to DAPORS. In 2007, one individual had consumed so much microwave popcorn he developed a case of bronchiolitis obliterans (the media had thusly named it ‘popcorn lung’), and won a $7.2 million verdict against several retailers and brand-owners for his condition (CBS News, 2012).

Regulatory Environment of Diacetyl

The United States National Institute for Occupational Safety and Health (NIOSH) conducted a thorough risk assessment using data from both animal and human inhalation studies. In October of 2016, NIOSH established a time weighted average recommended exposure limit (TWA REL/8hr) of 0.005ppm and a short-term exposure limits (STEL/15 min) of 0.025ppm for diacetyl (McKernan, 2016). The American Conference of Governmental Industrial Hygienists

(ACGIH) has also recently adopted Threshold Limit Values (TLV/8hr) of 0.01ppm and a STEL of 0.02ppm (Clark et al., 2015). However, some studies claim that exposures to flavoring chemicals in the workplace did not produce and increased risk of pulmonary health, as previous studies were biased based on the inherent correlation found within longitudinal spirometric testing (Ronk et al., 2013).

Although the cases presented deal with large volumes of popcorn oil, some claim that the amount found in individual units of microwave popcorn is enough to cause disease over a long period of time (Egilman and Schilling, 2012). Some of these claims may be justified given the high temperatures associated with cooking a bag of microwave popcorn; particularly, the inherent design of a temperature 'self-regulating' microwave susceptor to reach 200˚C (Figure 1.3) (Regier, 2014).

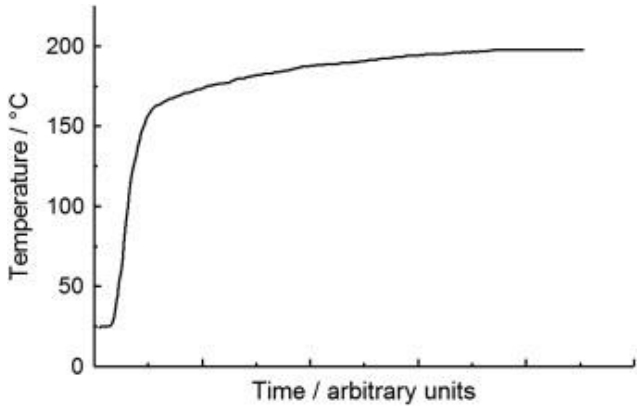

Figure 1.3. Temperature of self-regulating susceptor during cooking (Regier, 2014)

Microwave Popcorn Bag Composition

A microwave popcorn bag contains multiple laminations of various kinds of paper, adhesives, susceptors, and coatings (Jackson, 1995). Microwave popcorn bags are generally

made by laminating paper feedstock with an aluminum, vapor-deposed, Polyethylene Terephthalate susceptor embedded within the inner and outer layers of the package (Figure 2) (Moseley et al., 2000). The inner and outer layers can be coated with various hydro- and lipophobic coatings, which are generally made using perfluorinated chemicals (Jackson, 1995). Increased financial costs and the environmental impact of harvesting and processing have allowed some paper mills to operate exclusively on recycled feedstock, which can affect safety and quality (Biedermann and Grob, 2013; Morris, 2011). Thus, several sources of potential contamination are inherent within microwavable packaging.

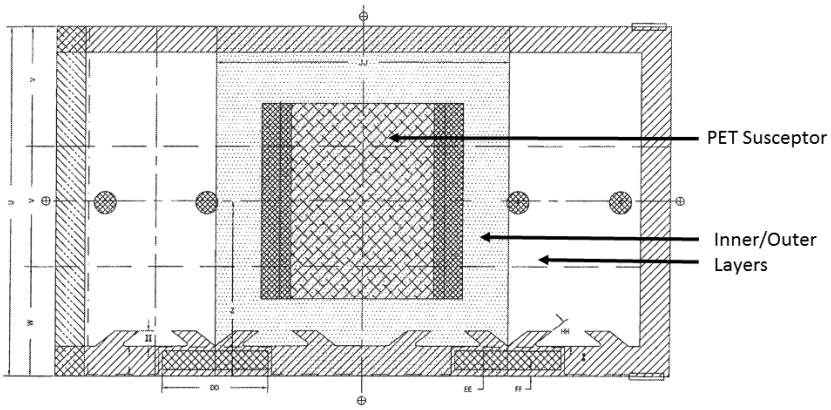

Figure 1.4. Structural Composition of a Microwave Popcorn Bag (Moseley et al., 2000).

Previous Works Measuring Diacetyl in Microwave Popcorn

Walradt and others (1970) conducted some of the first analyses on popcorn VOCs, and identified 36 volatile compounds with some certainty and 20 tentatively identified compounds

(Walradt et al., 1970). Buttery and others analyzed popcorn oil in an enclosed 5L round-bottomed flask equipped with a Tenax trap, which was then microwaved for 2.5 minutes. Results found levels of 170ppm of diacetyl, and 200 ppm of 2,3-pentanedione (Buttery et al., 1997). Identifying sources of VOC contamination within a converted microwave popcorn bag can be a difficult task due to its many layers and constituents.

Rosati and others (2007) has analyzed VOCs in microwave popcorn placed a microwave into an inert chamber and developed a purge and trap mechanism for collecting and analyzing VOCs of interest. The authors noted greater than 80% of VOCs released occur at the opening of the bag, post-cooking (Rosati et al., 2007). This work focused on a fully converted and filled bag and replicated end-use conditions. Furthermore, results showed that most of the VOCs analyzed were from flavor and oil constituents.

Recent work by Zhang and others (2014) characterized fine and ultrafine particle emissions from microwave popcorn using a water-based condensation particle counter (Zhang et al., 2014). The authors noted a significant increase in total and ultrafine particle emissions in microwave popcorn packaging with susceptors, as opposed to a brown paper bag. However, this study may have been biased since it incorporated a variable that may contain a recycled feedstock; and could thus contain a variety of VOCs that could be more toxic than particulate matter generated from the susceptor technology. Headspace temperatures of a bag of filled microwave popcorn can reach 200˚C and higher (Risch, 2009), depending on several parameters, such as the amount of oil present and the wattage of the microwave. Thus, when converted bag structures are subjected to elevated temperatures various volatile organic compounds (VOCs) within any part of the bag can be released during cooking.

The increased awareness of VOCs in high performance-packaged foods (i.e.-microwave

popcorn) has also highlighted gaps in monitoring and evaluation of materials throughout the production value chain. Furthermore, the cost of monthly testing and evaluation can be expensive and cumbersome to many stakeholders. Thus, there is an opportunity to develop and evaluate technologies to monitor certain foods and their packaging to provide a higher degree of traceability and tighter process controls on safety. This technology could also allow for the incorporation of a wider specification of materials, such as recycled feedstocks with varying degrees of contamination, into food packaging articles. This could further increase total recycling rates for plastic and paper feedstocks, and thus, increase sustainability.

Real-time Detection in Thermoplastic and Paper Packaging

The Impact of Unrecycled Paper and Plastics

In 2013, approximately 300 million metric tons of plastic were produced worldwide and less than half has ended up in landfills or was recycled (Markets and Markets, 2014; NAPCOR, 2016). Conversely, in 2014 approximately 68.6 million metric tons of paper and paperboard were generated in the United States, 64.7% of which was recycled (EPA, US Environmental Protection Agency, 2016). Un-recycled and un-landfilled packaging can degrade in the environment which can release potentially toxic organic and inorganic products (Bayer, 2002; Kirwan, 2012). Globally, recycling efforts have increased in recent years as social and political pressure accumulates. The National Association for Polyethylene Terephthalate (PET) Container Resources (NAPCOR) reports that approximately 1.8 billion pounds of PET (about 30.1% of the total produced) were recycled in 2015 (NAPCOR, 2016). NAPCOR also reports that producing new products from recycled polyethylene terephthalate (RPET) uses two-thirds less energy than

what is required to make products from raw virgin materials and additionally reduces greenhouse gas emissions as compared to products made with virgin material (Markets and Markets, 2014; Marsh et al., 2007). Unlike thermoplastics, paper and paperboard cannot be recycled an infinite number of times. Currently, society uses the term 'recycling' in place of 'recovery' or 'collection'. Additionally, because of many fillers and other additives in papers (i.e.- titanium oxide in white ledger), as much as 30% is unrecyclable (Kirwan, 2012).

Contamination associated with recycled materials

Many components of these materials can also pose potentially negative health and environmental effects (Freire et al., 1998; J. Vandenburg et al., 1999; Kang et al., 2011; Vandenburg et al., 1999; Welle et al., 2011). Particularly, migration of these compounds can also pose a health risk to the consumer (Bach et al., 2011; Haldimann et al., 2013; Keresztes et al., 2009; Piringer et al., 1998; Schmid et al., 2008). Residual catalyst, if leached into foods or the environment, could have potentially deleterious effects (Mihucz et al., 2017). Of particular concern in recent years is the widespread use of antimony in the polymerization of PET and impact on health and the environment as a result of migration and degradation processes (Carneado et al., 2015; Takahashi et al., 2008; Westerhoff et al., 2008). In paper and paperboard, residual coatings, inks, and other additives used in products not designed for food contact could pose negative health and environmental effects in recycled products, if proper process controls are not maintained. Additionally, certain components in secondary packages could migrate into foodstuffs via the gas phase (Jickells et al., 2005).

Migration of contaminants from packaging to items such as food can reduce food quality imparting off-odors and unintended flavors; additionally, some contaminants may be toxic if

ingested (Cheng et al., 2010; Jrup, 2003; Karayannidis et al., 2007; Widén et al., 2004). The degree of food contamination will depend on its initial concentration in the package, diffusivity within the package under the conditions of storage, duration and exposure temperature, and the contaminant solubility in the food (Cheng et al., 2010; Jrup, 2003; Widén et al., 2004).

The use of recycled fiber is an important facet of material and product sustainability, however, not all recycled feedstocks are processed with the intention of being incorporated into food contact materials. Recycled fiber feedstock may be sourced from many different sources: newspapers, journals, printed paper communications, paperboard boxes, corrugated board, and can contain a wide variety of chemicals used in the manufacturing and use of these products. Thus, they can contain any number of volatile organic contaminants, such as those used in adhesives, printing inks, varnishes, and thermo-printing (Biedermann and Grob, 2013).

Regulatory environment of packaging

In the United States, the FDA does not provide special regulation or preclearance for the use of paper or polymeric recycled materials or products used in food contact structures, but is rather treated as an indirect additive to food products. Although specific process by which these products are manufactured, or the source of their raw materials are unregulated by special provision in the CFR, the finished product must meet the same regulatory specifications as virgin material, with the exception of paper. Recycled food contact materials also must comply with Good Manufacturing Practices (GMP) requirements that apply to food contact materials (21 C.F.R. Section 174.5).

General EU legislation (EU No. 1935/2004) requires that all materials produced for food contact be systematically tested to ensure compliance at each stage of the supply chain; ensuring

the composition of the final material and the potential migrant is known or predictable and chemical analysis only must determine known critical compounds. However, specific legislation regarding virgin and recycled paper and board materials is not consistent within the EU, and adoption of national legislation varies from member state to member state (Grob et al., 2009). Thus, if articles are to be sold ubiquitously throughout the EU, they must comply with each member state's legislation, and is subject to the principal of mutual recognition.

Paper and paperboard

In paper and paperboard, the FDA permits the use of pulp from reclaimed fiber if it complies with 21 CFR 176.260 (Food and Drug Administration (FDA), 2016a). Reclaimed fiber can be sourced from both post-industry and post-consumer feedstock. Paper and paperboard products must not contain any "poisonous or deleterious substance" which is retained in the recovered pulp and migrates into the food. Regulatory thresholds have been established for certain compounds under sections 406 and 409 of Chapter 9 of the Federal Food, Drug, and Cosmetic Act (FD&C Act). These limits vary depending on the nature of the compound, application of the package (e.g. food packaging), and conditions of use (e.g., elevated or reduced temperatures, acidic or basic, liquid or solid) (Silva et al., 2006).

With these regulations in view, it becomes important to evaluate these structures for potential contaminants that could migrate from various components of the packaging and co-mingle with food products. Additionally, the use of microwavable packaging has grown in popularity over the last 50 years with the rise of busy lifestyles and ageing population. These structures require a higher degree of performance and consideration of safety from packaging materials and feedstocks (Regier, 2014). One aspect of safety that must be considered, is the

potential vaporization and migration of small organic molecules, or Volatile Organic Compounds, from packaging materials into foodstuffs.

Regulatory environment of recycled PET

For example, the US Food and Drug Administration (FDA) has established a 0.05 /in^2 (approximately 0.20 mg/dm^2) regulatory threshold of chloroform-soluble extractive migration from phthalate polymers into various food simulants. There are additional restrictions with respect to additive levels in virgin materials, and thresholds of regulation (TOR) for substances used in food-contact articles. Limitations for certain food contact substances (FCS) which have clearance for use are regulated within 21 CFR 170.39 (FDA 2016b; 2017). The European Union uses a different approach for plastics, where, overall limits are regulated to 10 mg/dm^2, and specific migration limits (usually in mg kg-1 or ng g-1) under (EC) 10/2011 (2011).

With all of this in view, it is difficult, if not impossible, to know if the molding/extrusion of resultant materials yields a product that meets the Code of Federal Regulations requirement without direct measurements of the final product. Current FDA policy mandates that manufacturers of food-contact packaging made from recycled plastic are responsible for manufacturing materials with specifications similar to packaging comprised of virgin material (2006). Furthermore, the recyclers must demonstrate that the contaminant levels in the packaging components are low enough to comply with the CFR and FDA requirements (2006).

Consumer demands for more sustainable packaging has led to the increase in PCR% content labels on many thermoplastic and paper packages. However, the safety of these recycled materials must be considered, and are regulated by many governments around the world by rules such as The Code of Federal Regulations (CFR) in the United States, which considers food

packaging as an indirect food additive. Therefore, risk assessment of packaging from a toxicology and regulatory perspective requires an understanding of both packaging-component toxicities and predicted levels of human exposure. Many large retailers and brand owners have launched initiatives in an attempt to increase the level of post-consumer recycled plastic and safety in packaging (Johnson, 2014), yet the responsibility of safety and environmental stewardship hangs on the converters and end users of these packaging materials.

Agricultural growers and packers utilize plastic packaging for retail convenience foods and food service products and are currently the largest users of plastic film and sheeting in the U.S. (Markets and Markets, 2014). Although the chemical and physical properties of packaging made from virgin materials may be known initially, externalities such as UV-degradation and exposure to other chemicals in the waste stream could significantly broaden the composition and overall quality of the material once recovered (Curtzwiler et al., 2011; Heckman, 2005; Markets and Markets, 2014; Nerin et al., 2003; Schwartz, 1988; Westerhoff et al., 2008). The suitability of a recycled material for direct food contact, therefore, is directly correlated to the source of the waste stream and the recycling method (Franz et al., 2004; Perring et al., 2001; Whitt et al., 2012).

The increase in single-stream recycling (i.e.- the incorporation of laundry detergent bottles and certain e-wastes) has made it difficult for thermoplastic to be conveniently sorted for food-contact materials (Fordham et al., 1995; Karayannidis and Achilias, 2007; Markets and Markets, 2014; Nerin et al., 2003; Westerhoff et al., 2008). Inadequate sorting can result in the incorporation of non-food-contact polymers into the recycling feedstock for direct food-contact packaging. The development of technology to monitor and evaluate the safety of plastic intended for food-contact materials can be a valuable tool to evaluate safety in real time, and can

allow for converters to adjust blend ratios of various streams of recycled and virgin feedstock to ensure safety and regulatory compliance. Additionally, these technologies have direct application to monitoring more than thermoplastic and paper materials, and can be used to monitor VOCs of interest in-situ.

Overall, the current state of microwave popcorn in both high and low-fat varieties needs to be evaluated, and a repeatable method for characterization and quantification should be established for microwave popcorn oil. Furthermore, the effects of elevated temperatures on DAPORS should be studied in further detail. Continuous monitoring of DAPORS and other forms of contamination in both food and packaging should be explored to provide solutions in-situ characterization in the manufacturing environment. This research has implications for stakeholders across the microwave popcorn value chain, including: customers, brand-owners, lawmakers, and researchers investigating the safety and sustainability of certain food products and their packaging.

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CHAPTER II

CHARACTERIZATION AND QUANTIFICATION OF DIACETYL AND POSSIBLE OTHER RELATED SUBSTANCES (DAPORS) IN HIGH-FAT AND LOW-FAT MICROWAVE POPCORN OIL BEFORE AND AFTER COOKING

Nathan Davis

Introduction

Microwave-popped popcorn is a commonly enjoyed snack food around the world. Popcorn Production in the United States is a $1.9 billion industry with $140 million in exports. 45.3% ($860.7 million) of this industry is comprised of un-popped popcorn most popularly sold in microwavable packaging (D'Costa, 2016). However, recent claims regarding the ability of common flavorings (diacetyl and 2,3-pentanedione) in popcorn oil formulations to cause a chronic lung condition, bronchiolitis obliterans (BO), has called the safety of microwave popcorn into question(Hubbs et al., 2012).

BO is an inflammatory condition that affects the bronchioles of the lung; the smallest airways within the organ. Many different chemicals can cause lung injury of this kind, such as nitrogen oxides, welding fumes, and ammonia. Symptoms of BO include a dry cough; shortness of breath; and/or fatigue and wheezing in the absence of a cold or asthma (King Jr, 2003; Yousem et al., 1992). Repeated inhalative exposures to 2,3-pentanedione have been shown to cause fibrosis of intra-pulmonary airways in rats, demonstrating symptoms similar to BO in humans (Morgan et al., 2012). Several studies have linked this disease to exposure to ketone-type flavoring compounds such as diacetyl Flake and Morgan (2016). And other studies claim microwave popcorn could pose a significant health risk to consumers. These claims have been

used to justify several considerable lawsuits of note in both plant workers in microwave popcorn facilities and consumers.

Microwave popcorn consists of un-popped popcorn kernels and flavored, salted oil in microwavable paper bags with a susceptor laminated between two layers of paper to maximize heat transfer. Popcorn oil for microwave applications is predominantly comprised of a semi-solid fat with additions of salt and butter-related flavorings. Palm and partially-hydrogenated soybean oil are the most commonly used fats in microwave popcorn. However, recent FDA ban on trans-fats in foods, popcorn oil formulations have shifted to palm oil (Carr, 2016). In 2008 the popcorn industry moved away from diacetyl in many popcorn formulations as a response to consumer complaints (Carr, 2016). However, compounds similar in chemical structure and taste to diacetyl remain in many formulations. There are seven such compounds that are generally related to diacetyl and butter-flavor formulations, which are often referred to within the microwave popcorn industry as DAPORS (Figure 2.1).

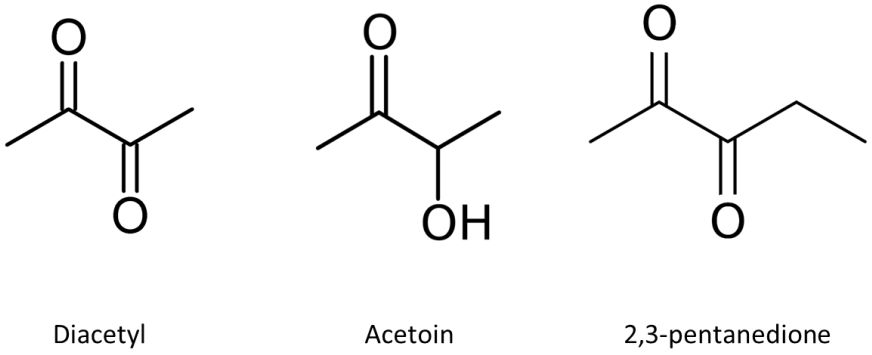

Figure 2.1. Structural identification of diacetyl, acetoin, and 2,3-pentanedione

Toxicology of DAPORS

Endogenous α-dicarbonyl compounds, such as those found in DAPORS (particularly diacetyl and 2,3-pentanedione) are among the reactive chemical species associated with the tendency to form cross-links with proteins (Miller and Gerrard, 2005). Furthermore, this same functional group has the capacity to form advanced glycation end products, or proteins or lipids that bind to sugar molecules (i.e.-glucose), specifically its electron-attracting carbonyl groups (Figure 1) (Roberts et al., 1999). 2,3-pentanedione has been reported to be even more reactive than diacetyl (Epperly and Dekker, 1989; Flake and Morgan, 2016; Hubbs et al., 2012). acetoin (3-hydroxy-2-butanone) is a structurally similar compound to diacetyl, and is often included in may butter flavor formulations. However, an α-hydroxyketone in acetoin replaces the reactive α-diketone associated with the toxicity of diacetyl and 2,3-pentanedione (Hubbs et al., 2012). To confirm this phenomenon, the US National Toxicology program (NTP) conducted a 90-day study on inhalative exposure to diacetyl and acetoin in Sprague-Dawley rats. Significantly higher levels respiratory tract lesions from exposures as low as 25ppm of diacetyl were found in both rats and mice when compared to acetoin (NIOSH, 2016).

Numerous studies have linked this disease to inhalative exposure to ketone-type flavoring compounds, such as diacetyl (Boylstein et al., 2006; Fedan et al., 2006; Flake and Morgan, 2016; Hubbs et al., 2008; Lockey et al., 2009; Rigler and Longo, 2010; Starek-Swiechowicz and Starek, 2014; van Rooy et al., 2007). One study claims microwave popcorn could pose a significant health risk to consumers (Egilman et al., 2011). This has led to several substantial lawsuits in both plant workers in microwave popcorn facilities and consumers. In lieu of official regulatory thresholds, a framework such as the threshold of toxicological concern (TTC) may be useful in conjunction with current toxicology data exposure levels found in microwave popcorn.

Threshold of Toxicological Concern

The Threshold of Toxicological Concern (TTC) a form of risk characterization originally developed by the FDA for packaging migrants in which a certain level of human exposure is considered to be of negligible risk based on toxicology data from other similarly structured compounds (Munro et al., 2008). These values are typically determined by obtaining the 5th percentile of cumulative probability distribution of NOELs for similarly structured chemicals (Escher et al., 2010). Frawley (1967) proposed that a general threshold of exposure to food packaging materials be employed to predictively identify chemicals which have toxic effects of low or negligible risk (Canady et al., 2013; Frawley, 1967). The TTC is designed for low-level oral exposures to chemicals. Therefore, quantitative data must exist on the levels of the compound in a food product, and the toxicological mode of action should be understood (Schrenk, 2016).

This model has also been applied to food contact materials, flavorings, and aerosols (Barlow et al., 2001; Carthew et al., 2009; Cheeseman et al., 1999; Cramer et al., 1976; Munro et al., 1996; Renwick, 2004; Schnabel et al., 2015). The TTC was developed by Munro et. al. (1996) who built upon Cramer's classification which divides chemicals into three classes based on structural properties suggestive of varying degrees of inherent toxicity:

Class I – Substances have simple structures, known metabolic pathways, and are of low potential toxicity.

Class II – Substances with structures less clearly benign than those in Class I, but do not have a positive identification of toxicity or are not well studied, which are typical of Class III substances.

Class III – Substances that contain structural features that have no strong initial presumptions of safety, or may even suggest significant toxicity.

A procedure was developed by Munro et. al. (1999) to apply the TTC framework to flavoring substances, which was then adopted by the joint FAO/WHO Expert Committee on Food Additives in 1996, and threshold values were proposed for each of the Cramer classes. (Munro et al., 2008). These threshold values were derived by dividing the fifth percentile of the distribution of no observed effect levels (NOEL) in each class (Table 2.1) (Canady et al., 2013).

Table 2.1. A selection of TTC values (oral) proposed in the scientific literature

Chemical class	Threshold value∗	
	µg/d	µg/kg body wt per d
Cramer class I	1800	30
Cramer class II	540	9
Cramer class III	90‡	1.5
Organophosphates	18	0.3
Nongenotoxic compounds	1.5	0.025
Genotoxic compounds	0.15	0.0025

Data from Kroes et al. (2004) and Felter et al. (2009). ∗For oral exposure, based on a body weight of 60 kg. ‡From Munro et al. (2008). (Canady et al., 2013)

Additionally, others have applied this framework to the inhalative toxicological risk of a variety of compounds. Work by Carthew and others (2009) developed corrected no observable adverse effect concentrations (NOAECs) and no observable adverse effect levels (NOAELs) for local and systemic adverse effects, respectively, to determine an appropriate threshold of toxicological concern (5^{th} percentile) for many aerosol ingredients in consumer products (Table 2.2).

Table 2.2. Corrected no observable adverse effect concentrations (NOAECs) and no observable adverse effect levels (NOAELs) for local and systemic adverse effects, respectively, to determine an appropriate threshold of inhalative toxicological concern (5th percentile) for many aerosol ingredients in consumer products (Carthew et.al., 2009)

Cramer Class	**Local Effects**	
	5th percentile for local effects NOAEC (mg/m3) for 6h day	*5th percentile NOAEL for local effects µg/g lung tissue/ day*
1	1.4	54
3	0.47	18
1+2+3	0.97	38
	Systemic Effects	
	5th percentile for systemic effects NOAEL (mg/kg/day)	*5th percentile NOAEL for systemic effects µg/kg/day* [a]
1	0.41	410
3	0.07	70
1+2+3	0.13	130
	Threshold of Toxicological Concern	
	TTC for local effects µg/g lung tissue/ day	*TTC for systemic effects µg/kg/day* [b]
1	2.1	16.4
3	0.73	2.8
1+2+3	1.6	5.1

[a] Assuming a rat lung weight of 1.4g.
[b] Assuming bodyweight of 60kg.

Thresholds are determined TTC could then logically be used to evaluate the potential level of toxicity of flavoring constituents emitted from microwave popcorn during cooking. TTC are first determined by gathering exposure data from the food product. This, combined with toxicology data yields the histological distribution for establishing the TTC.

Previous Works Measuring Diacetyl in Microwave Popcorn

Initial characterization of popcorn VOCs took place in the 1970's. Walradt and others (1970) conducted some of the first analyses on non-microwave popcorn VOCs, and identified 36 volatile compounds with some certainty and 20 tentatively identified compounds (Walradt et al., 1970). Work by Buttery and others found levels of 170ppm of diacetyl, and 200 ppm of 2,3-pentanedione (Buttery et al., 1997). Identifying sources of VOC contamination within a converted microwave popcorn bag can be a difficult task due to its many layers and constituents. Rigler and Longo (2004) used scanning electron microscopy to measure the particle size of diacetyl from various forms of popcorn flavorings (Rigler and Longo, 2010). Rengarajan and Seitz (2004) analyzed flavor compounds from microwave popcorn using a rather elaborate supercritical fluid CO_2 apparatus in conjunction with SPME and to GC-MS (Rengarajan et al., 2004). Rosati and others (2007) analyzed VOCs in microwave popcorn placed a microwave into an inert chamber and developed a purge and trap mechanism for collecting and analyzing VOCs of interest The authors noted greater than 80% of VOCs released occur at the opening of the bag, post-cooking (Rosati et al., 2007). This work focused on a fully converted and filled bag and replicated end-use conditions. Furthermore, results showed that most of the VOCs analyzed were from flavor and oil constituents. Xie et al. (2012), analyzed VOCs in disposable paper packaging at various temperatures, but did not characterize volatiles at temperatures higher than 90˚C. This work focuses on migration of various compounds into food matrices, and does not address the volatilization of these compounds into the consumer's cooking atmosphere (Xie et al., 2012). Recent work by Zhang and others (2014) characterized fine and ultrafine particle emissions from microwave popcorn using a water-based condensation particle counter (Zhang et al., 2014). The authors noted a significant increase in total and ultrafine particle emissions in microwave

popcorn packaging with susceptors, as opposed to a brown paper bag. However, this study may have been biased since it incorporated a variable that may contain a recycled feedstock; and could thus contain a variety of VOCs that could be more toxic than particulate matter generated from the susceptor technology. Headspace temperatures of a bag of filled microwave popcorn can reach 200˚C and higher (Risch, 2009), depending on several parameters, such as the amount of oil present and the wattage of the microwave. Thus, when converted bag structures are subjected to elevated temperatures various volatile organic compounds (VOCs) within any part of the bag can be released during cooking. Many flavorings found in microwave popcorn have been studied in other food products such as Dairy and other snack foods (Clark and Winter, 2015). However, literature is sparse that both characterizes and quantifies flavor volatiles in microwave popcorn, or seeks to understand how DAPORS are affected by the high temperatures of the microwave environment; a critical parameter for establishing a TTC.

Furthermore, there is no recent work evaluating temperatures reached using laminated PET susceptors in microwave popcorn bags. Initial patent filings of susceptor technology show the temperature of a bag of microwave popcorn reaching temperatures of 200ºC and above when using a Myglyol (synthetic triglyceride mixture) food simulant. However, there has been little work to establish the headspace temperature of a bag of microwave popcorn. The subsequent experiments seek to establish the temperature of the headspace of a bag of microwave popcorn during cooking; to characterize and quantify DAPORS potentially formed and emitted during cooking; and to establish a TTC for the compounds listed based on their concentrations in microwave popcorn, and mode of action.

Materials and Methods

Reagents

The identity of the compounds was verified using reference standards of 2-butanone (>99%), 2,3-butanedione (>95%), 2,3-pentanedione (>97%), 2,3-hexanedione (>90%), 3-hydroxy-2-butanone (neat), from Sigma-Aldrich (St. Louis, MO, USA); and 3,4-hexanedione (>96%), 2,3-heptanedione (>98%), and 5-methyl-2,3-hexanedione (>94%) from TCI (Portland, OR, USA). Retention times were matched on the multidimensional GC capillary column to the samples, and mass spectra were used to confirm m/z qualifying ions to the spectral library.

Palm oil was obtained from a local grocery store to matrix match standards to samples. Blanks were run and subtracted from subsequent calibration samples.

Samples

High-fat (movie theatre-style) and low-fat (healthy) varieties of microwave popcorn were obtained from a popcorn manufacturer. High-fat samples had a total fat content of 36g (12g/serving) as palm oil, and 990mg (330mg/serving) of sodium per bag. Low-fat samples had a total fat content of 5g (2g/serving) of fat as palm oil, and 625mg (250mg/serving) of sodium per bag. The label on both products claimed that neither product contained no added diacetyl butter flavorings.

Temperature Profile Analysis

Filled bags were punctured with a small hypodermic needle at the top of the bag, just beneath the manufacturers seam (Figure 3b). The bag was placed into a Sharp (Model: 1000 W/R-21LT) commercial microwave oven coupled to Tripp-Lite 1000W line conditioners

(Model: LR1000) (Chicago, IL). The wattage of each microwave oven was calibrated according to ASTM Standard F1317 (ASTM, 2012). Average wattage output was calculated to be around 700 Watts. Voltage output from the outlet was measured at 116 Volts (AC).

An OPTOCON fiber optic temperature sensor (Model P/N: TS3 – 10mm O2) was placed into the headspace of the bag with the tip of the probe fully immersed into the package to ensure that it would not dislodge during the cooking process. Each bag was microwaved for 2 minutes and 19 seconds. Cooking time was determined by calculating the average cooking time of six bags of each flavor according to the manufacturer's instructions (listening for 1-3 seconds between pops). The sensor recorded temperature values every second for the duration of the cooking time (Figure 2.3).

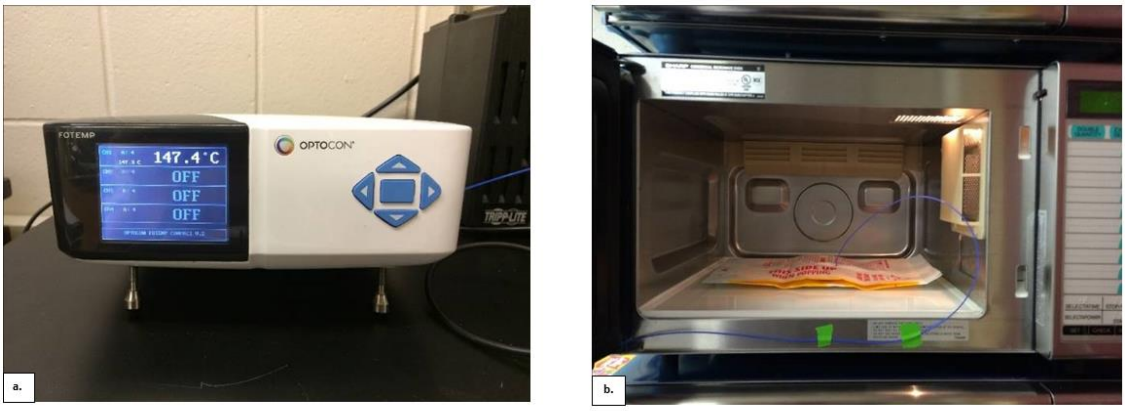

Figure 2.3. a.) Temperature probe reader used for temperature profiling; b.) Location of temperature probe in popcorn bag during cooking

Sample Preparation

High-fat (movie theatre-style) and low-fat (healthy) varieties of microwave popcorn were opened, and the contents were removed from the package into a glass jar with screw-top lid. Samples were heated at 40ºC just until the oil had melted. Oil was separated from the corn using

a glass Pasteur pipette directly into the 20mL amber headspace vials. Not enough oil could be removed from the package in the low-fat varieties, and thus, oil was obtained directly from the manufacturer. Five (5) grams of oil were weighed into 20mL headspace vials and immediately sealed.

One set of samples from each variety was heated in a 60L convection oven (Thermo Scientific, Waltham, Massachusetts, USA) until it reached a temperature of 190ºC; the average temperature reached in the headspace of a bag of low-fat microwave popcorn. The temperature of the oil in the sealed headspace vials was monitored by inserting an OPTOCON fiber optic temperature sensor (Model P/N: TS3 – 10mm O2) into a vial filled with 5 grams of palm oil.

Instrument Conditions

A Leap Technologies CombiPAL autosampler system (Trajan Scientific, Pflugervill, TX, USA) coupled to a multidimensional/gas chromatography - mass spectrometry (MD/GC-MS) (MOCON, Round Rock, TX, USA) was used for all analyses (Agilent 7890B GC/5977A MS; Santa Clara, CA, USA), and was fitted with two columns in series. The first column was non-polar (BPX-2, 83 m x 530 µm x 0.5 µm, SGE-Trajan Scientific, Pflugerville, TX, USA) and pressure balanced at the midpoint with a second polar column (DB-WAXETR, 30 m x 530 µm x 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). Effluent from the second column was split 1:3 by restrictor columns to the single quadrupole mass spectrometer and olfactometery sniff port, respectively. The GC run parameters used were as follows: the injector was held at 260°C in splitless mode; oven conditions held 40°C for 3 min, the ramped at 7°C/min to 105°C for 0min, then 10°C/min to 220°C and held for 1.2 min. Helium was used as a carrier gas at a rate of 8.6 ml/min^{-1}. The mass to charge ratio (m/z) range was set between 29 and 280. Spectra were collected in scan mode at 6 scans per second, and electron ionization energy was set at

70 eV. The MS source was held constant at 230°C and the quadrupole was held at 150°C. The instrument was tuned daily prior to analysis. MassHunter (v. B.07.00.1413, Agilent, Santa Clara, CA, USA) and a NIST11 spectral library were used for mass spectra data acquisition and analysis. Multitrax Multidimensional Control Software (v. 10.1, MOCON, Round Rock, TX, USA) was used for pressure balance programing.

SPME/GC-MS

Preliminary experiments were run to determine optimum fiber composition (Carboxen/polydimethyl siloxane/divinyl benzene, and carboxen/polydimethyl siloxane) and extraction temperature (30ºC, 35ºC, and 40ºC) using a 10ppm standard of diacetyl in palm oil. A 85 µm Carboxen/polydimethyl siloxane (CAR/PDMS) SPME fiber (57335-U, Sigma-Aldrich, St. Louis, MO, USA) was determined to be optimal for sensitivity and appropriate for the detection levels associated with the samples for extraction and pre-concentration (Figure 2; Appendix I). The CombiPal autosampler used for automated headspace sampling was set to the following parameters: 500 rpm agitation speed, 10 min incubation/extraction time at 40 °C, 260 °C desorption for 2 min directly into the GC inlet. To prevent carryover between samples, the SPME fiber also cleaned in a needle heater (260 °C for 2 min) under flow of ultra-high purity helium prior to each analysis.

Analysis of variance (ANOVA; $\alpha < 0.05$) and least squares means difference test (Tukey HSD; $\alpha < 0.05$) were conducted on data collected from experiments run with 100ppm standards adjusted for high-fat and low-fat sodium concentrations. Results from the ANOVA determined that adjusting for sodium concentration had no significant effect on peak area concentrations (p-value: 0.1488).

Analysis of variance (ANOVA; $\alpha < 0.05$) and least squares means difference test (Tukey HSD; $\alpha < 0.05$) were also conducted on data collected from experiments run with 100ppm standards with different fiber compositions and extraction temperatures. Results from the ANOVA determined that fiber composition had a significant effect on peak area concentrations (p-value: 0.0333), but the effect of extraction temperature was not significant (Figure 8).

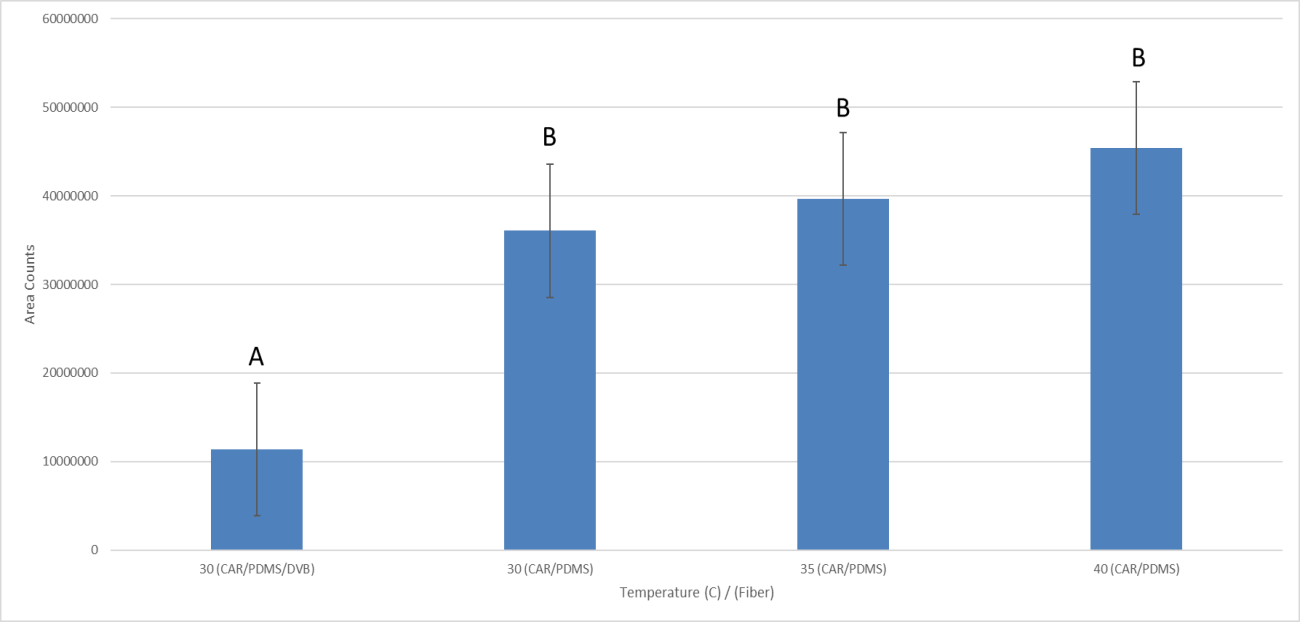

Figure 2.6. Analysis of variance on the effect of fiber composition and temperature in SPME extractions (N=3)

5g of palm oil was weighed into 20mL amber headspace vials. Quantitative analysis was done from calibration plots obtained from pure analytical standards (0.1-100µg/g). All tests and analyses were conducted in triplicate. Linear ranges, limits of detection (LOD) and quantification (LOQ) of the standards are presented in Table 1 of Appendix I.

Statistical Analysis

Data were presented as mean ± standard deviation. The Analysis of Variance (ANOVA) test was used to analyze the differences among group means and their associated procedures for each compound, and were blocked by fat content. Differences were tested by the student's t-test to find means that were significantly different from each other. The P-values that were < 0.05 were considered statistically significant. All data analyses were conducted using SAS JMP13 software (SAS, Cary, NC).

Results and Discussion

Temperature Profiling

Maximum temperatures achieved under the cooking conditions utilized here varied as a function of the amount of oil present in the sample. High-fat samples reached an average temperature of 162 ºC, which occurred approximately 20 seconds after the end of cooking (Figure 4). A rapid increase in temperature of approximately 130 ºC was observed within the first minute of cooking for all samples analyzed.

Low-fat samples yielded average maximum temperatures of 177 ºC, which occurred approximately 5-10s after the end of cooking. Maximum temperature observed in a single repetition was 205.9 ºC. A 23ºC decrease in temperature was observed from 1:01-1:36, likely due to an endothermic reaction by which the moisture from the popcorn was being vaporized causing evaporative cooking effects in the headspace of the bag. The continued increase in temperature after cooking is likely explained by the release of latent heat stored in the un-popped popcorn kernels and non-instantaneous heat transfer from the environment to the probe.

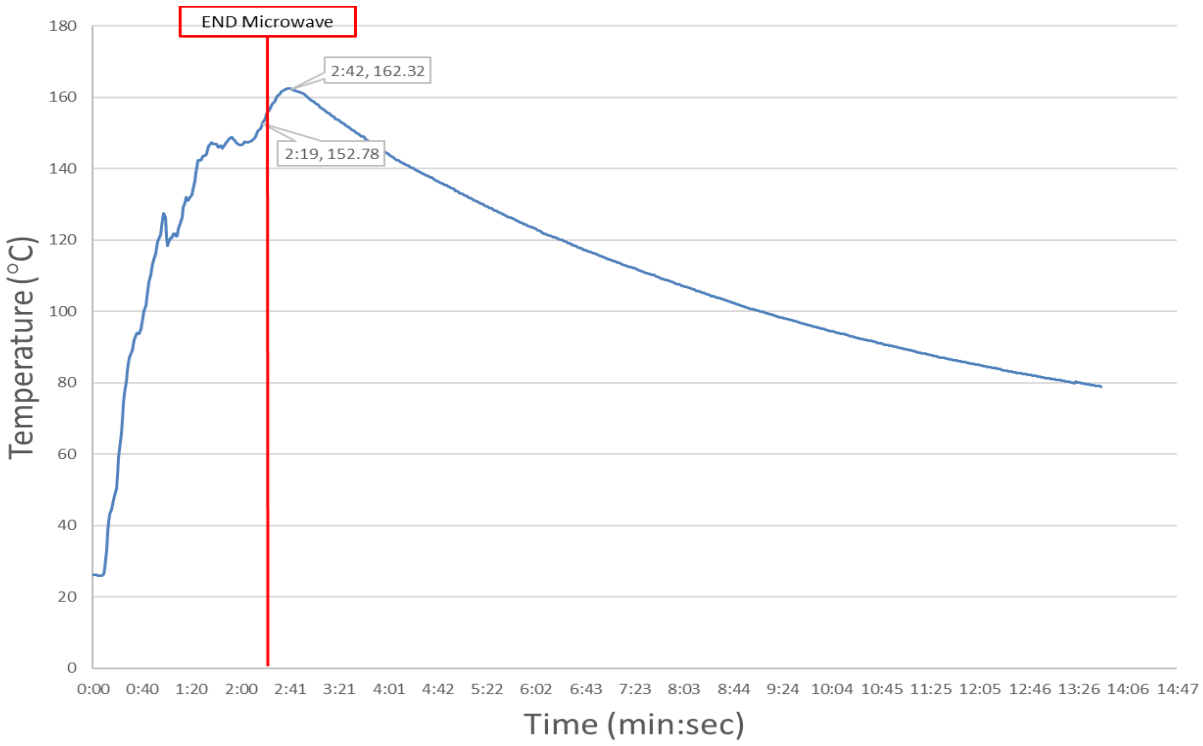

Figure 2.4. Average Temperature of the headspace of high-fat microwave popcorn during cooking

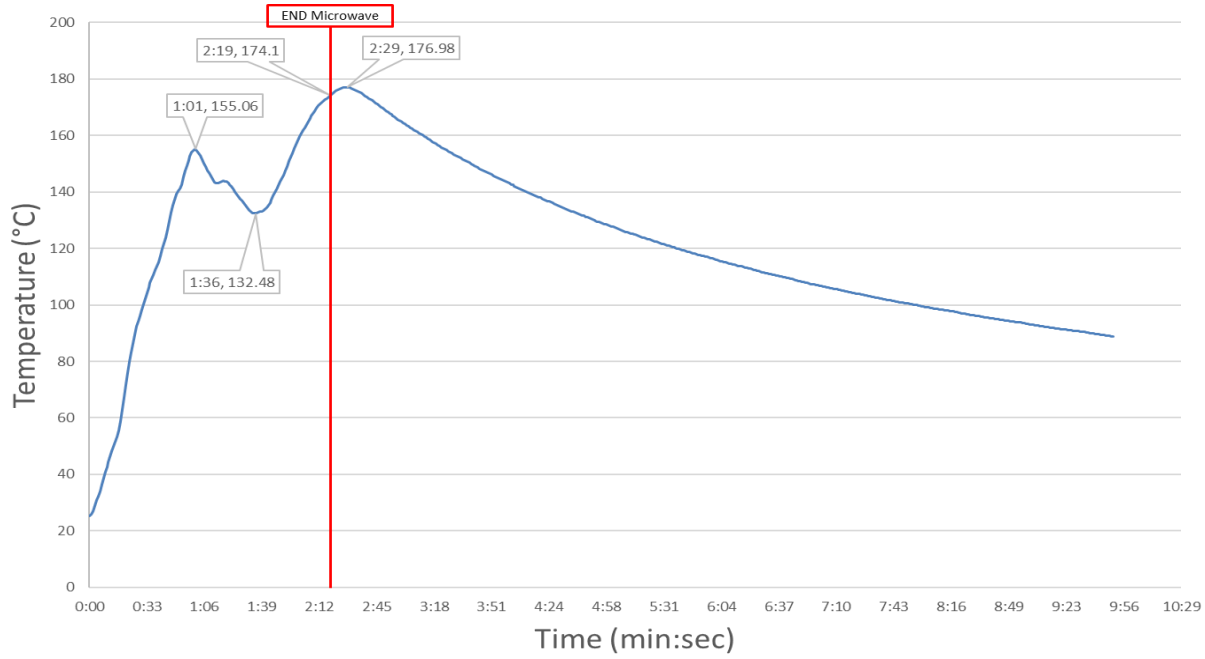

Figure 2.5. Average Temperature of the headspace of low-fat microwave popcorn during cooking

Higher temperatures observed in the low-fat samples could be due to the lower total fat content, thus more efficiently heating the headspace and generating higher temperatures. It is also worth noting that scorching and gas bubbles often occurred between the metalized susceptor and inner layers of the package in low-fat samples.

SPME GC-MS Analysis

The results of the quantitative analysis are outlined in Table 2.3 below with TTC values based on the Cramer Class (CC) of the compound. Of the seven compounds tested for in high-fat microwave popcorn oil samples, 2,3-hexanedione was found in the highest concentrations in high-fat samples, followed by acetoin, 2,3-pentanedione, acetyl valeryl, and diacetyl was found in the lowest concentrations. After exposing the high-fat samples to elevated temperatures, increased levels of diacetyl, 2,3-hexanedione, acetoin, and acetyl valeryl were observed. In low-fat samples, High concentrations of acetoin, 2,3-pentanedione, diacetyl, and acetyl valeryl were found. After heating, Increased concentrations of diacetyl were observed, while acetoin, 2,3-pentanedione, and acetyl valeryl showed decreasing concentrations. 2,3-pentanedione was the only compound found to have a significant decrease in concentration after heating (p-value: 0.0082) in both high and low fat samples. Additionally, there is moderate evidence to suggest that concentrations of diacetyl significantly increased after heating (p-value: 0.0284) in both high and low fat samples. This may be due to breakdown of higher carbon-chained DAPORS, or thermodynamically favorable chemistries reacting with other constituents within the bag during elevated temperatures, such as the Maillard reaction and Strecker degradation with dicarbonyl groups (Coultate, 2009).

Table 2.3. DAPORS Identified and concentration (mg/kg), retention time (RT; min), molecular formula, threshold of toxicological concern (TTC) proposed levels*, Cramer Class (CC)

RT (Min)	Compound	Molecular Formula	CAS No	Before Heating		After Heating to 190°C		TTC (µg/ day from inhalative exposure)**	TTC (µg/kg body wt. per day; oral)**	CC
				High-fat Amount (ppm)	Low-fat Amount (ppm)	High-fat Amount (ppm)	Low-fat Amount (ppm)			
4.5	diacetyl (2,3-butanedione)	$CH_3COCOCH_3$	431-03-8	0.28 ± 0.05^A	9.43 ± 0.56^B	0.67 ± 0.04^C	14.16 ± 1.90^D	5.1	9	II
6.3	2,3-pentanedione	$CH_3CH_2COCOCH_3$	600-14-6	9.04 ± 1.11^A	80.94 ± 1.64^B	5.47 ± 0.05^C	24.92 ± 1.55^D	2.8	1.5	III
8.3	2,3-hexanedione	$CH3(CH2)_2COCOCH_3$	3848-24-6	32.61 ± 8.09^A	< LOD	41.63 ± 8.44^A	< LOD	2.8	1.5	III
8.7	3,4-hexanedione	$CH_2CH_2COCOCH_2CH_3$	4437-51-8	< LOD	< LOD	< LOD	< LOD	2.8	1.5	III
9.3	acetoin (3-hydroxybutanone)	$CH_3COCH(OH)CH_3$	513-86-0	19.12 ± 2.31^A	348.71 ± 6.80^B	49.21 ± 1.36^A	253.92 ± 11.81^B	16.4	30	I
9.5	5-methyl-2,3-heptanedione	$(CH_3)_2(CH_2)_3COCOCH_3$	13706-86-0	< LOD	< LOD	< LOD	< LOD	2.8	1.5	III
11.1	acetyl valeryl (2,3-heptanedione)	CH3(CH2)3COCOCH3	96-04-8	4.90 ± 1.57^A	4.29 ± 0.35^A	5.91 ± 1.56^A	3.97 ± 0.23^A	5.1	9	II

*Different letters indicate significant differences between compounds. (N=12).
**Proposed levels are adapted from Carthew et.al. (2009). Inhalative thresholds assume a human bodyweight of 60kg.
***Linear Ranges, LOD, and LOQs are available in Appendix I.

Levels of diacetyl and 2,3-pentanedione have significantly decreased from levels previously measured in popcorn (170ppm and 200ppm, respectively) (Buttery et al., 1997). Discussions with suppliers indicated that total fat content is based on the total weight of the oil slurry (oil, flavor, salt, coloring, etc.). Thus, it was determined that the true amount of oil per bag for high and low-fat varieties was 33.3g and 2.4g, respectively. Based on the data collected, an approximation of DAPORS per bag, and their potential concentrations after cooking are organized in table 2.4 below. Assuming an individual inhales the entire vaporized contents of a bag of microwave popcorn, consumers could be inhaling considerable amounts of DAPORS

which may cause deleterious effects to pulmonary function. However, it is important to note the previous work of previous literature which notes that greater than 80% of Volatiles are released upon opening of the bag (Rosati, 2007).

Table 2.4. Determination of total DAPORS per bag of microwave popcorn in high and low-fat varieties.

DAPORS	Oil Content per Bag (g)		Total before heating (µg/bag)		Total After Heating (µg/bag)		Opening bag after cooking (80% loss)	
	H	L	H	L	H	L	H	L
diacetyl	33.3	2.4	9.3	22.6	22.3	34.0	4.5	6.8
2,3-pentanedione	33.3	2.4	301	194.3	182.1	59.8	36.4	12
2,3-hexanedione	33.3	2.4	1085.9	N/A	1386.3	N/A	277.3	N/A
acetoin	33.3	2.4	636.7	836.9	1638.7	609.4	327.7	121.9
acetyl valeryl	33.3	2.4	163.1	10.3	196.8	9.5	39.4	1.9

TTC Approach to Evaluating Inhalative Toxicity

Four of the eight chemicals of interest were found to be Cramer Class III compounds. Diacetyl was found to be a Cramer Class II compound. TTC and Cramer Class data was not available for 2,3-heptanedione. In low-fat popcorn, diacetyl and 2,3-pentanedione levels were found to be higher than proposed TTC levels. After adjusting DAPORS levels to account for total amount per bag of microwave popcorn, and adjust for loss upon opening of the bag, Diacetyl was found to be within the TTC limit of 5.1 µg, indicating Diacetyl may not be as serious of a health concern as some claim. However, levels of other DAPORS of interest are several times higher than the TTC in both high and low-fat samples.

Additionally, levels exceed the NIOSH proposed STEL values of 25ppb (15min), 75ppb (5min) and 375ppb (1min) for diacetyl; and 31ppb (15min), 93ppb (5min), and 375ppb (1min) for 2,3-pentanedione by several orders of magnitude (NIOSH, 2016). The results obtained, as well as samples corrected for serving size and loss of volatiles to the atmosphere, were much higher than NIOSH recommended levels. However, many consumers are not exposed to the same concentrated levels of DAPORS as those found in many microwave popcorn production facilities. Therefore, careful consideration and account for the context of the NIOSH regulations should be taken when attempting to extrapolate these levels to the average consumer.

Conclusion

This study characterized and quantified DAPORS found in high-fat and low-fat microwave popcorn, and the impact of the high microwaving temperatures on such compounds. Elevated amounts of 2,3-pentanedione, acetoin, 2,3-hexanedione, and acetyl valeryl were found, and exceeded proposed TTC inhalative thresholds as well as NIOSH recommended standards. Levels of diacetyl were found to be in amounts that fell below the threshold of toxicological concern. Of the seven compounds tested for in high-fat samples, 2,3-hexanedione (32.61 ± 8.09) was found in the highest concentrations in high-fat popcorn samples, followed by acetoin (19.12 ± 2.31), 2,3-pentanedione (9.04 ± 1.11), acetyl valeryl (4.90 ± 1.57), and diacetyl was found in the lowest concentrations (0.28 ± 0.05). After exposing the high-fat samples to elevated temperatures, increased levels of Diacetyl (0.67 ± 0.04), 2,3-hexanedione (41.63 ± 8.44), acetoin (49.21 ± 1.36), and acetyl valeryl (5.91 ± 1.56) were observed. In low-fat samples, high concentrations of acetoin (348.71 ± 6.80), 2,3-pentanedione (80.94 ± 1.64), diacetyl (9.43 ±

0.56), and acetyl valeryl (4.29 ± 0.35) were found. After heating, increased concentrations of diacetyl (14.16 ± 1.90) were observed, while acetoin (253.92 ± 11.81), 2,3-pentanedione (24.92 ± 1.55), and acetyl valeryl (3.97 ± 0.23) showed decreasing concentrations.

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CHAPTER III

SENSOR ARRAY FOR DETECTION OF ORGANIC AND INORGANIC CONTAMINANTS IN POST-CONSUMER RECYCLED PLASTICS FOR FOOD CONTACT

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ABSTRACT

Post-consumer recycled (PCR) plastic material is made by collecting used plastic products (e.g., bottles and other plastic packaging materials) and reprocessing them into solid-state pellets or flakes. Plastic recycling has positive environmental benefits, but may also carry potential drawbacks due to unwanted organic and inorganic contaminants. These contaminants can migrate into food packaging made from these recycled plastic materials. The purpose of this research was to identify economically viable real-time monitoring technologies that can be used during the conversion of virgin and recycled resin feedstocks (i.e., various blends of virgin pellets and recycled solid-state pellet or mechanically ground flake) to final articles to ensure the safety, quality and sustainability of packaging feedstocks. Baseline analysis (validation) of real-time technologies was conducted using industry-standard practices for polymer analysis. The data yielded supervised predictive models developed by training sessions completed in a controlled laboratory setting. This technology can be employed to evaluate compliance and aid converters in commodity sourcing of resin without exceeding regulatory thresholds. Furthermore, this technology allowed for real-time decision and diversion strategies during the conversion of resin and flake to final articles or products to minimise the negative impact on human health and environmental exposure.

CHAPTER IV

GENERAL CONCLUSIONS AND FUTURE WORK

The overall objective of the work was to characterize and quantify levels of diacetyl and possible other related substances (DAPORS) in microwave popcorn and estimate their potential health risk to the consumer using a threshold of toxicological concern framework combined with proposed regulatory thresholds. Secondarily, this work established the theoretical basis for real-time monitoring applications in certain food and food packaging applications; namely detection of volatile organic compounds (VOCs). Table 4.1 summarizes the results of the analyses detailed in chapter's 2 and 3. The first study (Chapter 2) focused on identifying key cooking temperatures and heating rates in current microwave popcorn packages to determine the temperatures achieved during cooking. These parameters were then used to heat the oil to observe any changes in composition or quantity of DAPORS being released. Results indicated that initial levels of diacetyl and 2,3-pentanedione were well above proposed regulatory thresholds, and the inhalative threshold of toxicological concern proposed by Canady and others (2013). After heating, Increased levels of diacetyl were found in low-fat popcorn varieties. 2,3-pentanedione levels also increased.

The second study (Chapter 3) identified economically viable real-time technologies to monitor and evaluate the safety and performance of virgin and recycled thermoplastics. Baseline analysis was conducted using industry-standard practices for packaging analysis in a controlled laboratory setting. Predictive models were generated via a series of training sessions of known variables to generated the model. This technology allowed for real-time decision and diversion strategies during the conversion of virgin and recycled feedstocks to mitigate the introduction of

excessively toxic materials into the environment; thus improving safety and end-of-life sustainability. This technological framework has applications in monitoring the safety of microwave popcorn and its packaging. Sensors such as FT-IR can be used to continuously characterize and quantify DAPORS in real-time to monitor changes in formulations, deviations in process controls, and improve the safety of these foodstuffs for both the plant employee and the consumer.

Overall, this research has shed light on the current state of microwave popcorn in both high and low-fat varieties, developed a repeatable method for characterization and quantification, and studied the effects of elevated temperatures on DAPORS. Additionally, this work proposed solutions for continuous monitoring of DAPORS in-situ with the ability to characterize in-plant. This research has implications for stakeholders across the microwave popcorn value chain, including: customers, brand-owners, lawmakers, and researchers investigating the safety and sustainability of certain food products and their packaging.

Table 4.1 Summary of Diacetyl Characterization and Real Time Monitoring Results

Thesis Chapter	Analysis or Technique	Notable Results
2	Temperature Profiling	Temperatures of the headspace of microwave popcorn can reach average temperatures of 180ºC and higher.
2	SPME/GC-MS	Levels of 2,3-pentanedione, action, 2,3-hexanedione, and acetyl valeryl were found in elevated levels in both high and low-fat popcorn oil. These concentrations were above both the TTC levels and NIOSH proposed levels.
2	SPME/GC-MS	Levels of diacetyl were within the threshold of toxicological concern (TTC) in both high and low fat varieties of popcorn, after correction for serving size and loss of volatiles to the atmosphere.

Table 4.1 Continued. Summary of Diacetyl Characterization and Real Time Monitoring Results

3	Real-time Monitoring	Applications for monitoring levels of DAPORS in-situ are feasible, and could be incorporated into a filling line for continuous monitoring.
3	Real-time Monitoring	Real-time monitoring technology is a valuable tool that can be incorporated into regular testing schema to enhance and improve the safety and quality of the manufacturing and end-user environment.

Future Work

Future work in Chapter 3 objectives should focus on extending the core concepts of real-time monitoring to paper packaging materials, and monitoring of DAPORS in the manufacturing environment using an array of sensors and combined predictive neural network. Technology to both spectate and quantify volatile compounds of interest in real-time is scant. Given the large and dynamic range of VOCs, speciation is also an important parameter of continuous monitoring to ensure proper process control and identify continual 'bad actors' in the process. An example of one sensor (out of a plurality) is Fourier Transformed Infrared Spectroscopy (FT-IR). Vibrational spectroscopy has been used for a wide variety of applications, and has become a universally accepted method for both qualitative and quantitative analysis of a multitude of compounds. Several DAPORS have also been studied, both in isolation and in application, using FT-IR techniques (Figure 4.1) (Gómez-Zavaglia et al., 2003; Povolo, 2011). The development and employment of this technology could have a positive impact on the microwave popcorn industry and potentially revive the segment. Moreover, real-time monitoring technology can be applied to a wider variety of VOCs of interest; such as those regulated by the State of California in Proposition 65, and can be tailored to individual requirements and performance metrics.

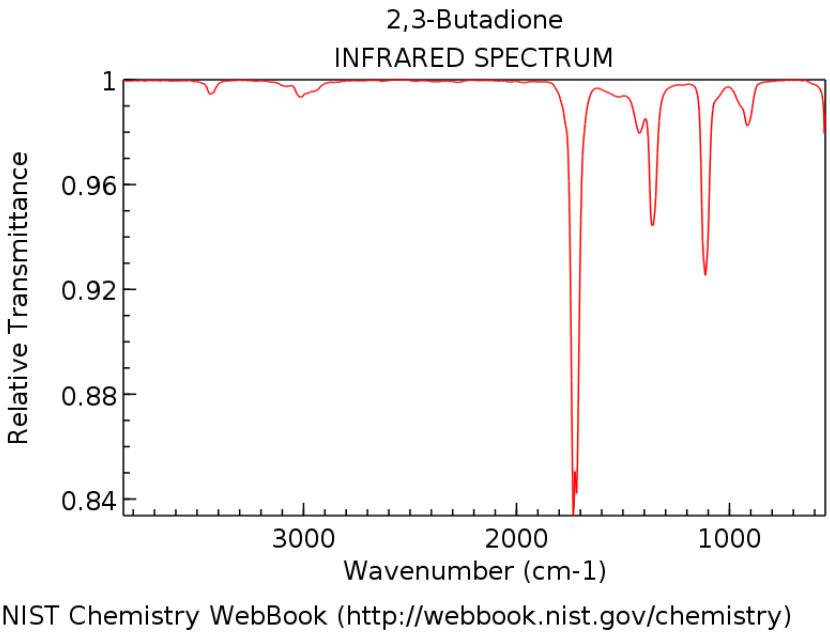

Figure 4.1. Sample Infrared Spectrum of diacetyl (2,3-butadione) collected in the gas phase using Gas-Chromatography/ Mass Spectrometry/ Infrared Detection (GC/MS/IRD) (NIST, 2009)

References

Gómez-Zavaglia, A., Fausto, R., 2003. Matrix-isolation and solid state low temperature ft-ir study of 2,3-butanedione (diacetyl). Journal of Molecular Structure 661, 195.

Povolo, M., 2011. Study on the use of evolved gas analysis ft-ir (ega ft-ir) for the evaluation of cheese volatile fraction. Tofsj 5, 10.

APPENDIX DIACETYL SUPPLAMENTAL INFORMATION

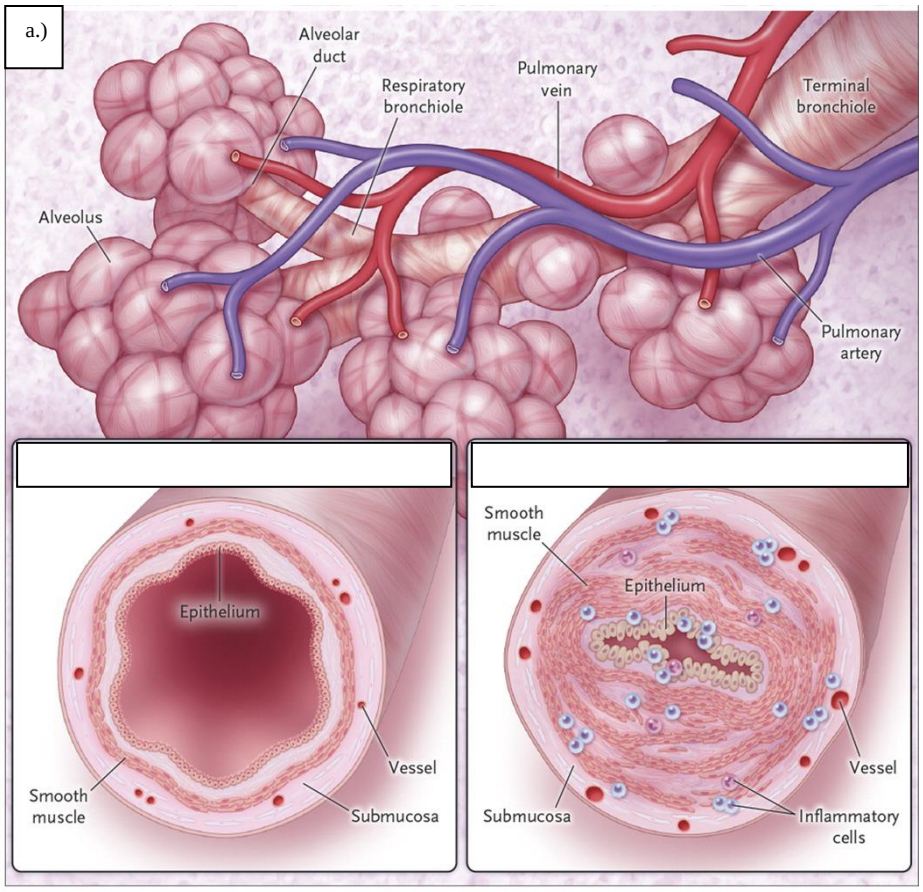

Figure A1. Schematic Views of Normal Bronchioles and Bronchioles with Bronchiolitis Obliterans (Barker et al., 2014).

Table A.1. Calibration standards for linear range, limits of detection (LOD) and quantification (LOQ) (mg/kg).

Compound	Linear Range	LOD	LOQ
2,3-butanedione	0-10	0.454	0.999
2,3-pentanedione	0-50	0.010	4.844
2,3-hexanedione	0-50	0.487	0.844
3,4-hexanedione	0-100	0.098	0.101
3-hydroxy-2-butanone (acetoin)	0-100	4.106	11.023
2,3-heptanedione	0-100	0.429	0.506

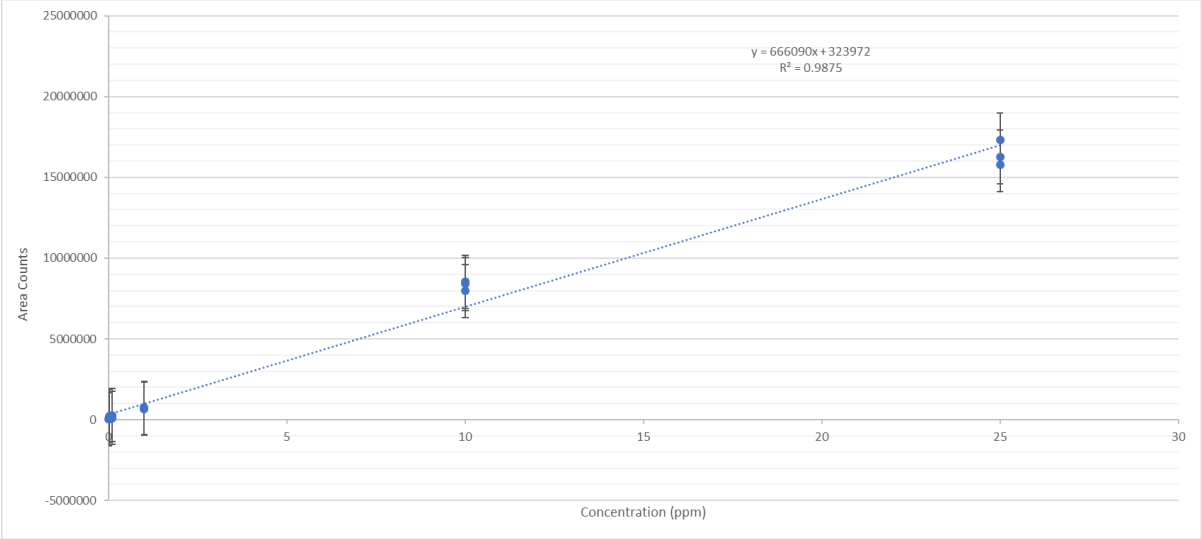

Figure A.2. Area counts versus concentration for 2,3-butanedione (diacetyl)

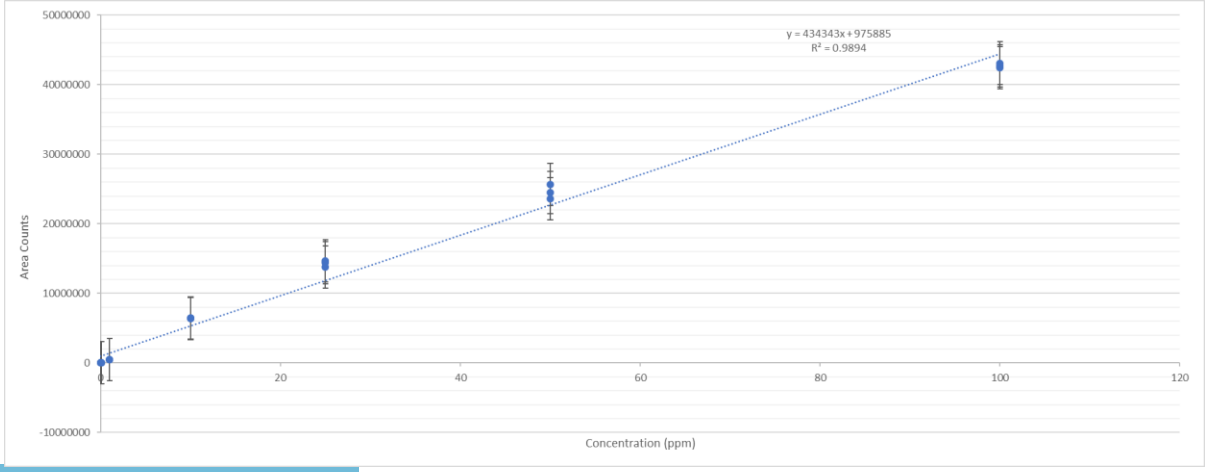

Figure A.3. Area counts versus concentration for 2,3-pentanedione (acetyl propionyl)

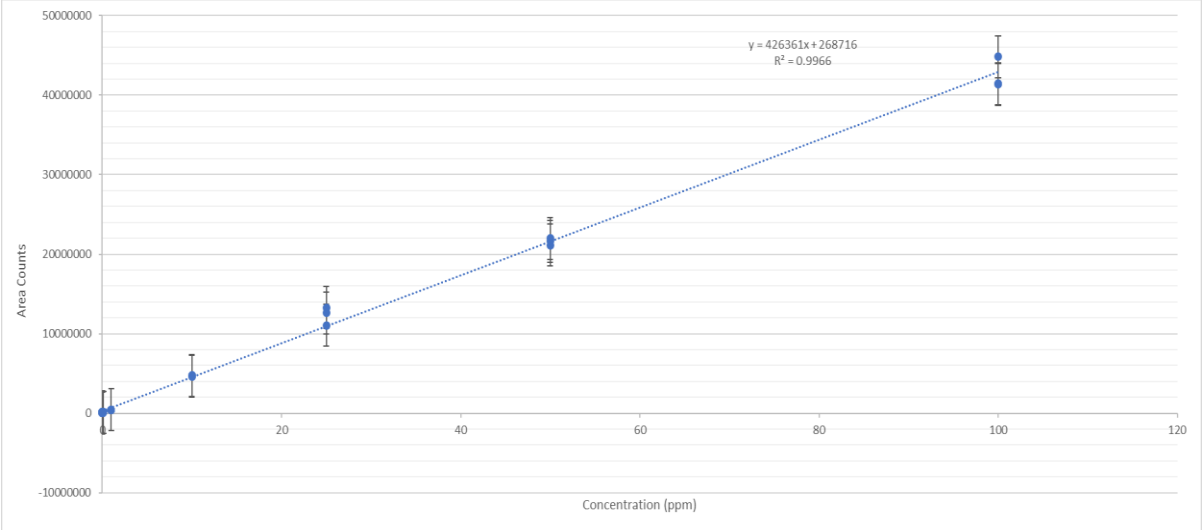

Figure A.4. Area counts versus concentration for 2,3-hexanedione

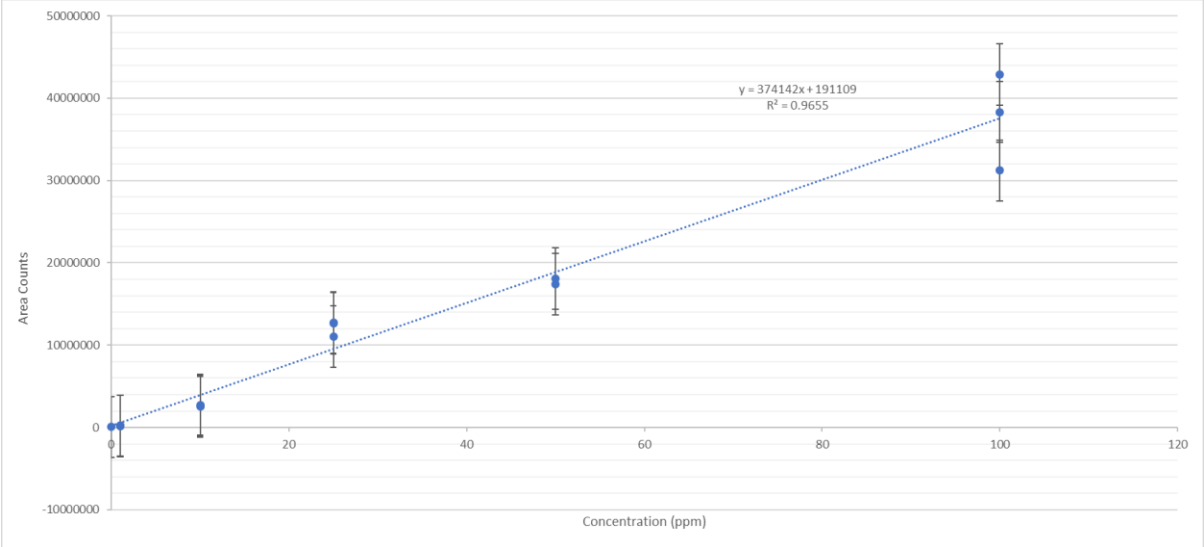

Figure A.5. Area counts versus concentration for acetoin (3-hydroxy-2-butanone)

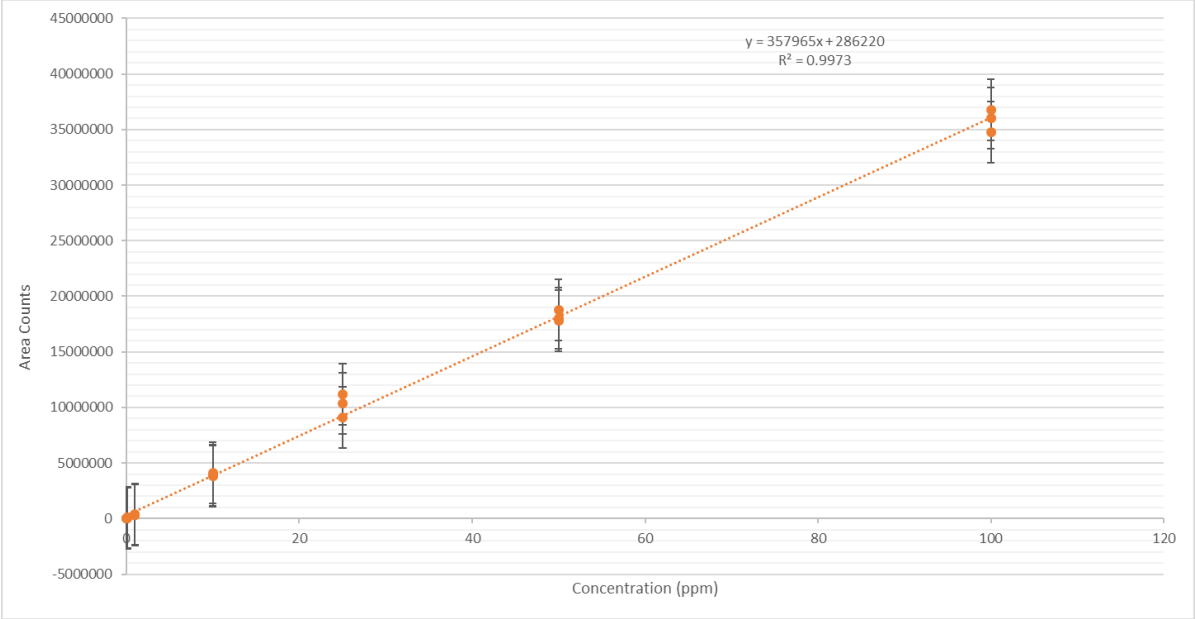

Figure A.6. Area counts versus concentration for 3,4-hexanedione

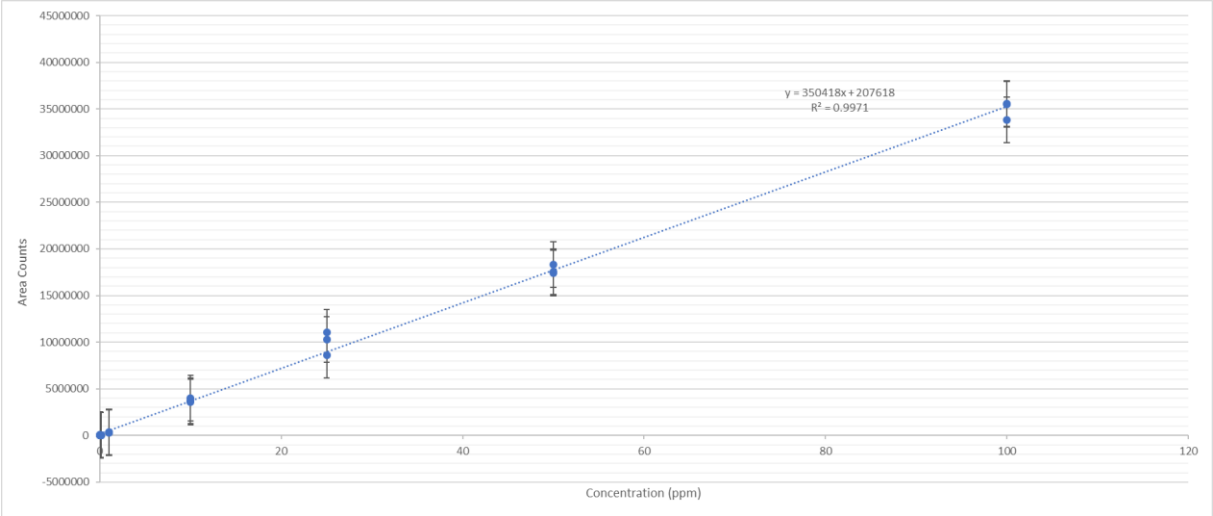

Figure A.7. Area counts versus concentration for 5-methyl-2,3-hexanedione

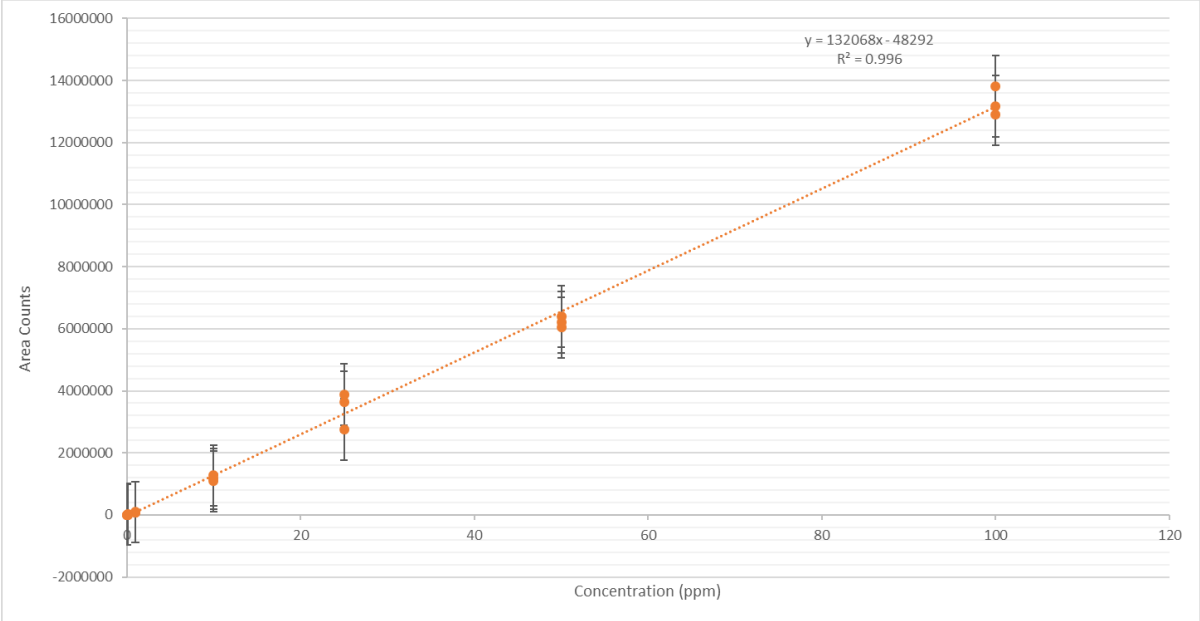

Figure A.8. Area counts versus concentration for 2,3-hepanedione

References

Barker, A.F., Bergeron, A., Rom, W.N., Hertz, M.I., 2014. Obliterative bronchiolitis. New England Journal of Medicine 370, 1820.